ENDEMIC GOITER

Report of the Meeting of the PAHO Scientific Group on Research in Endemic Goiter held in Puebla, Mexico, 27 to 29 June 1968

COMPILED AND EDITED BY

JOHN B. STANBURY, M.D.

PAN AMERICAN HEALTH ORGANIZATION
Pan American Sanitary Bureau, Regional Office of the
WORLD HEALTH ORGANIZATION

1969

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Report of the Meeting of the PAHO Scientific Group on Research in Endemic Goiter held in Puebla, Mexico, 27 to 29 June 1968

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PREFACE

Magnificent ceramic sculptures dating from pre-Colombian times are unassailable witness to the existence of goiter in the western world before the European settlement. Among the many figurines found in museums of archeology in the Republics of Central and South America, those with goiter are rare. The prevalence of goiter among the American Indians before Pizarro can only be conjectured. In the years which followed the Conquest numerous travelers on both sides of the spine of the Andes commented in their journals on the high frequency of the disease among the indigenes. Did the degradation and improvishment of a great people which certainly followed the arrival of the Spanish perturb some ecological balance which necessitated growth of the thyroid? We would like to know.

The first demonstrations of the effectiveness of iodine in eliminating endemic goiter were the trials of Marine and Kimball in Akron, Ohio, in 1917. The results were startling and were soon confirmed in Switzerland and later in Guatemala, where endemic goiter has now virtually vanished. No serious doubt remains concerning the effectiveness of iodized salt in prophylaxis of goiter, and as a public health measure salt iodization is economical, safe, and accepted. Indeed many of the governments of Latin America have taken heed and legislated iodization of salt, but with few exceptions programs have not been implemented effectively, nor the endemics reduced in scope. The reasons for these failures are in part economic, in part political, and in part sociogeographic. Endemic goiter remains today a major medical problem of rural Latin America, with an incidence and impact which is probably as significant as it was a century or two ago, and even more important because of the extraordinary rapid population growth of that part of the world.

In 1961, the Scientific Advisory Committee of the Pan American Health Organization (PAHO), a panel of distinguished medical scientists from the Western Hemisphere, set priorities for health research in Latin America. High on the list was endemic goiter. It was abundantly evident that the challenge of endemic thyroid disease warranted this degree of concern. It was also clear that much remained to be learned about the thyroid in endemic goiter. It also seemed possible that application of available research methods not only might yield some practical dividends, but also might provide a nidus in various academic centers around which research enterprise in medical science would crystallize.

The recommendations of the Advisory Committee were soon implemented by selection of several investigators in universities of Central and South America who were already experts in thyroidology and who worked in proximity to foci of endemic goiter. Choice of necessity was arbitrary and limited in order that the program could have a manageable size and realistic goals. The initial participants were Dr. Jorge Maisterrena from the Institute of Nutritional Diseases in Mexico City; Dr. Rodrigo Fierro from the University of Quito and the Technicologic Institute of Quito, Ecuador; Dr. José Barzelatto of the Salvador Hospital and the University of Chile; Dr. Yaro Gandra of the University of São Paulo, Brazil; Dr. Luis Carlos Lobo then of the University of Rio de Janeiro and now Dean of the Faculty of the University of Brasilia; and Drs. Marcel Roche and Karl Gaede of the Venezuelan Institute of Scientific Research in Caracas. Each of these investigators

commanded a laboratory which was close enough to patients with endemic goiter so that pathophysiological studies with contemporary techniques could be conducted. Each enthusiastically agreed to participate in the PAHO program. Plans were made, specific research programs suitable for each laboratory were identified, and problems of logistics, personnel and financing were engaged.

The first meeting of the group was held from 22 to 26 April 1963 under the sponsorship of PAHO at the Venezuelan Institute of Scientific Research in Caracas, with Dr. Marcel Roche as host. In addition to the scientists from the participating Latin American laboratories and their colleagues, several advisors from elsewhere attended. Among these were Dr. Herbert Vetter of the International Atomic Energy Agency, who had made many contributions to the application of radioisotopes to the study of endemic goiter in Austria, and Professor Andries Querido, then of the University of Leiden, the Netherlands, and now of the University of Rotterdam. Dr. Querido had just returned from a study of endemic goiter in the highlands of New Guinea. Also attending as advisors were Drs. Andre Ermans and Jacques Dumont of the Free University of Brussels and Dr. Christian Beckers of the University of Louvain, who had had extensive experience in endemic goiter in the northern and eastern zones of the Congo, and Dr. Robert Vought of the U.S. National Institutes of Health, Bethesda, Maryland.

Much was accomplished at this meeting. The consultants described the disease as they had seen it in various parts of the world. They demonstrated the kinds of studies which had proved profitable and which had clarified certain aspects of the problem and had raised new questions begging for research. In turn the participants from Latin America described the disease in their countries and the kinds of studies which they had been able to accomplish. It emerged with great clarity from this meeting that many aspects of endemic goiter remained for investigation and that the resources both in patient material and scientific personnel of Latin America lent themselves particularly well to further research both into the nature of the disease and into alternative programs of prevention and treatment.

The Caracas meeting concluded with an assignment of research programs and responsibilities to each of the Latin American participants. Dr. Maisterrena would establish a two-bed metabolic unit in a field station in the village of Tepetlixpa, not far from Mexico City. Dr. Fierro would continue his inventory of endemic goiter in selected villages of rural Ecuador, would continue to gather detailed demographic data from Tocachi and La Esperanza (villages 70 miles north of Quito), and would do a thorough in-hospital study of a group of typical cretins. Dr. Barzelatto would expand his genetic and physiological studies on the isolated goitrous Pewenche Indians of Pedregoso in southern Chile. Dr. Gandra was to continue his epidemiological studies among schoolchildren in the villages of São Paulo State and to begin studies on thyroid function and the effect of iodine prophylaxis under controlled conditions. Dr. Lobo's plans included extensive genetic tabulations in the Mato Grosso and in Goiás in central Brazil, combined with neurological investigations on a group of cretins transported from these regions to Rio de Janeiro. Drs. Roche and Gaede planned further observations on the thyroid function of primitive isolated Indians of southern Venezuela.

A second PAHO meeting took place in Cuernavaca, Mexico, from 4 to 8 October 1965, with Dr. Maisterrena as host. By this time, Drs. Eduardo Pretell and Federico Moncloa of the Cayetano Heredia Medical School in Peru had joined the program. The group was priviledged to have as an additional consultant the late Dr. Richard Follis. Reports were heard of research progress from each of the participating laboratories, and plans were again made for future work. The meeting was highlighted by the emergence of several unresolved problems. There was no consensus on the precise definition or classification of endemic cretinism. This remains to a degree unresolved, as may be seen in Chapters 7, 8, and 27 of this volume. Various communications indicated the unresolved question of the

relationship between endemic goiter and cancer of the thyroid. Several papers pointed out our lack of knowledge of the relationship of endemic goiter to certain physical infirmities which appear in association with endemic goiter, such as endemic deafmutism, short stature, and mental deficiency. The consensus was that the time had come for pilot studies on the prevention of endemic goiter by means of injection of iodized oil. This technique had been used successfully in New Guinea. The reason for considering its use in Latin America was the failure of iodized salt programs either to be implemented or because in many areas they were impractical because of the economics of salt distribution in isolated and impoverished communities. Plans were made for pilot projects in Ecuador and Peru, and these began in Ecuador in March of 1966 and in Peru in October of the same year.

The present volume comprises the papers presented at the third meeting of the PAHO Study Group on Endemic Goiter. This meeting was held from 27 to 29 June 1968 in Puebla, Mexico. Dr. Maisterrena was again the host. By this time investigators from Argentina had become participants in the program and Dr. Geraldo Medeiros-Neto of São Paulo had also joined. An additional consultant was Dr. Ian Buttfield of the University of Adelaide, Australia, Dr. Buttfield had had much experience with iodized oil in New Guinea and with iodine prophylaxis in Tasmania. Interest centered particularly on the results of the iodized oil prophylaxis programs in Ecuador and Peru (see Chapters 26 and 34). These results were compared with those obtained in New Guinea (Chapter 11) and in the Idjwi Island of the Kivu Lake in the eastern Congo (Chapter 10). As a result of these findings the consensus was that prophylaxis of endemic goiter with iodized oil is economically and technically feasible and is safe and effective.

As these results were presented it became clear that there are still no answers to many questions. The relationship of the disease to other co-existing aspects of the public health of endemic goiter regions is unclear. Thus, while the findings in Ecuador are consistent with a preventive role of the prophylaxis program for cretinism, the numbers so far are too small for certainty, and there is little reason thus far to believe that significant effects will be achieved in terms of mean stature, growth, or boney development. Evidence on the prevention of endemic deafmutism is not yet available.

The observations of Delange in the Congo (Chapter 10), of Gaitán and his colleagues in the Cauca Valley of Colombia (Chapter 25), and of Barzelatto et al. in Chile (Chapter 22) pointed out that all is not well with the simplistic view that environmental iodine deficiency is the only factor causing endemic goiter. It is necessary to look for other environmental factors which, working in concert with iodine deficiency or even alone, may ensure the persistence and even provoke the emergence of endemic goiter. Still another problem was the realization of the difficulty and importance of assessment of intellectual development in relation to endemic goiter. Observations in rural Ecuador made it quite clear that there are important deficits in intellectual development. They also made it clear that assessment of these deficits in quantitative terms is difficult, as is relating these alterations to endemic goiter and preventive programs. The origin of these defective persons and of overt cretins and their relationship to endemic goiter and to iodine deficiency and prophylaxis are perhaps an outstanding problem of the whole enterprise.

This volume, then, is an assemblage of communications of work in progress on endemic goiter in Latin America, with relevant studies from Africa and the Far East as indicators of what the disease is like elsewhere and what may be expected of prophylactic programs under other circumstances. These papers are testimonial to the rising elegance and scientific sophistication of medical research in the thyroid field in Latin America. One may see here the results of planning for research on a defined medical problem and the value of international research cooperation. It has

been a source of particular gratification to note that the scope of investigative activities and the numbers of persons involved has grown as the research program has progressed.

From the beginning, the participants have enjoyed the support of the Pan American Health Organization. Dr. John Kevany of PAHO and recently Dr. Joginder Chopra have been instrumental in the planning and execution of the meetings and the field trials. The meetings could not have been held without the time and attention given their organization by Professor Roche in Caracas in the first occasion and by Dr. Maisterrena for the two held in Mexico. They and their associates and staff are due the gratitude of all the participants for the pleasure and profit which these have afforded. The scientific activities of the group have been materially assisted by generous grants from the Williams-Waterman Fund. Individual investigators have had financial assistance from the National Association for Retarded Children, the United States Public Health Service, and the International Atomic Energy Agency. Individual laboratories have also been assisted by grants from their own governments. Finally, it is a pleasure to acknowledge the skillful secretarial assistance of Mrs. Elizabeth Kelleher in the preparation of the manuscripts for this volume.

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SECTION I

ENDEMIC GOITER AND CRETINISM: GENERAL ASPECTS

CHAPTER 1

INTRATHYROID IODINE METABOLISM IN GOITER

A.M. Ermans[1]

Goiter corresponds to a reactive mechanism linked with an increased secretion of thyrotropic hormone. In man this mechanism is usually triggered by a deficient iodine supply. The adaptative nature of endemic goiter is now clearly demonstrated (35). As regards sporadic goiter, it is generally admitted that the same mechanism is responsible, although in this case the decisive role of iodine deficiency has not been established (2, 25).

Apart from a similarity of morphological and metabolic characteristics (12), the two diseases have in common the absence of any known congenital defect affecting the utilization of iodine (21, 28). The balance between thyroid hormone needs and iodine supply is obtained only at the expense of radical changes in the functioning of the gland (17, 18). These anomalies are capable of influencing per se the development of the disease, and of evolving independently of the causal factor (19).

The purpose of this paper is to examine the anomalies of thyroid metabolism in goitrous glands. Three aspects will be considered in turn:

1) Iodine content, changes in the iodination level of the thyroglobulin, and the kinetic characteristics of the gland;
2) Changes in intrathyroidal hormonogenesis;
3) The relation between metabolic and morphological changes.

MODIFICATIONS OF THE IODINE STORES

Iodine Content in Goiter

As shown in Table 1, the iodine content of the thyroid gland is about 10 mg in normal adult man (10, 16, 20, 23, 24, 37). In a goitrous gland, iodine reserves vary in proportion to the iodine supply. If there is no marked iodine deficiency, as in sporadic goiter, the size of exchangeable iodine in the gland is often considerably increased (10, 19, 23); in endemic goiter, the iodine pool generally attains very similar levels to those observed in

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Table 1. Thyroid iodine stores and iodine environment.

Country	Type	Thyroid gland		Urinary excretion ug per day	Reference
		Qg* (mg)	^{127}I concentration (mg per gm)		
	Normal				
Belgium		12.1	.62	59	(16, 20)
Colombia		17.5	.70	229	(37)
Sweden		12.9(+)	-	-	(23)
United States		9.4	-	221	(24)
		6.3-14.7	-	80-225	(10)
	Sporadic goiter				
Belgium		22.0	.17	-	(20)
Sweden		20.9(+)	-	-	(23)
United States		4.7-55.6	-	31-262	(10)
	Endemic goiter				
Chile		12.2	-	33	(3)
Colombia	(tr)	15.3	.29*	360	(37)*
Congo	(ST)	8.6-15.8	.08	27	(17, 18)
	(FT)	1.9			
	(tr)	6.8-36.4	.16	41	
Mendoza (Argentina)		0.3-12.6	-	23	(35)
Misiones (Argentina)	(ST)	9.4	-	-	(38)
	(FT)	4.7	-	-	(38)

Notes:

Qg: Exchangeable organic iodine determined by kinetic methods.*
+: Estimation by spectrophotometric method of Heedman et al. (23).
tr: Iodine prophylaxis or pretreatment with iodine.
ST and FT: "slow" and "fast" turnover rates of the exchangeable organic iodine pool of the thyroid.
**: Personal communication.*

normal glands, even if there is a marked iodine deficiency (3, 25, 35, 37, 38). Very low values have been recorded by some authors (17, 35), but the data of iodine kinetics (17) suggest that, in these cases, the thyroidal pool of exchangeable iodine did not reflect adequately the actual iodine content of the gland.

The acquisition of these iodine reserves, in spite of a very low iodine supply, is achieved by means of a mechanism of adaptation described in 1954 by Stanbury et al. (35), which is characterized by a drastic reduction in urinary excretion of iodine. A number of observations have since confirmed that normal or even above-normal quantities of stable iodine are accumulated by these goitrous glands, even in the most severe conditions of iodine deficiency (8, 18, 35).

Iodine Concentration

Table 1 also shows a constant reduction of iodine concentration in the thyroidal tissue of sporadic and endemic goiter (17, 19, 37). In the normal gland, this concentration attains approximately 600 µg per gm of fresh tissue; it falls to a third of this level in glands of 75 to 100 gm (19). Figure 1 demonstrates the existence of a close relation between the changes in iodine concentration and the size of the goiter expressed in grams.

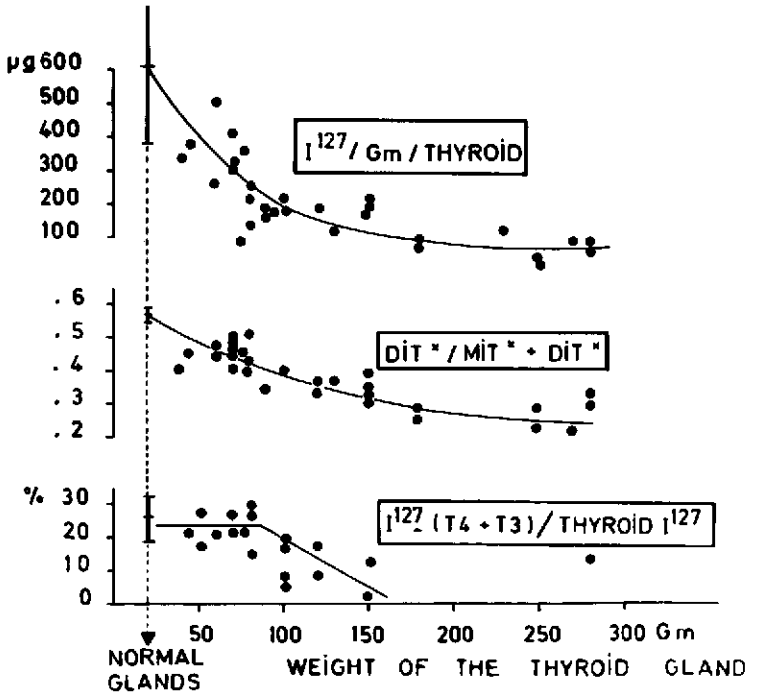

Figure 1. Modifications in iodine content and distribution in human thyroid glands as a function of their estimated weight. Data for normal glands are indicated by the mean values and the standard errors of the mean; normal weight was assumed to be 20 gm. (From Ermans et al., 19)

Given that normal quantities of stable iodine are accumulated and stored by these goitrous glands, it may be deduced that the drop in intrathyroidal iodine concentration is not the direct consequence of a deficient iodine supply but of the increase in the volume of the gland and the subsequent dilution of the iodine reserves. The drop in iodine concentration does not affect the various constituents of the thyroidal tissue equally; the percentage of stable iodine linked with the "particulate" fraction of thyroid homogenates is markedly reduced when iodine concentration of the tissue is decreased (Camus et al., unpublished data).

Iodination Level of Thyroglobulin

Iodination levels of normal human thyroglobulin vary between 0.2 to 0.5 per cent (9, 19). Separation of thyroglobulin was achieved by ultracentrifugation in sucrose gradients or by Sephadex G200. In 12 samples of thyroid tissue taken from patients living in the Brussels area and showing no known thyroid disorder, the average level was 0.23 ± 0.05 per cent, with a range of 0.12 to 0.60 per cent. In the same area the average iodination level observed in 20 samples of goitrous glands attained only 0.06 ± 0.01 per cent. Figure 2 shows that the iodination level of thyroglobulin in normal and goitrous glands varies in direct proportion to the iodine concentration in the gland.

Exchangeable Organic Iodine

Levels of plasma $PB^{131}I$ are generally found in the normal range in endemic goiter (3, 15, 17); this tallies with the fact that the exchangeable intrathyroidal pool usually attains a sufficient size. However, measurements

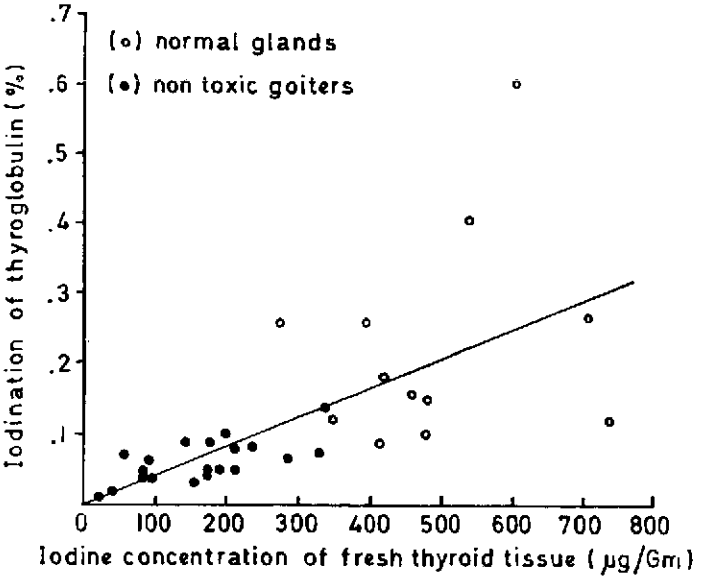

Figure 2. Relationship between the iodination level of thyroglobulin and the corresponding total iodine concentration of fresh tissue in 32 human thyroid samples. (From Ermans et al., 19)

of plasma $PB^{131}I$ performed in the Uele region (Figure 3) showed in some cases of goiter much higher values for which the mean was about ten times higher than observed in the rest of the population. This difference was found (18) to correspond to radical modifications of the kinetic characteristics of intrathyroidal metabolism.

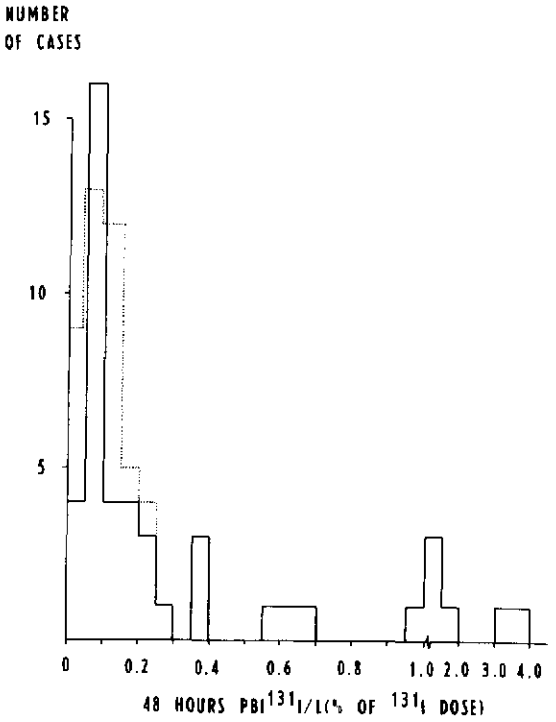

Figure 3. Distribution of the levels of the 48 hours plasma $PB^{131}I$ in 45 random-selected subjects of the Uele goiter endemic. The dotted lines represent the corresponding values obtained from Belgian nongoitrous controls.

In so-called "slow turnover" glands, the relation between specific activities of extra- and intrathyroid organic iodine fits in with the hypothesis that there is a single homogeneous compartment of the organic iodine in the gland (Figure 4). By contrast, in "fast turnover" glands, the specific activity of thyroidal iodine was strikingly lower than that of plasma PBI. This implies that the labeled iodine was secreted from a separate iodine pool functionally distinct from the rest of thyroid iodine stores.

Similar differences have also been observed for the fractional rate of hormonal secretion (17), as well as for the release rate of ^{131}I under perchlorate block. Among 13 goitrous subjects of the Uele region, 11 showed remarkably stable fractional release rates under perchlorate block (mean: 0.020 ± 0.005* with a range of 0.014 to 0.032). By contrast, corresponding values obtained in the two other subjects were 0.099 and 0.111.

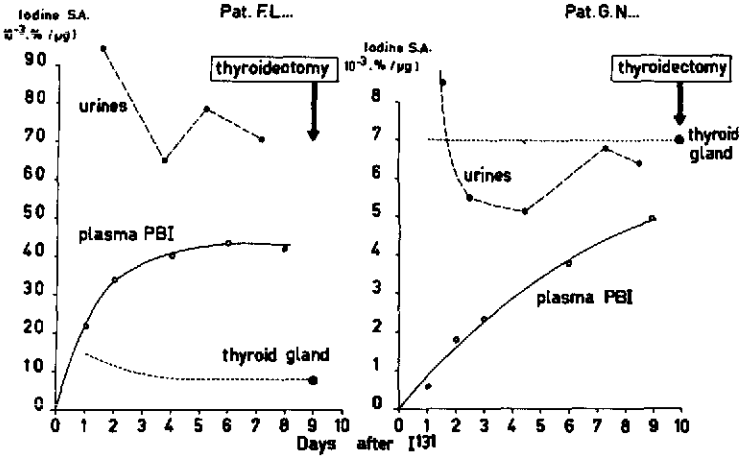

Figure 4. Specific activities of iodine in the homogenized thyroid gland, in plasma PBI, and in urine after the administration of ^{131}I in 2 goitrous patients. Patient F.L. belongs to the "fast" ^{131}I secretion group and patient G.N. to the "slow" group. The specific activities of thyroid iodine before thyroidectomy are estimated from the ^{131}I concentrations measured on the homogenized thyroid glands, taking into account the in vivo modifications of the thyroid ^{131}I content. The ^{127}I content of the glands was assumed to remain unchanged. (From Ermans et al., 18)

Clearcut differences of the plasma $PB^{131}I$ levels have also been reported in other endemics (3, 6) and in particular by Weinstein et al. (38). These authors found levels of plasma $PB^{131}I$ in a proportion of 1 to 10, in two groups of goitrous subjects studies in Misiones, Argentina.

The physiological significance of the considerable acceleration observed in the thyroid turnover of certain patients remains obscure. Choufoer et al. (6) reported that in New Guinea the phenomenon is observed mainly in small thyroid glands and in young subjects; Barzelatto et al. (3) and Weinstein et al. (38) have found it most frequently in nodular goiter.

An interesting aspect of the glands with a fast turnover rate has been revealed by chromatographic study of labeled iodoamino acids (17); the content of labeled T_4 and T_3 of these glands is much higher than in slow-turnover glands. This suggests that hormonogenesis in these conditions gives a far better yield (Figure 5).

HORMONOGENESIS IN GOITER

After enzymatic hydrolysis of normal human thyroid tissue, the distribution of stable iodine between the various iodoamino acids is 33 per cent for MIT, 33 per cent for DIT, 16 per cent for T_4, and 8 per cent for T_3 (20); the

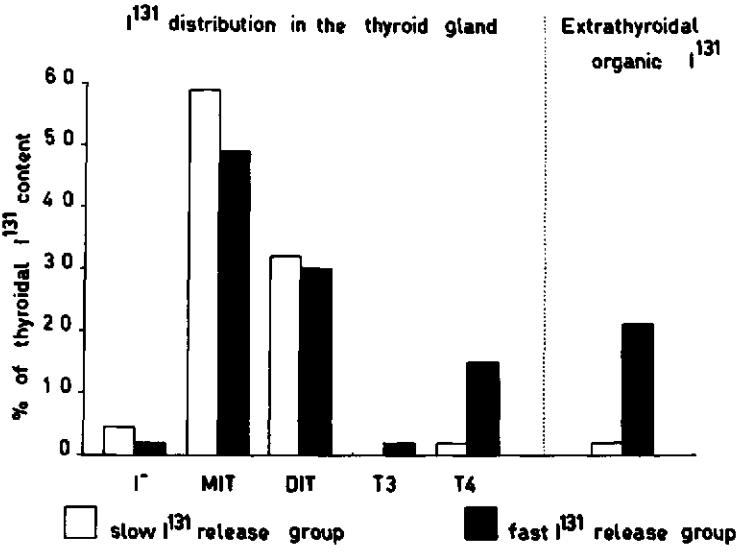

Figure 5. Average distribution of ^{131}I among iodide and the iodinated amino acids of the homogenized thyroid glands, with the corresponding values of the extrathyroidal organic ^{131}I pool at the time of thyroidectomy (8-10 days after ^{131}I administration). Results are given in percentage of the ^{131}I content of the thyroid gland at the time of thyroidectomy. Open columns indicate the results found in patients with a slow ^{131}I release from the gland (8 cases); black columns indicate the results in patients with a fast release (4 cases). (From Ermans et al., 17)

rest of the intrathyroidal organic iodine is to be found in the form of a polypeptide resistant to conventional digestive processes. For each molecule of T_4, it may be deduced that the normal human thyroid gland contains about eight molecules of MIT and four molecules of DIT. If one admits that this pattern of distribution characterizes each thyroglobulin unit, the lowest iodination level of thyroglobulin would be 0.4 per cent, assuming a molecular weight of 650,000 for this protein.

In normal glands, after labeling with radioiodine, the specific activities of MIT and DIT reach an equilibrium almost immediately (20). By the second hour, the ratio between the specific activities attains a stable value approaching one (1.10 ± 0.02). The incorporation of radioiodine in the form of T_4 is a much slower process. From the relation between the specific activities of DIT and T_4 during the first five days, it would appear that the renewal rate of the T_4 iodine pool is only 0.06 per day.

Systematic abnormalities in the distribution of iodoamino acids have been reported in sporadic goiter in the form of a relative reduction in the iodine-rich compounds, namely DIT and T_4, in favor of MIT. These anomalies were originally attributed to a defect in the mechanism of synthesis (13, 32), but it was demonstrated subsequently that they could be induced experimentally

in animals (27, 33) or even in vitro, by reducing iodine availability (11). Identical anomalies have been found in endemic goiter (Figure 5).

Iodotyrosine Metabolism

As regards iodotyrosines, the modifications observed in goitrous glands consist of a rise in the MIT/DIT ratio, or according to the terminology used by Bois and Larsson (4) by a drop in the DIT/IT ratio (i.e., DIT/MIT + DIT). The latter, which in normal men attains an average of 0.53, varied between 0.53 and 0.11 (mean value: 0.38 ± 0.11) in 46 samples taken from nontoxic goiter in Belgian subjects.

Figure 6 shows that the reduction in the DIT/IT ratio is closely linked with the drop in iodine concentration of the thyroid tissue, both in sporadic and endemic goiter. Considerable differences in the DIT/IT ratio were observed in various samples taken from a single goiter, depending on the iodine concentration of the sample. In endemic goiter, Wahner et al. (37) observed similar differences between nodular and paranodular tissues.

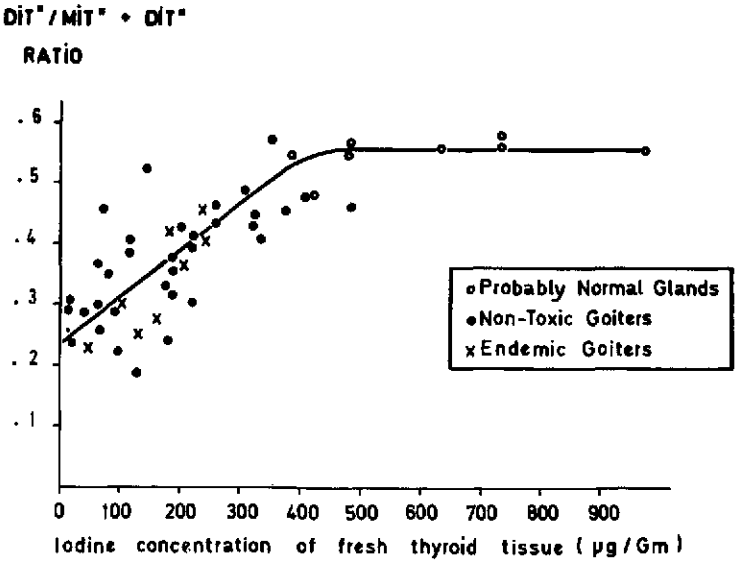

Figure 6. Comparison of the distribution of radioiodine between MIT and DIT with the corresponding iodine concentration of the tissue in 50 thyroid samples. 34 samples were removed from sporadic nontoxic goiters and 8 from presumably normal thyroid glands of patients living in the Brussels area. 8 samples were from endemic goiters of the Uele region in the Congo (8). (From Ermans et al., 19)

Anomalies observed in the distribution of iodotyrosines in goiter are thus, as suggested by Bois and Larsson (4) and DeGroot (11) in experimental studies, a reflection of the degree of iodination of the thyroglobulin. In the human thyroid these anomalies occur when iodine concentration falls below 400 µg, which corresponds to an iodination level of about 0.15 per cent.

It is interesting to note that for concentrations of more than 400 µg, i.e., physiological levels, the DIT/IT ratio continues to remain remarkably constant. Investigations conducted by Lachiver (26) revealed the existence of a similar critical iodination level for iodotyrosine distribution during physiological modifications in the thyroid size of dormice.

The specific activity of DIT rapidly reaches equilibrium with that of MIT, and their ratio after 24 hours is identical (1.10±0.02) to that observed in normal glands. However, as Figure 7 shows, this equilibrium is achieved with a slight delay in comparison with data obtained for normal thyroid glands.

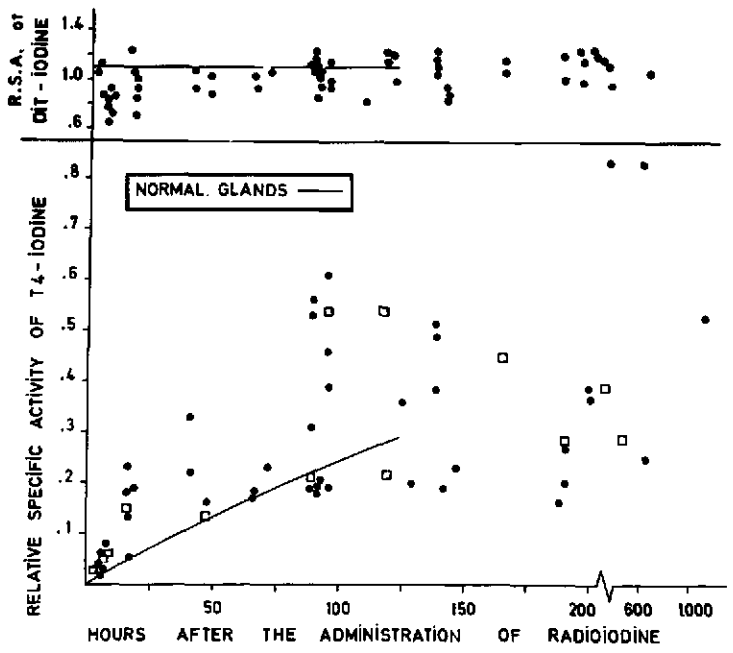

Figure 7. Relative specific activities of iodine in the form of DIT and T_4 in hydrolysates of nontoxic goiters as a function of the time elapsed from the administration of radioiodine. R.S.A. is estimated by the ratio between the S.A. of each compound and the S.A. of MIT at the same time. Black dots and open squares correspond to the R.S.A. of T_4 iodine of thyroid samples with a ^{127}I concentration respectively higher or lower than 250 µg per gm. Continuous lines correspond to the computed mean values obtained in normal glands (10). (From Ermans et al., 19)

Iodothyronine Metabolism

In sporadic goiter the percentage of T4 iodine is not diminished in the first phase of thyroidal hypertrophy. In small goiters, whose iodine concentration is more than 250 μg per gm, the percentage of T_4-^{127}I is about the same as that observed in normal glands, (i.e., about 16 per cent), but it is markedly less in glands with a concentration of less than 250 μg per gm. The percentage of T_3 recorded in normal and pathological glands varies enormously, and no systemic modification could be shown in goitrous glands.

The specific activity of T_4 iodine is given in Figure 7 as a function of the time of administration of the radioiodine. The values are very similar to those observed in normal glands. This finding seems to be in contradiction to the hypothesis that the coupling process slows down. The reduction in the T_4 content of these glands seems to be due chiefly to a diminution in the diiodotyrosyl groups available for the process of synthesis. This diminution is however not confirmed if one considers the concentrations of DIT present in these glands.

The question arises if, at such low iodination levels, the availability of the diiodotyrosyl groups could be limited by critical conditions independent of their relative concentration. There is the possibility that, at very low iodination levels, the reacting diiodotyrosyl groups might be spaced farther apart within the thyroglobulin molecule so that the condensing process could not take place. It might even be suggested that under this condition the thyroglobulin might not contain enough iodine to permit the coexistence of two diiodotyrosyl groups, i.e., of four atoms of iodine in the same molecule. To some extent, findings obtained in nontoxic goiter seem consistent with such a view since the lowest iodination level at which a normal T_4 content is found in the thyroglobulin is approximately five atoms of iodine per thyroglobulin molecule. However, as pointed out previously, this interpretation is questionable because of the probability of a heterogeneous distribution of iodine among the various thyroglobulin units (5, 20).

An alternative possibility is a modification of the tertiary structure of the thyroglobulin in relation to its diminished iodine content. Strong evidence of such modifications has recently been afforded by several authors (22, 30). Some of them have observed associated modifications of the iodoamino acid distribution in the substrate (9, 31). Furthermore, it has recently been reported that thyroglobulin purified from nodular goiters shows sedimentation properties analogous to those of the so-called "prethyroglobulin" (9). However, these modifications reflect changes in the thyroglobulin density on account of the decreased iodination; they do not imply changes in structure (9).

Finally, the distribution of iodoamino acids observed in goiter agrees with recent in vitro studies of thyroglobulin. When thyroglobulin is iodinated in vitro, its iodination level appears as the determining factor of the distribution of iodine among the iodotyrosyl groups (9, 14, 34). Relative amounts of DIT and T_4 are consistently increased by augmenting the iodine content of the substrate.

RELATION BETWEEN METABOLIC AND MORPHOLOGICAL CHARACTERISTICS

The relationship between morphological and metabolic modifications has taken on particular interest since Nadler et al. (29) showed that the size of the follicles has a decisive influence on iodine exchanges. Since iodine uptake by the follicle is a function of its surface, the surface to volume ratio will decrease with an increasing diameter and the turnover of iodine per unit of volume will therefore decrease.

Observations made recently by Decostre (7) suggest that this process could play a determining role in changing iodine metabolism in goitrous glands. On the basis of planimetric studies, he worked out a colloid involution index representing the percentage of the surface of the follicles constituted by follicles with a diameter over the maximum diameter observed in normal tissue, i.e., 200 microns. In goiter samples where the index was less than 5 per cent, i.e., in tissue of the microfollicular type, the DIT/IT ratio was the same as that observed in normal glands. On the other hand, as soon as the involution index increased, the DIT/IT ratio was reduced in proportion. A similar relation is obtained between the colloid involution index and the iodine concentration of the thyroid tissue.

The increase in size of the thyroid follicles may thus constitute one of the mechanisms responsible for the drop in iodine concentration in the thyroidal tissue and subsequent metabolic anomalies.

SUMMARY

An initial consequence of thyrotropic stimulation is an increase in thyroidal clearance which enables sufficient or above-normal quantities of iodine to be accumulated in the gland. In most patients submitted to a constant iodine deficiency, this mechanism of adaptation may be obtained with no appreciable increase in the volume of the thyroid gland. In these conditions, as suggested by Choufoer et al. (6), "The performance of the thyroid of approximately normal size is just as good (or as bad) as that of the obviously enlarged thyroid. This raises the possibility that goiter has no useful part in the adaptation to iodine deficiency. It may rather be an unpleasant by-product of this adaptation..."

The study of intrathyroidal metabolism shows that the enlargement of the gland is indeed the starting point for a series of anomalies which result in a slowing-down of thyroid hormone synthesis.

The first anomaly is probably an increase in the volume of the follicles with a consequent diminution of the iodine exchange of the colloid. The second is the decrease of the iodination level of the thyroglobulin which leads to a drop in its thyroxine content.

The only way the gland has to compensate for inadequate production of thyroid hormone is to increase thyrotropic stimulation, which in turn induces an enlargement of the follicles, and thus a further dilution of the iodine stores. It may be suggested that this self-maintained mechanism contributes directly to the development of goiter. Furthermore, reported data stress that,

under physiological conditions, the level of thyroglobulin iodination in man may be only slightly higher than the iodination level at which the intrathyroidal metabolism slows down. The iodination level of thyroglobulin thus probably constitutes a fairly critical condition for the normal activity of the gland in human beings. This could explain the fact that any additional stimulation of thyroid activity by physiological or dietary factors may play a decisive role in the development of goiter.

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CHAPTER 2

RECENT ADVANCES IN THE KNOWLEDGE OF THE CONTROL OF THYROID GROWTH AND FUNCTION

J.E. Dumont, P. Neve, and J. Otten[1]

Important progress has been made during the last few years in knowledge of the regulation of thyroid growth and function. In this review, we will not attempt to cover all the recent researches on the subject; rather we shall endeavor to summarize the present state of knowledge of the physiology of the thyroid regulatory systems, and to use this knowledge to discuss the pathogenesis of endemic goiter.

THYROID REGULATION

Thyroid Regulation by Thyrotropin

In the classical negative feedback theory, the anterior pituitary secretes thyrotropin, which induces thyroid hormone secretion, and the plasma thyroid hormone inhibits the secretion of thyrotropin. This simple system, which has become more complex and sophisticated, is now known to be controlled by the hypothalamus and central nervous system (Figure 1) (17, 54, 67, 71, 72).

Neurophysiological evidence indicates that the anterior hypothalamus exerts a tonic control over the thyrotroph cells of the anterior lobe of the pituitary (54, 72). Nerve fibres from the anterior hypothalamus secrete a releasing factor, TRF, into the primary capillary plexus of the hypothalamo-hypophyseal portal circulation. This substance is carried to the sinusoids and the thyrotrophs of the anterior pituitary, where it stimulates the secretion of thyrotropin. The secretion of TRF by the hypothalamus may be influenced by various stimuli on the central nervous system. In some animals, for example, the activation of central thermoreceptors in the anterior hypothalamus

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Abbreviations: HTF: heterothyrotropic factor; LATS: long-acting thyroid stimulator; PTU: propylthiouracil; T_4: l-thyroxine; T_3: l-triiodothyronine; TRF: thyrotropic hormone releasing factor; T/S ratio: Thyroid to serum concentration ratio for radioiodide.

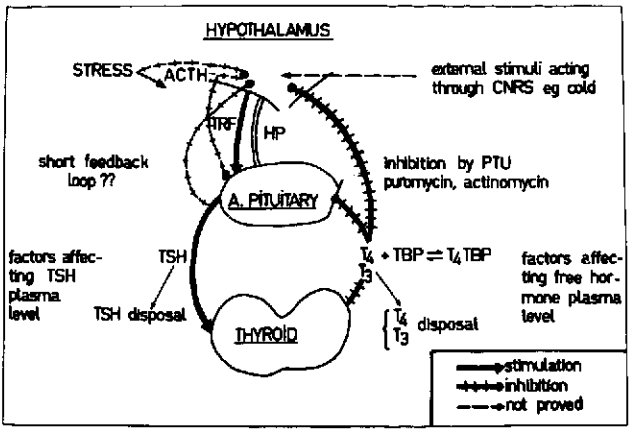

Figure 1. Regulation of thyroid function by the hypothalamo-pituitary system.

induces, presumably by this mechanism, a stimulation of TSH secretion and an activation of the thyroid. This effect, observed in rats, guinea pigs and goats, has not yet been demonstrated in man.

TRF has been extracted and purified (47, 78). It stimulates the secretion of TSH by the anterior pituitary both in vivo and in vitro (14, 25). This effect is not inhibited by inhibitors of protein synthesis such as cycloheximide and puromycin, and is therefore not dependent on new protein synthesis (12). However, TRF stimulates in vitro the synthesis of TSH by rat pituitary explants (81). The secretory effect of TRF is blocked, in vivo and in vitro, by T_3 and T_4, but this inhibition may be overcome by increasing the dosage of TRF (15, 48, 87). T_3 is much more active than T_4 (15). The in vitro inhibitory effect of T_3 is prevented by inhibitors of protein synthesis, puromycin and cycloheximide (15) and by a preincubation in the presence of actinomycin D, which inhibits RNA synthesis (79). These data suggest that the inhibitory effects of thyroid hormones on the thyrotrophs depend on the synthesis of new proteins; in the case of the thyrotrophs, these proteins may perhaps be compared to bacterial repressors.

Studies with TRF, results of microinjection experiments, and the fact that normal thyroid feedback responses are retained when the hypothalamic component of TSH control is inactivated, point to the anterior pituitary as the major site of thyroid hormone feedback inhibition. Recent evidence for thyroxine-sensitive neurones in the hypothalamus and for the accumulation of TRF in the median eminence of thyroidectomized rats also suggests a hypothalamic feedback (72).

The feedback inhibition of TSH secretion in vivo depends on the level of free rather than total thyroid hormone concentration in the plasma. Thus variations in the PBI levels concordant with variations in the level of thyroxine-binding proteins in the plasma or with changes in the availability of

the binding sites of these proteins can be found in the absence of modification of TSH secretion.

Propylthiouracil, a drug which decreases both the metabolic effectiveness of T_4 and its deiodination, inhibits the suppressive effect of this hormone on TSH secretion *in vivo* (29, 61). Thus the factor involved in the negative feedback is sensitive to metabolic activity rather than to the available amount of hormone (29, 61). That the system is sensitive to the action and not to the concentration of hormone is further suggested by the inhibition by actinomycin D of the suppressive effect of T_4 (7). The data of Mouriz et al. (61) indicate a close correlation between the deiodination of T_4 and its physiological activity. There is some suggestion that T_3 may be the intracellular active compound in the feedback inhibition of the thyrotrophs (17). The very close parallel of the *in vivo* experiments with the results obtained on pituitaries *in vitro* is compatible with the previous conclusion that the site of feedback inhibition is the pituitary thyrotrophs. This conclusion could be confirmed by experiments on the effects of propylthiouracil on thyroxine metabolism and action in anterior pituitary preparations *in vitro*.

TSH synthesis in the thyrotrophs seems on the whole to follow the same regulation as TSH secretion. This regulation is more sluggish, as shown by the transient increase and decrease in the TSH pituitary content at the beginning of treatment with thyroxine and PTU respectively (6, 89).

Secretion of certain tropic hormones, such as LH and ACTH, is inhibited by intrahypothalamic placement of the tropic hormones themselves (72). These observations have led to the short feedback loop theory of pituitary control through the direct action of pituitary hormones on the hypothalamus. It is not known whether a similar mechanism operates in the regulation of TSH secretion.

Recent studies have suggested a coordinated regulation of TSH and ACTH secretion (17, 26). Acute stress in the rat causes a rapid increase in ACTH secretion and a rapid decrease in TSH secretion (17), while chronic stress leads to a concomitant increase in both secretions (26). The mechanism of this coordinated regulation is not yet known, but there is evidence that acute inhibition of TSH secretion may result from ACTH inhibition of TRF action on TSH secretion (17).

The normal serum TSH concentration in man is less than 3 mμg of human TSH standard per ml (mean 11 euthyroid persons: 1.8 mμg/ml) (63). It is greatly increased in hypothyroidism (7 to 156 mμg/ml), it increases in euthyroid subjects treated with methimazole as soon as a slight decline in PBI is observed, and decreases abruptly immediately after an intravenous injection of thyroxine (63, 74). Data obtained with endogenous TSH metabolism are consistent with those obtained previously with exogenous TSH (8). In the latter studies it was shown that the rate of disappearance of TSH follows first order kinetics, with an apparent distribution volume 1.38 times the plasma volume and a half-time of 35 minutes in euthyroid subjects. The calculated secretion rate per day corresponds to 75 per cent of the pituitary content of TSH. The fractional disappearance rate of TSH is increased in hyperthyroidism and decreased in hypothyroidism. In rats, the kidney has a major role in the disposal of TSH. This seems to apply to man also (8).

The time constant of the regulation of TSH secretion is very small. Plasma TSH level in the rat is already increased 15 minutes after electrical stimulation of the hypothalamus and reaches a peak value after 30 minutes of excitation (5). The T_4 negative feedback is also rapid; the TSH serum level in a patient treated with methimazole drops to normal less than one hour after an injection of thyroxine (63). Similarly, the disappearance rate is fast (8). The whole suprathyroidal mechanism for the regulation of thyroid function responds rapidly. This rapid regulation of the secretion of a slowly acting hormone such as T_4 seems curious. A preferential release of the more rapidly acting T_3 by the stimulated thyroid or the regulation by thyroid hormone of other hormone secretion could perhaps justify it.

Thyroid Regulation by Other Pituitary Factors

Pituitary factors other than TSH have been implicated in the regulation of thyroid function. Heterothyrotropic factor (HTF) is a substance in the mammalian hypophysis which stimulates intensely the thyroids of teleosts while increasing only slightly the activity of the mammalian thyroid (37). Since it has not been possible to separate HTF activity from FSH, it is now believed that this activity is a property of mammalian FSH (36).

α MSH, β MSH, lysine, and arginine vasopressin and corticotropin A have been reported to stimulate directly thyroid secretion in mice and rabbits (13, 19, 39, 92), and MSH and vasopressin are said to stimulate the thyroid uptake of ^{131}I in mice (13). These results have not been constant, the relationship between effect and dose is variable, and the effects are not reproduced in other species (55, 92). Although these effects are interesting in that they may provide clues to the TSH receptor in the thyroid, they are not believed to be of physiological significance (92).

According to the simple model of thyroid regulation by negative feedback inhibition, hypophysectomy, and thyroid hormone administration should equally depress thyroid function. The findings of Halmi et al. (51) that the uptake of iodide by the thyroid is more depressed in T_3-treated than in hypophysectomized rats, and the observation of Khazin and Reichlin (58) that the thyroid release rate of iodine was lower in T_4-treated than in hypophysectomized rats seem at variance with the model. The inhibition of iodide uptake by T_3 is not in fact due to a decreased transfer rate of iodide from the blood to the thyroid but to an increased transfer rate of iodide from the thyroid to the blood (42). This effect requires a functional hypophysis (51); it is not due to an extrapituitary secretion of TSH (43).

Other differences between hypophysectomized and T_3-treated animals have been demonstrated. Among these are a more efficient binding of iodide to proteins and an increased sensitivity to TSH in the T_3-treated rats (42, 43). On the other hand, suppression of TSH stimulation by anti-TSH antibody, thyroid hormone treatment, and hypophysectomy equally depress thyroid hormone synthesis and, contrary to earlier evidence (58), the secretion of thyroid hormone (73). These results could be interpreted by postulating the secretion of a thyroid inhibitor by the pituitary, but no such inhibitor has ever been demonstrated. Moreover, these results could also be explained as direct effects of thyroid hormones on the thyroid which would require some pituitary dependent factor, or by effects of the other pituitary hormones on the thyroids of the T_3-treated rats (42, 43). Since cortisol stimulates the binding

of iodide to proteins (52) and inhibits thyroid anabolism (93), the presence of this hormone could perhaps explain the findings in T_3-treated rats. There is, therefore, no support for the hypothesis that the pituitary may regulate the thyroid by specific hormones other than TSH. It is possible that other well known hormones such as growth hormone, insulin, cortisol, etc., might influence thyroid metabolism and growth directly.

Thyroid Regulation by Long-Acting Thyroid Stimulator

LATS is a serum 7S γglobulin which is responsible for maintaining, and possibly for initiating, hyperthyroidism (60). An antibody to an unknown antigen in thyroid tissue, LATS induces all the effects of TSH on thyroid tissue which have been looked for so far: early effects such as secretion and its metabolic concomitants (increased glucose oxidation and phospholipid turnover, etc.) and delayed effects (increased iodide uptake, etc.). This factor obviously can mediate thyroid activation and growth; its concentration is not regulated by feedback inhibition by thyroid hormones. LATS does not seem to be involved in the pathogenesis of endemic goiter or cretinism since no LATS activity has been found in the gamma globulin concentrates of sera of such patients in New Guinea (2). The role of LATS in human physiopathology has been excellently reviewed (60).

Direct Nervous Regulation of Thyroid Function

Vagal stimulation and acetylcholine injection increase the thyroid blood flow, while sympathetic stimulation and adrenaline or noradrenaline decrease it (33, 57, 83, 84). Such variations can influence the uptake by the thyroid of substrates (amino acids), of iodide, and of TSH. These effects are of short duration and are therefore not likely to influence significantly the balance of thyroid function (84). In the rabbit vagal and sympathetic stimulation, acetylcholine, adrenaline, noradrenaline, and serotonin enhance the TSH stimulation of thyroid hormone release (83). In the dog noradrenaline also enhances the TSH secretory effects (35), while vagal stimulation increases thyroid secretion (57). All these effects are either transient or of low magnitude. Although they may explain some of the effects of central nervous system stimulation on thyroid secretion (3, 33, 34), their overall contribution to this secretion remains uncertain.

Intrathyroid Regulation by Iodine

During recent years the evidence that the thyroid contains mechanisms for modifying its own function has increased. Various aspects of thyroid metabolism have been shown to be autoregulated by means of iodine derivatives.

Iodide transport and organification: The inhibition of iodide binding to proteins in the thyroid by iodide ("Wolff-Chaikoff effect") provides a rapid homeostatic mechanism by which hormonal synthesis is accelerated at low intrathyroidal levels of iodide, and retarded when thyroidal iodide levels exceed a critical maximum (68, 80, 90). The acute inhibitory effect of large doses of iodide on iodide organification is overcome during prolonged administration by an adaptation mechanism called the "escape phenomenon."

Braverman and Ingbar (16) have shown that this escape is due to a reduction of the thyroid iodide transport capacity. "Qualitatively similar data

were obtained in studies of the thyroids of control and adapted hypophysectomized rats. Administration of TSH to adapted rats did not alter their in vitro iodine metabolism, or their ability to resist the inhibitory effects of high concentrations of extracellular iodide. These data suggest that adaptation to the inhibitory effects of large doses of iodide occurs through an intrinsic thyroidal mechanism that reduces the thyroidal iodide transport capacity and thereby allows intrathyroidal iodide to decline to concentrations insufficient to sustain the Wolff-Chaikoff effects" (16). In molecular terms, the Wolff-Chaikoff effect suggests an allosteric effect of iodine in the iodide binding system; the escape phenomenon suggests a more complex regulation involving protein synthesis. It would be of interest to know if the latter phenomenon is sensitive to inhibitors of protein and nucleic acid synthesis.

The autoregulation of iodide transport not only operates at high iodide levels in the diet; indeed, the iodide uptake in mice and rats remains high when animals submitted to a low iodine diet are hypophysectomized (40, 49, 86, 91). A rapid and sensitive mechanism of regulation of iodide uptake in the human thyroid has been demonstrated in man (9). Acute depletion of extracellular iodide was followed within a few hours by an increase in the clearance rate and uptake of iodide by the thyroid. The mechanism involved is apparently not mediated by the pituitary but resides within the gland itself (9).

The mechanism of thyroidal self-regulation of iodide transport has been analyzed by Halmi (49). Hypophysectomized rats not given TSH show a rise of the T/S ratio when they receive PTU or are fed a low iodine diet long enough, but the dietary iodine levels do not affect the T/S ratios in rats receiving a goitrogen. On the basis of these and other data, Halmi hypothesized that thyroidal I^- transport is stimulated by TSH but inhibited by an intrathyroidal substance which contains iodine in organic form (49). Our finding that actinomycin D and Fluorouracil stimulated uptake of radioiodide in mice thyroids led us to suggest that the inhibitor postulated by Halmi could be an iodinated protein which would have, like its mRNA, a rapid turnover (28). Part of our findings could be explained by an altered food intake (76), but similar results were obtained in experiments in which such artefacts were avoided (22, 50, 53). The effects of actinomycin were obtained in hypophysectomized rats, and they were observed in incubated thyroids from treated rats. This suggests that these were direct thyroid effects (50). The data therefore fit Halmi's and our hypothesis, but they are still far from proving it. Whatever the nature of the inhibitor, the fact that this inhibition is relieved by PTU, certainly suggests that it is an organified form of iodine and not iodide.

The experiments of Barakat and Ingbar (9) clearly show that the thyroid self-regulation of iodide transport must contribute to the increased iodide uptake which is a main component of the adaptation of man to iodine deficiency.

Growth: The evidence in favor of self-regulation of thyroid growth is scanty. In rats given propylthiouracil and a low iodine diet, injection of iodide for eight days significantly depressed the increase in thyroid weight, despite unchanged serum and pituitary TSH concentrations (1). The value of this negative evidence is, of course, limited by the precision of the determinations of plasma levels of TSH. On the other hand, thyroid weight decreases similarly in hypophysectomized animals on a low iodine diet and in similar animals having a normal diet (86).

Secretion: It is well known that iodide decreases the fractional disappearance of ^{131}I from human toxic goiter. This inhibition is counteracted by TSH (41, 45). Iodide also decreases the fractional disappearance rate of ^{131}I from the thyroid of the rat treated with propylthiouracil, and as a consequence, depresses the plasma thyroid hormone level (64, 65).

The inhibitory effect of iodide on thyroid secretion may be ascribed to different causes: decrease of TSH secretion, inactivation of circulating TSH, interference with TSH secretory action, and a direct effect of iodide on the secretory mechanism. Three types of evidence show that the inhibition by iodide of thyroid secretion is not due to decrease in TSH secretion:

- the iodide effect is obtained in Graves' disease in which LATS and not TSH is the plasma thyroid activating agent;
- iodide fails to decrease TSH concentration in thyroidectomized or PTU-treated rats (64);
- the iodide effect is obtained in hypophysectomized rats which receive TSH daily (64).

In order to inhibit secretion, iodide must penetrate the thyroid cell. This is shown by the fact that the effect is completely inhibited by $KClO_4$ (65). Iodide also depresses the LATS-induced secretion of Graves' disease. These observations show that iodide acts directly on the thyroid and not on TSH availability. Since iodide inhibits the secretion of autonomous thyroid nodules in patients whose TSH secretion is suppressed, this effect does not even require the presence of TSH (44). The intrathyroidal inhibitory agent is iodide and not an organified derivative since the inhibition takes place in thyroids of rats and in nodules of patients treated with blocking doses of PTU (64) and of methimazole (44). Intrathyroidal iodide has therefore a direct depressive effect on thyroid secretion in hyperactive glands. Whether it has a similar effect in normal glands is uncertain. The locus of this action of iodide in thyroid metabolism is unknown.

The self-regulation of thyroid secretion by intrathyroidal iodide may be important in the pathogenesis of goiter, since it is a mechanism by which secretion can be selectively inhibited, thus permitting a dissociation of thyroid metabolism and thyroglobulin synthesis on one hand and thyroid secretion on the other hand.

Other Modes of Regulation of the Thyroid

Non-neoplastic thyroid tissue growth can occur independently of TSH action. Thus reparative growth of thyroid grafts after implantation is not prevented by thyroxine in dosage sufficient to suppress TSH secretion and takes place normally in hypophysectomized animals (24). The resultant tissue responds normally to TSH stimulation but survives in its absence. Such self-limited growth may be seen in human thyroids near a hemorrhage or adenoma or in subacute thyroiditis. Its cause is bound up with local trauma or ischemia or cell death, but its mechanism is unknown. Similarly the rapid growth of thyroid tissue in newborn rats, which is not associated with increased thyroid function, may also be independent of TSH (24). Reparative thyroid growth is a self-limited process and infantile growth occurs at a definite period of life; neither seems to be involved in the pathogenesis of endemic goiter.

However, the existence of these phenomena shows that thyroid growth should not be considered as necessarily caused by increased TSH secretion.

THYROID REGULATION AND ENDEMIC GOITER

In the second part of this review, we shall consider the adaptation of man to iodine deficiency and the pathogenesis of endemic goiter as problems of thyroid regulation. More questions will be asked than answers provided. The available methods for studying iodine metabolism and for measuring plasma TSH levels by radioimmunoassay, give to the investigator the tools which are required to provide these answers. Some of these may be forthcoming in the near future.

Adaptation to Iodine Deficiency

The first adaptation to an iodine-deficient diet should be a more complete use of the available iodine. Such an adaptation can occur quite independently of TSH, as we have seen, by intrathyroidal regulatory mechanisms. The iodide trapping would be enhanced, while iodide organification would be maximal. It is, of course, well known that the first adaptation to iodine deficiency in man is an increase in the uptake of iodide by the thyroid (85). It is not known, however, to what extent this adaptation is dependent on TSH. As TSH itself stimulates iodide trapping, but also induces iodide release by the thyroid (77), the thyroid self-regulatory mechanism could be of more benefit to the iodine-deficient thyroid. Increased iodide uptake without goiter has been observed in several endemias (18, 21, 75), which would be consistent with, at least to a degree, mechanisms independent of TSH. Moreover, in the New Guinea endemia TSH levels did not seem to be increased in nongoitrous patients, even though these patients had high uptakes (18). On the other hand, studies on experimental iodine deficiency in rats have not shown any dissociation between increased iodide uptake by the thyroid and increased thyroid weight (86). To test the importance of thyroid self-regulation of iodide uptake in the adaptation to iodine deficiency, it would be interesting to compare plasma TSH levels in areas where iodine supply is rich (U.S.), and sufficient and mildly deficient (Europe); one could perhaps, in this way, establish whether the relation between iodine supply in the diet and thyroid iodide clearance, is solely a function of the thyroid-pituitary negative feedback loop.

Endemic Goiter

Whether or not TSH is involved in the primary response of the thyroid to iodine deficiency, the literature seems to admit that it is responsible for the secondary growth of the gland. Indeed, the combination of enhanced growth and iodide trapping in the thyroid with lower or normal plasma thyroid hormone levels, suggests that the stimulatory TSH part of the feedback mechanism operates normally, while thyroid hormone secretion or action tends to fall below the normal level. Thyroid suppressibility by T_3, and in severe iodine deficiency increased plasma TSH levels and decreased PBI levels, confirm this hypothesis (2, 18, 23). However, the possibility remains that in man iodine deficiency per se could induce some thyroid growth by a thyroidal self-regulatory mechanism. If such is the case, the high plasma TSH levels found in goitrous patients in severe endemias could be ascribed to the hypothyroidism caused by thyroid decompensation (18). One wonders what the TSH plasma levels might be in areas where endemic goiter coexists with normal PBI levels.

Two possible mechanisms for increased TSH secretion in endemic goiter will be considered. The first has been demonstrated by Studer and Greer (86) in rats fed an iodine-deficient diet. "The amount of hormone secreted from the gland into the circulation is not constant, but depends primarily on the quantity of hormone stored in the gland, a given fraction of the thyroidal store being released each day. Therefore, when an iodine-deficient program is begun, the quantity of thyroid hormone secreted will diminish as intrathyroidal stores tend to decrease. This change will occur more promptly in animals with rapid thyroidal turnover, such as the rat, than in man, where turnover is much slower. The initial decrease in serum PBI produced by the decline in thyroidal secretion of hormone is probably too small to be measured accurately chemically, but the fall is adequate to cause increased TSH secretion because of reduced negative feedback to the adenohypophysis. Increased TSH secretion will in turn increase the rate of thyroidal secretion", the trapping of iodide, and will induce tissue growth (86). The primary phenomenon in the pathogenetic sequence would therefore be a deficient thyroid hormone biosynthesis. This deficiency could be caused by iodide lack in the thyroid, or by goitrogenic agents acting at the level of iodide trapping, iodide organification, or oxidative coupling of iodotyrosines. The studies on iodine metabolism reported in this symposium and previously (30, 31, 32) thoroughly demonstrate a deficiency of thyroid hormone biosynthesis in endemic goiter and thus support the validity of the proposed mechanism.

The studies of Morreale de Escobar and Escobar del Rey suggest another mechanism (29, 56, 61). Any factor which might decrease the effectiveness of thyroid hormone at the pituitary level without decreasing in parallel the fractional disposal rate of these hormones would induce a stimulation of the thyroid by TSH. If the metabolic effectiveness of thyroid hormone at the pituitary level and at the periphery were decreased similarly, a new steady state would be achieved in which the individual would be euthyroid with higher TSH and thyroid hormone plasma levels, and with higher thyroid hormone production and disposal. Under conditions of limited thyroid hormone synthetic capacity, as in the case of iodine deficiency, such a situation could easily bring about a state of uncompensated TSH stimulation. Thiouracil derivatives which inhibit thyroxine metabolic effectiveness could act as the postulated goitrogenic factors. However, these compounds also decrease T_4 deiodination, i.e., one of the pathways for thyroid hormone catabolism. The proposed model would require that the decrease in T_4 deiodination would not decrease the thyroid hormone fractional disposal rate as much as thiouracil decreases thyroid hormone effectiveness, i.e., that because of a decreased T_4 deiodination rate the total disposal of thyroid hormone would not be kept at a normal level. The applicability of this model to the pathogenesis of endemic goiter certainly deserves consideration.

PTU decreases thyroxine degradation in man as in the rat (38, 82), but it seems to impair the pituitary feedback mechanism very little (11). If the mechanism of goitrogenesis exhibited in PTU-treated rats would apply to endemic goiter, one would expect patients with normal PBI to be already hypothyroid, and to have a slow thyroxine degradation rate. On the contrary, endemic goitrous patients with low PBI do not present signs of hypothyroidism (18) and tend to have high thyroxine fractional turnover rates (10). There is therefore little evidence that impairment of thyroid hormone effectiveness is a significant factor in the pathogenesis of endemic goiter. A good way to demonstrate this mechanism would be to show that endemic goitrous patients submitted to iodine prophylaxis would exhibit the triad: euthyroidism, high

plasma thyroid hormone levels, and normal plasma binding of thyroxine.

Several other factors may influence TSH secretion by interfering with the thyroid pituitary feedback mechanism. For instance, a change in plasma free thyroxine level may be caused by an increased thyroxine disposal rate or by an increase in the T_4 plasma protein binding capacity. An increased thyroxine disposal rate due to increased fecal excretion has been induced experimentally in the rat (88), but has not been demonstrated in man. Increased serum TBP level is a physiologic consequence of pregnancy; in normal subjects the decrease in free thyroxine levels is rapidly compensated by increased secretion and to some extent by decreased degradation of thyroxine. This compensation may not occur when the supply of thyroid hormone is very limited. The data of Choufoer et al. (20) suggest that this mechanism may cause hypothyroidism in pregnant endemic goitrous women.

Thyroid Growth and Goitrogenesis

Experimental iodine deficiency, treatment with antithyroid drugs, and chronic stimulation by TSH induce an increase in the thyroid weight. In the stimulated thyroid, the cell mass is considerably increased while the follicular luminal spaces are reduced (Figures 2, 3, 4, 5). The thyroid contains more cells; these cells are taller and larger than normal, and contain more RNA per unit cell mass (59, 62). In short, chronic thyroid stimulation causes tissue hyperplasia, cellular hypertrophy, and a relative reduction of the follicle space (59, 62, 86). TSH stimulates both the secretory processes and the synthesis of proteins, including thyroglobulin (27, 66). In chronic stimulation, however, the relative, but not absolute, reduction of the storage space indicates that the increase in synthesis does not outpace the stimulation of secretion.

Pathological examination of human endemic goiters has demonstrated hyperplasia in infancy and childhood, followed only later by stages of colloid involution (69). The early stages of goiter thus correspond to the picture of experimental chronic thyroid stimulation. Focal increase of the hyperplastic process will lead to nodule formation (69).

Colloid involution of goiter indicates a dissociation between the synthesis and the degradation of thyroglobulin. The pathogenesis of this process has been elucidated by Greer et al. (46). They produced hyperplastic goiters in rats by feeding a low-iodine diet. Refeeding a high-iodine diet after a hyperplastic goiter has been present for only a short time resulted in return of the thyroid gland to normal size and histological appearance. If the hyperplastic goiters had been present for several weeks or months, the same changes in thyroid histology were produced but the thyroid always remained of large size. Changes nearly identical with those produced by refeeding a high-iodine diet followed hypophysectomy. Iodide decreases the secretion of thyroid hormone by a direct effect on the thyroid, iodide could therefore induce a temporary dissociation between synthesis and hydrolysis of thyroglobulin and in this way cause refilling of the follicular luminal space. However, while iodide failed to do so, hypophysectomy induced colloid involution in rats that were fed a low-iodine diet combined with PTU. The conclusion of these studies was that the most important etiologic factor in the production of colloid goiter is a reduction of TSH stimulation of a thyroid gland previously hyperplastic for a prolonged period of time. A correlation between the onset of colloid involution and a decrease in the TSH plasma levels in an endemic goitrous population would prove that this mechanism applies to human endemic goiter.

Figure 2. Normal dog thyroid after three days of preparation with thyroid extracts PAS stained x 1,400.

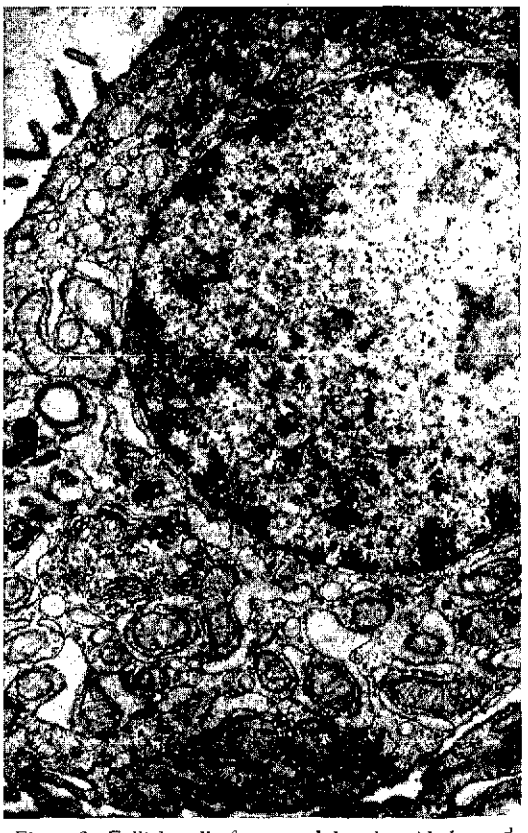

Figure 3. Follicle cell of a normal dog thyroid observed with electron microscope. Gluturaldehyde/osmium tetroxyde fixed by Karnowski's method stained x 23,000.

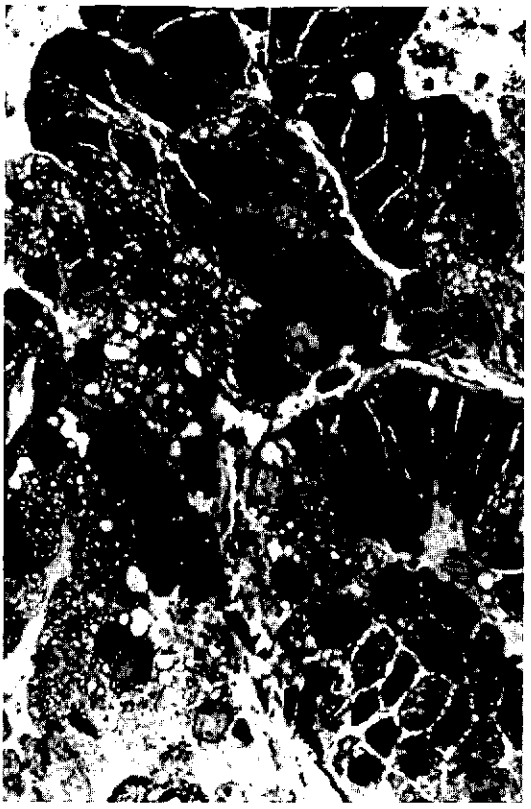

Figure 4. Semithin section through dog thyroid follicles after six days of repeated daily thyrotropin injection. The follicle cells are so high that the colloid lumen has nearly disappeared. Light vacuoles are scattered through the cytoplasms of the follicle cells. Methylene blue at pH = 12 stained x 1,400.

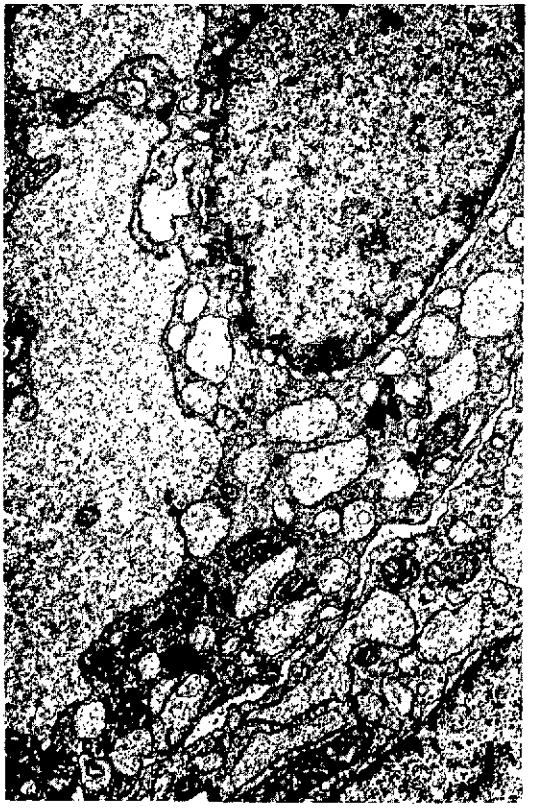

Figure 5. Electron micrograph of two adjacent follicle cells in a dog thyroid after six days of daily stimulation by thyrotropin. The ergastoplasmic cisterni are strongly dilated. Free polysomes are numerous. Glutaraldehyde/ osmium tetroxyde fixed by Karnowski's method stained x 23,000.

If this should be found, it would be of great interest to know if factors other than iodide repletion might be responsible for temporary lapses in TSH secretion and consequent phases of colloid involution.

Experimental studies have shown that protein deficiency per se enhances the increase in iodide trapping but depresses the growth response of thyroids in rats submitted to a low-iodine diet or to administration of PTU (4, 70). The effects of protein deficiency on the regulation of the thyroid may influence the pathology of endemic goiter, since protein and iodine deficiency are sometimes combined. In particular, these effects could perhaps explain in part the high uptake of radioiodide in nongoitrous patients in some endemias. It would be interesting in this regard to observe if correction of protein deficiency in some areas will result in an increase of goiter incidence in these areas.

SUMMARY

Present knowledge of the mechanisms of thyroid regulation is reviewed with regard to its implications in the pathogenesis of endemic goiter. Six regulations are considered: the preponderant pituitary thyroid feedback mechanism and its control by the hypothalamus; regulation by other pituitary factors; LATS stimulation of thyroid function; direct regulation of thyroid function by the nervous system, and neural transmitters; self-regulation of thyroid function by means of iodide or iodine derivatives; and regulation of thyroid growth by local factors. The role of these regulatory mechanisms in the adaptation to iodide deficiency and in the pathogenesis of endemic goiter is discussed.

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CHAPTER 3

PATHOPHYSIOLOGY OF NONTOXIC GOITER

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During the last ten years a major effort has been made by several groups of investigators to evaluate the importance of endemic goiter in different countries and to better understand the pathophysiology of this disease. Very few studies have dealt with the preliminary stages of the disease. In long-standing goiter no major difference has been observed between endemic and sporadic nontoxic goiter (or colloid goiter), either from the morphological or the physiological point of view (8, 50, 193).

This paper reviews briefly the recent data which have been reported in the study of nontoxic goiter. At the same time it focuses attention on the unsolved problems which most need investigation in the light of our actual knowledge of thyroid physiology.

GENERAL PATTERN OF IODINE METABOLISM IN NONTOXIC GOITER

Many investigations have been performed on the kinetics of iodine in man. The most common model used is composed of three parts: inorganic iodine compartment, thyroidal iodine compartment, and compartment of extrathyroidal hormonal iodine (8, 49, 143, 151, 167). In such a model the iodide is taken up by the thyroid and released back into the blood as hormonal iodine. The thyroid hormones in turn are degraded and the released iodide is either excreted by the kidneys or accumulated in the thyroid (Figure 1). The thyroid compartment is, in fact, a multi-compartmental one, as demonstrated in the normal thyroid gland (20, 122, 160, 165, 178, 179, 199) and in goitrous glands (11, 16, 41, 55, 56, 167, 191, 194). For studies on peripheral metabolism it is useful to subdivide the extrathyroidal hormonal iodine compartment into two subcompartments corresponding to the hormones, thyroxine (T_4) and 3,5,3'-triiodothyronine (T_3). These are bound to the binding proteins or circulate as free iodoamino acids (131, 143, 153). It is likely that one should also

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Abbreviations: I: iodine; LATS: long-acting thyroid stimulator; PBI: protein-bound iodine; PII: plasma inorganic iodine; TBG: thyroxine-binding globulin; TBP: thyroxine-binding proteins; TBPA: thyroxine-binding prealbumin; Tg: thyroglobulin; TRF: TSH-releasing factor; TSH: thyroid-stimulating hormone.

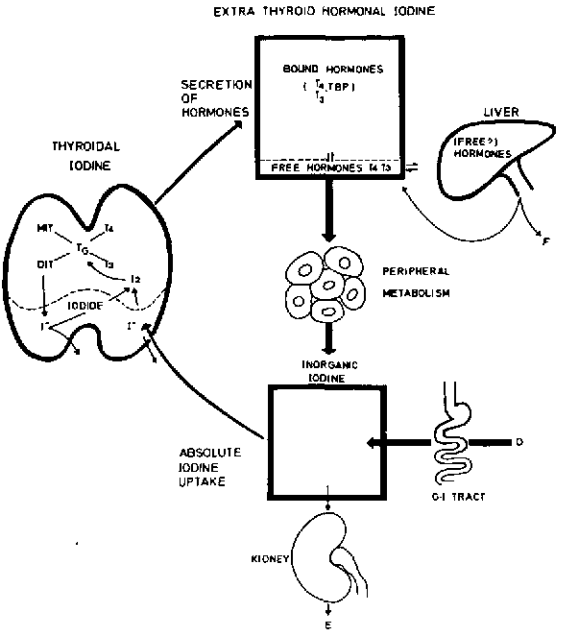

Figure 1. Diagram of iodine metabolism.

consider a third subcompartment made by the liver. Indeed, this organ contains significant amounts of thyroid hormones, probably in a loosely bound state, and possibly acts as a system to buffer any significant variation in the level of the free circulating hormones (18, 24, 125, 131).

Cavalieri and Searle (24) have quantitated the rate of exchange of radiothyroxine between plasma and liver in normal subjects. As much as 118 µg of thyroxine are accumulated per hour in the liver from the plasma. As Osorio and Myant (132, 133) pointed out, this thyroxine is accumulated as "free" thyroxine and bound reversibly to cellular sites. Rat liver perfusions in vitro have confirmed the bi-directionality of thyroid hormone flow and the smooth endoplasmic reticulum has been implicated in this phenomenon (65, 76). No information is available on the role of liver on iodine metabolism in patients with nontoxic goiter. It should be remembered that patients with endemic goiter often suffer from malnutrition and cirrhosis, which in turn may affect the synthesis of the thyroxine-binding proteins and the accumulation of T_4 in the liver (85, 181). In experimental animals, a liver factor inhibiting thyroid uptake has been described (91).

Very recently Berman et al. (17) have proposed a more complex model of iodine metabolism. The model includes three thyroidal phases (i.e., rapid initial accumulation of iodine, delay, and storage), iodide feedback from the thyroid, and release of T_4 and T_3. Iodide release by the thyroid is independent of the thyroid iodide trap. The application of this model to thyroid physiology still requires further study, particularly as far as T_3 contribution and the so-called iodide escape are concerned (cf. Chapter 4).

THE INORGANIC IODINE POOL AND ITS EXCHANGES TO THE THYROID AND THE KIDNEYS

Most of the patients with endemic goiter have a low urinary excretion of iodine. This has been considered as evidence of low iodine intake (6, 8, 9, 46, 55, 104, 106, 107, 167). It has been clearly demonstrated that within the physiological range in euthyroid subjects there is a direct relationship between the dietary iodine intake, the urinary excretion of iodine, and the plasma inorganic iodine (101, 186). Conflicting results have been reported on this relationship in endemic goiter (102, 106).

In cases of sporadic nontoxic goiter, the urinary iodine excretion rate is usually lower than in normal subjects of the same population (10, 37, 41, 86, 167).

There is little doubt that severe iodine deficiency is not the only factor involved in the pathogenesis of colloid goiter. The incidence of goiter is not necessarily proportional to the urinary excretion rate of iodine, e.g., in Venezuela (158), in New Guinea (25) or in the Congo (8, 49a). In all the endemic areas there are goitrous and nongoitrous subjects living in the same environment. Recently in an iodine-deficient endemic area of Greece, no difference was found between the mean dietary iodine intake of the goitrous and nongoitrous inhabitants (106). In the Idjwi Island, Delange et al. (45) surveyed 17,000 inhabitants and found that in the northern part of the island, the mean per cent of goiter was 54.4 (31.4 to 71.4 per cent) and the urinary iodine excretion rate 13.1 µg per 24 hours, while in the southern part of this area, the goiter incidence averages 5.3 per cent (3.7 to 8.8 per cent) with an iodine excretion of 18.3 µg. These authors have postulated the presence of a supplemental goitrogenic factor in the diet. Such a possibility cannot be ruled out at the present time, although no clear-cut evidence in favor of a role of dietary goitrogens in endemic goiter in man has yet been presented (27, 29, 54, 70).

These observations do not dismiss the theory of iodine deficiency in nontoxic goiter (and especially in endemic goiter) but rather suggest the possibility of some "permissive" or "aggravating" action such as intrathyroidal disturbances of iodine metabolism and exogenous goitrogenic factors (8, 9, 45, 56, 106).

In experimental animals iodine deficiency appears to be the starting point of thyroid hyperplasia. If stimulation continues for long periods of time, nodules will appear in some parts of the gland (48, 174, 175). The diffuse goiter becomes then multinodular, generally called "colloid goiter," the colloid areas usually coexisting with hyperplastic zones (48).

Relatively few new experimental data on the production of endemic goiter in animals are available (48). In line with Marine's (111) original postulate on the pathogenesis of colloid goiter, some interesting observations have been made on rats with hyperplastic goiters produced by feeding cyclically low-iodine and high-iodine diets, with or without propylthiouracil (71, 171). From these observations it appears that the most important etiologic factor in the production of colloid goiter is a reduction of TSH stimulation of a thyroid gland previously hyperplastic for a prolonged period of time. This observation may explain why no increase in the level of TSH has been reported in endemic or

sporadic goiter when the protein-bound iodine has been normal (compensated goiter). It also may explain why iodine prophylaxis has only a limited influence on the thyroid size of previously goitrous patients.

Several studies on iodine kinetics in nontoxic goiter have appeared. Most have dealt either with the plasma inorganic iodine concentration (PII) and the absolute iodine uptake by the thyroid (AIU) or with the secretion of thyroid hormones and their metabolism. Normal values for PII vary from one country to another according to iodine intake. In the United States the PII level averages 0.5 µg per 100 ml (60, 147, 188, 190). In Europe, the PII concentration varies from 0.1 to 0.3 µg per 100 ml (3, 5, 10, 37, 38, 86, 108). It has been suggested that a value of PII below 0.08 µg per 100 ml should be considered as evidence for iodine deficiency (5, 8, 10, 86, 193).

In some endemics the PII is indeed very low. In the Uele (Republic of Congo), the PII averages 0.04 µg per 100 ml (8). A similar value has been found in Greece, but with no statistical difference between goitrous and nongoitrous subjects (106). Reduced PII and values of urinary iodine excretion have been reported from New Guinea (25) and the Idjwi Island (45), but in other endemics a low-normal (6, 9), a normal (187), or even a high PII concentration (173) has been observed.

Within limits there is an inverse relationship between the PII and the thyroidal clearance (Figure 2) (5, 8, 10, 86, 87, 108, 167). As a result the absolute iodine uptake (AIU) is often normal, if not higher, in goitrous subjects than in nongoitrous (9, 106). This observation suggests that iodine

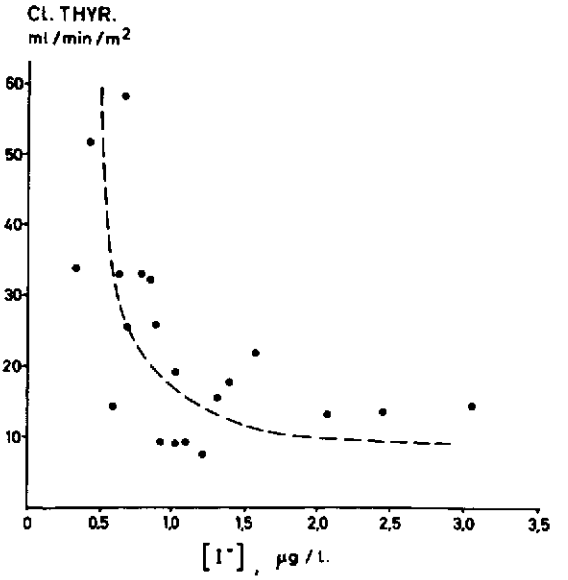

Figure 2. Relationship between plasma inorganic iodine and thyroid clearance of iodine.

deficiency is an important factor in goitrogenesis by acting as an "initiating" or "permissive" factor when the disease starts, and that when goiter is well established (and especially nodular goiter) a state of iodine deficiency arising from a reduced AIU is responsible for a depletion of the thyroidal iodine stores is no longer a major factor. At this stage the thyroid cells are probably working less efficiently and the disturbances are inside the thyroid gland itself (8, 50).

Except for the reports of Cassano et al. (23) and Ackerman et al. (3), no significant increase in renal iodide clearance has been reported in nontoxic goiter. No significant increase of fecal iodine excretion has been observed in endemic goiter (25, 106), although it has been demonstrated in sporadic goiter (83, 138, 182).

Recently Costa et al. (32) have reported that muscular and adipose tissues contain more iodine in goitrous patients than in normal subjects. This observation requires confirmation and further study.

THYROID SECRETION

Isotopic studies have demonstrated heterogenicity of the thyroid gland. First observed in animals by metabolic or autoradiographic experiments (122, 160, 178, 179, 199), this concept has been extended to man, particularly when goiter is present. From in vivo studies it has been demonstrated that there are usually at least two organic iodine pools in the gland, one characterized by a relatively slow turnover rate and the other by a relatively high turnover rate (8, 9, 11, 55, 56, 167, 191, 194). The heterogeneity of the distribution of iodine in the thyroid has been mostly studied in goitrous glands, but this concept should also be extended to normal thyroids. Thyroid secretion often is normal, even when there is evidence of very low iodine intake. A major difficulty is that it is generally assumed that the thyroid secretion corresponds exclusively to thyroxine, whereas this is not correct. It is urgent to validate many kinetic studies by measuring not only thyroxine but also the absolute amounts of triiodothyronine secreted by the thyroid or circulating as bound or unbound thyroid hormone (124, 143, 195). Despite the small contribution of T_3 to the level of thyroid hormones circulating in the blood, it accounts for 20 per cent of hormonal iodine secretion in euthyroid subjects (152) and the high "calorigenic" effect of T_3 as compared to T_4 is well recognized. Any shift in the thyroid secretion from T_4 to T_3 in nontoxic goiter has never been established. Changes of the T_4/T_3 ratio in animals by measuring only the radioactivity may be misleading but represent an attractive explanation for the adaptive mechanism (25, 49a).

The total iodine content of goitrous glands is usually higher than normal, but the concentration per gram of tissue is usually reduced. This observation is not restricted to endemic goiter, and is encountered in sporadic nontoxic goiter as well (8, 12, 13, 15, 55, 56, 119). The main component of thyroid follicles--thyroglobulin--has a low iodine content (12, 13, 40, 154).

Long-term thyroid studies in which radioiodine has been given as a tracer dose confirm the concept of an iodine "leak" from the gland, as originally proposed by Hickey and Brownell (81). This conclusion is based on the differences between the absolute uptake of iodine by the thyroid and the secretion rate of hormones, or on the fact that the specific activity of urinary iodide consistently exceeds the specific activity of plasma inorganic iodine

(9, 16a, 41, 55, 56, 123, 130, 167, 194). Data supporting these conclusions have often been obtained indirectly and analyses omit the possibility that significant amounts of triiodothyronine might be secreted by the thyroid (143). According to Werner (195), as much as 50 μg of T_3 may be degraded per 24 hours (124). A level of T_3 secretion equivalent to that degraded could account for all of the "iodine leak" by the thyroid. Several reports have suggested a preferential secretion of T_3 as compared to T_4 in endemic goiter (6, 8, 49a, 134). Also, arguments in favor of an "iodine leak" are often based on the assumption that isotopic equilibrium has been reached (9, 41, 56). Some observations, such as those of Loewenstein and Wollman (98) in rats on a moderately low iodine diet, indicate that equilibrium takes longer to be reached than is usually assumed. Thus after 99 days the concentration of radioiodine in the rat thyroid was still increasing slowly. After 40 days the ratio of specific activity in the diet was only 0.8. It must take several months to reach equilibrium in man, because of the low iodine turnover rate and the large variations in the daily iodine intake, even in iodine-deficient endemics (106). In euthyroid subjects, not much iodine is left for any "iodine leak" according to kinetic calculations (143). A similar conclusion has been reached in our laboratory in normal euthyroid adolescents (15a).

PERIPHERAL METABOLISM OF THYROID HORMONES

Studies on peripheral metabolism of thyroid hormones in nontoxic goiter can give information on the degradation rate. If equilibrium between the degradation and the secretion rate is assumed, the data permit an estimate of the secretory activity of the thyroid. Some of the assumptions made in the calculations of thyroid hormone kinetics may be misleading. As pointed out by Rall et al. (143) and Werner (195), it may be fallacious to assume that the PBI corresponds only to T_4. Methods for the determination of T_3 in the serum are elaborate (82, 124). Determinations of T_3 in nontoxic goiter are not available, but a shift from T_4 to T_3 has been claimed in endemic goiter. Radioactive T_3 concentration often remains low, but this may not be surprising in view of the short biological half-life of T_3.

Several reports on T_4 metabolism in endemic goiter have demonstrated a significant increase in the fractional turnover rate (6, 16). In all these studies, the PBI has been assumed to be comprised of T_4. Relatively normal values of T_4 degradation rate were calculated. In the Uele endemic, for instance, the T_4 degradation rate averaged 65 μg per day per 70 kg (16). Observed increases in T_4 fractional turnover rate have probably been related to alterations in the thyroxine-binding protein capacity. In the Uele endemic we observed a significant decrease in TBPA binding capacity (181), but TBG values were elevated. It is unlikely that this was on a genetic basis, since similar observations have been made in the Indianas of the Pedregoso endemia in Chile (6). There is no evidence of any relationship between the alterations of the TBP and the goitrous process itself. TBPA reduction could be a consequence of the "chronic sickness" of these patients, who often suffer from parasitic or chronic infection. Modification of TBPA probably explains the increase in the fractional turnover rate of thyroxine (172), although the increase in TBG capacity should decrease the free T_4 level, and thereby explain the frequent observation of clinical signs of hypothyroidism in the Uele patients.

Pregnancy is an aggravating factor in the development of goiter. Recent data from euthyroid pregnant women indicate that peripheral T_4 metabolism is normal (52), but there is an increase in renal clearance of inorganic iodine which in turn decreases the plasma inorganic iodine and stimulates the thyroid pump (2, 34, 35).

Information on the free thyroxine and triiodothyronine of patients with endemic goiter would permit an evaluation of the real lack of thyroid hormones for the tissues, and also an estimation of any relationship of the disease with the hypothalamo-pituitary mechanism responsible for the secretion of TSH.

ENZYMES AND THYROID PROTEINS IN NONTOXIC GOITER

Apart from environmental factors, goiter could result from defects in the glandular machinery responsible for the synthesis and the secretion of the thyroid hormones. Errors of thyroid metabolism are well recognized, but their occurrences in so-called "simple goiter" has never been demonstrated. Certain disturbances have been observed but if these anomalies were present at the origin of the disease, or if they were the result of the colloidal transformation of the gland is not known (8, 50).

Enzymatic Activity

Few enzymatic studies have been performed with goitrous tissue. Progress in this respect must await a better understanding of normal hormone synthesis itself. A familial aggregation of goiter (73, 104, 112) and a higher concordance rate for thyroid hyperplasia in monozygotic than in dizygotic twins (105) has been reported. Iodotyrosine-deiodinating activity has repeatedly been found normal, or in the lower range of normal (6, 13).

Thyroid Proteins

Since nontoxic goiter is characterized by accumulation of large quantities of colloid filling the enlarged follicles, a major effect has been made to characterize components of the colloid. Goitrous glands contain less thyroglobulin than normal, and a similar pattern has been observed repeatedly in endemic goiter (9, 33, 97), in sporadic goiter (15, 42, 140, 144, 157, 164, 169), and in some cases of congenital thyroid diseases in man or animals (44, 47, 57, 92, 93, 155, 156, 157, 192). It has also been observed that the thyroid content of thyroglobulin is rapidly reduced in rats receiving goitrogenic substances (95, 136). Decrease in thyroglobulin content is probably related to reduction in the synthesis of thyroglobulin itself. Studying congenital goiter in sheep, Falconer (57) failed to observe any significant incorporation of labeled amino acids in thyroglobulin. More recently, Thomson and Goldberg (176) observed in rats that chronic administration of goitrogenic substances altered the thyroidal protein pattern. A 3 to 8S peak predominated. The uptake of tritiated leucine was increased in the presence of goitrogens, the subunit labeling being similar to control rats. There was never any formation of 19S thyroglobulin. In iodine deficiency radioactive leucine is rapidly incorporated in 19S protein. These results are somewhat different from those reported by Vecchio et al. (183) and Cavalieri and Searle (24a). The decrease in thyroglobulin content of goitrous glands is not related to stimulation of the proteolytic system (11, 15, 57, 58, 139).

The 4S peak which is significantly increased in goitrous tissue shares immunological properties with serum albumin (9, 92, 157). This does not exclude a thyroidal origin of the 4S component (92, 176, 177). There is no relationship between the thyroid content of the 4S component and hemoglobin used as an index of blood contamination of the thyroid tissue (12, 15, 42). More recently, both in a rat transplantable thyroid tumor (47) and in congenital goiter of cattle (155), protein other than thyroglobulin possibly related to the 4S component has been shown to form a soluble complex with antithyroglobulin antibodies.

Gel chromatography on agarose columns indicates that there is a relative decrease in the 27S and 32S polymers of thyroglobulin from goitrous tissue; the 27S content is inversely related to the diameter of the follicles.

The iodine content of thyroid extracts from goitrous tissue is lower than normal (8, 12, 45, 55). Purified thyroglobulin preparations from goitrous glands display the same differences from normal (40, 152, 159). In our laboratory deCrombrugghe (36, 39, 40) has investigated the molecular properties and stable iodoamino acid content of human thyroglobulin isolated from nontoxic goiter. The effect of the degree of iodination of thyroglobulin in its iodoamino acid content and sedimentation properties has also been evaluated. Some of these observations may be relevant to the pathophysiology of nontoxic goiter and the effect of iodine prophylaxis. Thyroglobulin from euthyroid sporadic goiter was purified on Sephadex G-200. The iodine content was as low as 0.05 per cent, as compared to values averaging 0.5 per cent in normal human thyroids and 0.7 per cent in normal bovine thyroids from Belgium. Spectrophotometry of thyroid and iodoamino acid residues of thyroglobulin revealed the presence of monoiodotyrosine; diiodotyrosine and T_4 residues were very low.

These findings confirm those of others (12, 15, 51, 55, 56, 97, 140, 144). Similar modifications in the distribution of iodoamino acids have been observed in iodine deficiency or after goitrogenic drugs (19, 43, 84, 89, 115, 142, 150, 166, 171).

Using iodine-poor thyroglobulin from goitrous glands and substrate, deCrombrugghe et al. (40) carried out experiments involving iodination in vitro. A progressive increase in the DIT and T_4 content was observed when thyroglobulin from goitrous tissues was iodinated. Normal values of T_4 were obtained when 95 moles of iodine per mole of thyroglobulin were added. Iodine content reached 0.95 per cent by chemical analysis. It is interesting to note that Marine (110) had concluded from his observations that thyroid hormone production is inadequate when thyroglobulin iodine content is below 1 per cent.

Sucrose gradient centrifugation and velocity sedimentation measurements have demonstrated that thyroglobulin preparations from goiter have slower sedimentation rates than normal thyroglobulin. Iodination of thyroglobulin from goiter increases the sedimentation rate. These observations are in harmony with those of Seed and Goldberg (161), Nunez et al. (126), Lissitzky et al. (94) and Robbins et al. (155).

Thus it appears that the degree of iodination is a major condition for a normal yield of thyroxine, and no enzyme for coupling of DIT residues to form T_4 appears to be necessarily required. Preliminary observations on thyroglobulin from goiters in the presence of a high concentration of guanidine or urea

8M are consistent with the possibility of some modification of the primary structure of thyroglobulin (36).

MECHANISMS OF GOITROGENESIS

Thyrotropic Hormone

The negative feedback involved in control of thyroid activity involves a hypothalamo-pituitary system with two essential hormones, the thyrotropin-releasing factor (TRF) and thyrotropin (TSH). Several pieces of evidence indicate that TSH secretion is inversely related to the free thyroid hormone level (31, 127, 128, 143, 146, 180). It is therefore not surprising that Adams (4) reported a high TSH concentration in the blood of goitrous patients of the endemia of New Guinea (25). No other reliable TSH determinations have been reported from other goiter endemics. In sporadic nontoxic goiter, no increase of the blood TSH was reported by Pimstone et al. (137), Munro (120), or by Major and Munro (103), but Bates and Condliffe (7) and Lemarchand-Beraud (90) found values slightly higher than normal. Inconclusive results were recently reported by Kirkham (88) and also by Odell et al. (127) using immunoassays with human TSH antibodies. No LATS-like substance has been observed in nontoxic goiter. These findings favor a normal hypothalamo-pituitary control of TSH secretion in patients with nontoxic goiter.

Nevertheless, it must be recalled that no systematic assays have been performed during the first stages of the disease, and that the bioassays generally used are probably not sensitive enough to detect minor modifications. Negative results do not exclude that the iodine-deficient gland could be unusually sensitive to TSH. Iodide excess in the rat does not modify the TSH concentration in the blood (1).

Iodine Intake

There is little doubt that iodine deficiency is an important factor in producing thyroid hyperplasia. Recognition of iodine deficiency is generally based on the finding of a low urinary iodide excretion rate and a high radioiodide uptake in the thyroid. In this respect goiter has been considered as an adaptive reaction to iodine deficiency to cover the needs for normal thyroid secretion (167).

Surprisingly enough, the amounts of iodine necessary to cover the average needs in adults are not well known. Various indirect calculations lead to an estimated requirement amounting to 150 to 200 μg of iodine per day (193). According to Greenwald (67) the human requirement is less than 100 μg per day. Few investigations have been carried out in goiter endemics in order to estimate directly the real iodine intake. Apart from the data obtained in New Zealand by Hercus and Roberts (80), and more recently in Greece by Malamos et al. (106), little other information is available. Usually the severity of iodine deficiency has been based on the determination of the urinary iodine excretion rate. Lack of correlation between the dietary iodine intake and the urinary iodine has been reported in goitrous patients by Vought et al. (99, 189).

In normal individuals living in nonendemic areas, there may be tremendous variations in the iodine intake from day to day and from one patient to another. Vought and London (184) observed in the U.S.A. a daily dietary iodine intake which varied from 18 to 1540 μg in 19 healthy subjects followed over a period of six weeks. In euthyroid adolescents living in Belgium, the mean iodine intake (log-normal distribution, Figure 3) was found to be 32 μg per day (109). In view of the fact that there is no problem of endemic goiter in Belgium and comparing the data obtained in the U.S.A. and in Belgium, no answer can be given with certainty regarding the optimal iodine intake of healthy individuals (121).

Iodine balance studies have been carried out in euthyroid subjects from a nonendemic area in order to estimate the needs for iodine. Data have been collected from normal children or adults on free-diet (53, 109, 184, 185), in pregnant women (53), and in normal or thyroid patients on a free or a preselected iodine intake (75, 106, 184, 186, 187, 189). A considerable variation in the daily iodine intake has been observed even in iodine-deficient goiter endemics (7 to 642 μg I per 24 hours), as shown by Malamos et al. (106).

A number of iodine balance studies performed in euthyroid subjects have shown a negative balance (Figure 4) (109, 186). This observation indicates that to recognize the exact dietary iodine intake and needs in a population, long-term periods (i.e., months, if not years) of observation may be essential. Periods of negative iodine balance are not necessarily critical for normal thyroid owing to the large iodine stores present in the thyroid gland and the minute amounts of iodine used for daily thyroid secretion. On the other hand, if a state of iodine deficiency has developed for some time, the thyroid clearance increases, permitting accumulation of large amounts of iodine, if iodine becomes available. (Note: Regarding the finding of negative iodine balance, further studies should take advantage of making a clear distinction between "exogenous" iodine balance (i.e., dietary iodine versus urinary and feed iodine) and "endogenous" iodine balance (i.e., dietary iodine versus obligatory excretion of iodine) (41a, 109, 186).

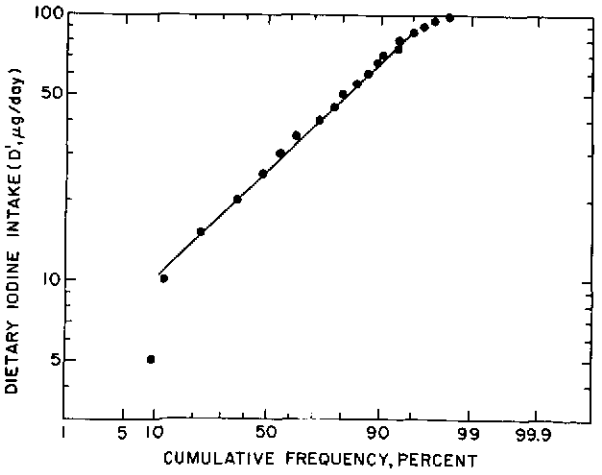

Figure 3. Plot of the accumulated per cent of iodine intakes on lognormal probability paper, indicating lognormal distribution of dietary iodine intake (reprinted with kind permission of "The Journal of Clinical Endocrinology and Metabolism").

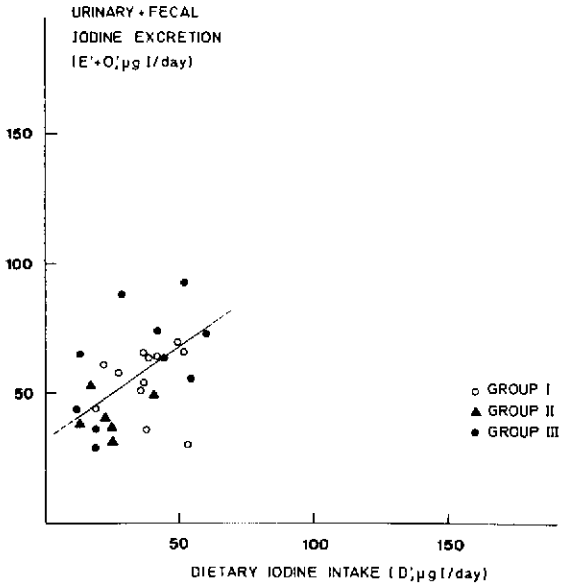

Figure 4. Plotted points are the means of I intake (D') and excretion (E'+ O') during the balance period (reprinted with kind permission of "The Journal of Clinical Endocrinology and Metabolism").

Ample experience has been accumulated to prove beyond doubt that iodine prophylaxis is an effective step in preventing endemic goiter. Because of the difficulties encountered in some countries in the use of iodized salt, iodized oil has been proposed (26). Lipiodol iodinated hydrolized poppy seed oil (containing ~ 40 per cent I) has been used (22, 45, 79, 117). Aside from the decrease in the thyroid size, the PBI increased from 1.8 to 4.8 µg per 100 ml (79). No T_4 determinations have been performed and the increased PBI (or BEI) possibly could be explained by some nonhormonal material from the iodized oil.

Iodide goiter has occasionally been reported after absorption of large quantities of iodine (more than 18 mg) for various periods of time (197). A reduction of the thyroid size has customarily been reported from endemics where iodized oil was administered. More information is still necessary for a complete evaluation of the indications for iodized oil as prophylaxis of endemic goiter. It may be noted that iodine crosses the placenta and that goiter (with tracheal obstruction) has been observed in mothers treated with large doses of iodine (61, 96, 113). Endemic iodide goiter has been reported in the north island of Japan, Hokkaido (173). Iodine in amounts up to ~ 200 mg per day are consumed by these patients (118). Surprisingly, as compared to sporadic cases of iodide goiter, all these patients are euthyroid. Plasma inorganic iodide ranged from 13 to 44 µg per 100 ml (123).

The mechanism of thyroid-blocking caused by pharmacological doses of iodide remains poorly understood (196, 197). Biological iodinations within the thyroid are inhibited by iodide (198). Rats on a relatively low iodine diet (with a hyperactive thyroid) are more sensitive than rats on a higher

iodine intake (62, 198). In euthyroid man, PII averaging 10 to 12 μg per 100 ml are inhibitory (168). The Wolff-Chaikoff effect is only transitory and the escape from iodide inhibition involves a loss of normal iodide-concentrating ability of the gland (21). Another striking effect of excess iodide is to inhibit the release of organic iodine from the gland, but the mechanism remains poorly understood.

Antithyroid Substances

Environmental factors other than iodine deficiency have been considered in the etiopathology of nontoxic goiter. Among them, the role of goitrogenic substances has often been proposed to explain some impairment in thyroidal hormone synthesis responsible for the development of goiter. Most of these data have been obtained in experimental animals (28, 29). No clear-cut evidence has yet been obtained in man in favor of a role for antithyroid compounds, such as goitrin, in goiter endemics. Such physiopathological mechanisms require further attention in field studies.

Microbial Factors

Another etiologic hypothesis has been an infective mechanism (66, 68, 116). Unfortunately, no microbiological agents have been isolated. In Germany Hechte (77, 78) has described an association between goiter and polluted shallow wells. Similar relationships have been reported by Vought et al. (187) in Northern Virginia (U.S.A.). In many endemics the drinking water comes from superficial (and easily polluted) sources. The mechanism by which water pollution might modify iodine metabolism remains obscure. Some new perspective may come from recent observations. It has been shown that Clostridium perfringens shares thyroid stimulating properties with TSH (100, 135). Some bacteria (e.g., Paracolobactrum) produce an enzyme (myrosinase) which converts progoitrin into the active goitrogen, goitrin (129). Harden and Adams (74) have shown that changes in intestinal flora following gastrointestinal surgery can influence iodine metabolism. Bacterial infection and bacterial exotoxins acutely or chronically given to experimental animals alter thyroid function (63, 64, 72, 145, 162), and modify the normal turnover rate of the extrathyroidal organic iodine compartment (72, 162). In man alterations of thyroid function during acute exposure to Pasteurella tularensis or after vaccination against encephalomyelitis virus have been described (163). It is generally well known that most of the goiter endemics are characterized by poor sanitary conditions.

SUMMARY

1. The pathophysiology of nontoxic goiter has been carefully studied by many investigators; many of the results are doubtless relevant to endemic goiter. Many parallels in intrathyroidal metabolism of iodine have been observed between the two conditions.

2. While there can be no doubt that iodine deficiency is a major factor in the origin of endemic goiter, it seems certain that there are other factors. Colloid or nodular goiter may in the last analysis be due primarily to iodine deficiency, but the deficiency may result from internal causes. Endemic goiter also may be a result of internal causes acting in concert with iodine deficiency, but this remains to be proved.

3. In this review the synthesis and turnover of thyroid hormones and the metabolism of iodine are reviewed in respect to sporadic and endemic goiter. Mechanisms of goitrogenesis are discussed, including iodine deficiency, antithyroid substances, and parallel microbiological factors.

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CHAPTER 4

A THYROID MODEL AND ITS ANALYSIS BY COMPUTER[1]

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Since it was described by Riggs (18), a three-compartment model of iodine kinetics has been widely used in the analysis of normal and pathological thyroid physiology (3, 7, 8, 12, 13, 22). In order to fit data obtained by newer techniques, this model has been progressively altered (6, 19, 22). Recently Berman (2) described an 11-compartment model derived from mathematical analysis of data from patients with a variety of thyroid conditions.

It is now clear that the interpretation of iodine kinetics in man is complicated by several aspects of intrathyroidal iodine metabolism not appreciated when the three-compartment system was developed.

1) There is a functional heterogeneity of thyroid iodine, evidenced by the "last come, first served" mode of secretion (21, 23) and by the existence in the gland of a newly-labelled TSH-dischargeable iodine compartment (6).

2) There are two distinct sources for intrathyroidal iodide (9, 11, 14, 20); exogenous iodide trapped from plasma by the thyroidal iodide pump, and endogenous iodide derived from intrathyroidal deiodination of iodotyrosines.

3) A fraction of newly trapped iodide diffuses back to the plasma (15, 25).

4) There is intrathyroidal recirculation or direct spill of endogenous iodide from the gland to the plasma (5, 17, 20).

5) The thyroid gland secretes two hormones having very different rates of metabolism.

In order to include these characteristics in the simulation of iodine kinetics, a 20-compartment model has been designed. The model has been

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established primarily along physiologic rather than mathematical lines. Thus, the various amino acids have been separated into distinct compartments, and the physiologic sequence of iodide trapping, binding, coupling, storage, intrathyroidal recirculation of iodide, and secretion has been included. The distribution spaces for iodide, T_3 and T_4 have been divided into intravascular and extravascular compartments, and urinary and fecal excretory routes are provided. The "last come, first served" secretion has been included, following the model proposed by Ermans (6).

This system is able to reproduce with accuracy the iodine kinetic data of normal man. It is also certain, after considering the model, that new interpretations must be given to some of the parameters of human iodide kinetics which have previously been evaluated on the basis of the three-compartment model.

METHODS

Description of the Model

The general network of the model is represented in Figure 1. It is characterized by the following parameters:

Q_i (μg) - quantity of iodine^{127} in compartment i.
V_i (liters) - distribution space of iodine in compartment i.
$\rho_{j,i}$ (μg/day) - flux of iodine^{127} from compartment i to compartment j.
$\lambda_{j,i}$ (fr/day) - rate constant of transfer of iodine from compartment i into compartment j.

Ingested iodide enters the plasma iodide compartment (cpt. 1) and rapidly equilibrates with the extravascular iodide compartment (cpt. 2). Plasma iodide is taken up by the thyroid ($\rho_{3,1}$) or excreted in the urine ($\rho_{19,1}$). From the first thyroid iodide compartment (cpt. 3) some iodide is rapidly bound to thyroglobulin ($\rho_{4,3}$ and $\rho_{5,3}$) to form MIT (cpt. 4) and DIT (cpt. 5) in the "binding pool," and a portion diffuses back to the plasma ($\rho_{1,3}$). Some of the iodotyrosine groups are then coupled to form T_3 and T_4 in the "coupling pool" (cpt. 6=MIT, cpt. 7=T_3, cpt. 8=T_4, cpt. 9=DIT). In a sense these four compartments may be visualized as an average molecule of thyroglobulin. From this coupling pool, iodinated thyroglobulin is either directly hydrolized ($\rho_{18,6}$, $\rho_{14,7}$, $\rho_{15,8}$, $\rho_{18,9}$) or stored in the "storage pool" ($\rho_{10,6}$, $\rho_{11,7}$, $\rho_{12,8}$, $\rho_{13,9}$). This "storage pool" (cpt. 10=MIT, cpt. 11=T_3, cpt. 12=T_4, cpt. 13=DIT) represents mixing compartments in continuous exchange with the coupling pool ($\rho_{6,10}$, $\rho_{7,11}$, $\rho_{8,12}$, $\rho_{9,13}$). T_3 and T_4 liberated by proteolysis enter their respective plasma compartments (cpt. 14 and cpt. 15), and equilibrate with their extravascular compartments (cpt. 17 and cpt. 16). The MIT and DIT are deiodinated in the gland and their iodide enters a second thyroid iodide compartment (cpt. 18). From this compartment, iodide is spilled to plasma ($\rho_{1,18}$) or reutilized in the binding compartment ($\rho_{4,18}$, $\rho_{5,18}$). From their extrathyroidal compartments, T_3 and T_4 are either excreted in the feces (cpt. 20) or degraded to liberate iodide, which re-enters the plasma iodide compartment.

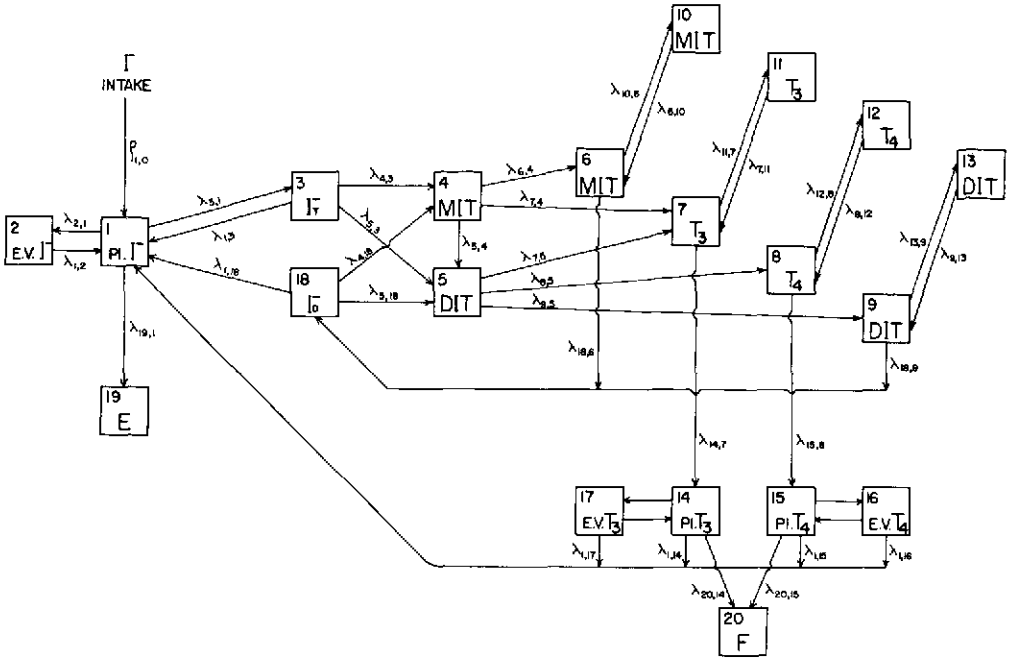

Figure 1. Detailed diagram of the model. The squares represent the compartments, the arrows connecting them, the various pathways of iodine metabolism. Compartments 1 and 2 are the extrathyroidal iodide compartments. Compartments 3 and 18 are the thyroidal iodide compartments (exogenous and endogenous, respectively). Compartments 4 and 5 represent the "binding" pool; 6, 7, 8, and 9 represent the "coupling" pool; and 10, 11, 12, and 13 the "storage" pool. Compartments 14 and 15 represent extrathyroidal hormones with their distribution spaces (compartments 17 and 16, respectively). Compartments 19 and 20 represent urinary and fecal excretions.

Basic Assumptions for Calculation of the Parameters

Calculation of the various parameters was based on the following assumptions:

1) In the steady state, the compartment sizes (μg of I^{127}) remain constant. The input of each compartment is equal to its output.

2) The proportion of total thyroid iodine^{127} in the binding pool is 2 per cent, coupling pool 50 per cent, and storage pool 48 per cent. These proportions have been determined by visual fit of the computed curves to experimental data.

3) The typical iodoamino acid distribution in the normal gland is taken to be MIT:25 per cent, DIT:50 per cent, T_3:5 per cent, and T_4:20 per cent.

4) The specific activities (quantity of radioiodine per μg stable iodine) of MIT and DIT show a parallel evolution with time after administration of radioiodine. A similar parallelism is observed for T_3 and T_4 (6).

5) Exogenous and endogenous iodide compartments have been separated because of the different evolution of their specific activity. However, the binding rate is considered to be the same from both iodide compartments.

6) Hydrolysis of thyroglobulin from the coupling pool is complete, and liberates all the iodotyrosines and iodothyronines present in the molecule. The iodothyronines are directly secreted into the blood. The iodotyrosines are completely deiodinated and iodide undergoes either intrathyroidal recycling or spillage into the plasma.

7) The T_3 to T_4 ratio in the secretion is equal to the intrathyroidal ratio.

8) The overall turnover rates are the same for the two components of the binding pool, for the four components of the coupling pool, and for the four components of the storage pool.

The basic numerical values used for calculation are given in Table 1.

Method of Calculation of the Various Parameters

On the basis of the preceeding assumptions and numerical values, the parameters were evaluated by the following method (Table 2).

A. Intrathyroidal Compartments

1) Organic iodine compartments: The total thyroidal I^{127} compartment (10,700 μg) (5) was divided on the basis of the relative distribution in binding, coupling, and storage pools, and in the various iodoamino acid compartments, MIT, DIT, T_3 and T_4.

2) Secretion of hormonal iodine: Assuming the total daily secretion of hormonal iodine to be 60 ug, the relative amounts of T_3 ($\rho_{14,7}$) and T_4 ($\rho_{15,8}$) secretion were evaluated on the basis of the intraglandular T_3/T_4 ratio, i.e., 1/4. The fractional secretion rates from the coupling compartments for T_3 and T_4 were calculated on the basis of flux to pool size ratio. The identity of overall turnover rates of the four coupling compartments assumption 8) ($\lambda_{18,6}=\lambda_{18,9}=\lambda_{14,7}=\lambda_{14,7}=\lambda_{15,8}$) leads to the estimation of the total daily iodide input into compartment 18, i.e. ($\rho_{18,6} + \rho_{18,9}$).

3) The total daily iodine turnover by the gland is given by ($\rho_{18,6} + \rho_{18,9} + \rho_{14,7} + \rho_{15,8}$).

4) Estimation of iodide spill: ($\rho_{1,18}$). In order to evaluate this flux, three different methods were available:

a) The daily secretion of 60 μg of iodine from a total thyroidal iodine compartment of 10,700 μg determines a secretion rate constant of .0056/day. The difference between the rate of hormone secretion and the experimentally observed net thyroid radioiodine release rate, .01/day, may be interpreted as nonhormonal iodine secretion (65). In the present case, this difference of .0044 fr/day determines a spill of 47 μg per day. With a total input of 180 μg in

Table 1. Independent parameters.

	UNITS	
Daily iodide intake ($\rho_{1,0}$)	μg/d	145
Total thyroidal iodine (Q_G)	μg	10,700
Binding pool (B.P.)	Fract. Q_G	.02
Coupling pool (C.P.)	Fract. Q_G	.50
Storage pool (S.P.)	Fract. Q_G	.48
Monoiodotyrosine (MIT)	Fract. Q_G	.25
Diiodotyrosine (DIT)	Fract. Q_G	.50
Triiodothyronine (T_3)	Fract. Q_G	.05
Thyroxine (T_4)	Fract. Q_G	.20
Thyroidal organic iodine secretion (H)	μg/d	60
Binding rate (K_B)	fr/d	720
Iodide spill ($\rho_{1,18}$)	μg/d	5
Backward diffusion	Fract.	.20
Iodide distribution space (V_{1+2})	Liters	26.6
Intravascular iodide space (V_1)	Liters	4.8
Plasma iodide concentration (I)	μg/1	2.82
Half-time of iodide diffusion in iodide space	Days	.0417
Fecal excretion (F)	μg/d	17
Half-time of T_3 diffusion in T_3 space	Days	.11
Half-time of plasma T_3 metabolism	Days	1.3
Half-time of T_4 diffusion in T_4 space	Days	.50
Half-time of plasma T_4 metabolism	Days	6.9
T_3 distribution space ($V.T_3$)	Liters	31
T_4 distribution space ($V.T_4$)	Liters	10
Plasma T_3 or T_4 space (V_{14} or V_{15})	Liters	2.5

Table 2. Calculated parameters.

	Q_i in μg	$\lambda_{j,i}$ in fr/d		$\lambda_{j,i}$ in fr/d	
Q_1	13.5	$\lambda_{2,1}$	16.6	$\lambda_{12,8}$	.0224
Q_2	61.5	$\lambda_{1,2}$	3.6	$\lambda_{8,12}$	.0234
Q_3	.0903	$\lambda_{19,1}$	9.5	$\lambda_{13,9}$	.0224
Q_4	71.4	$\lambda_{3,1}$	6.02	$\lambda_{9,13}$	.0234
Q_5	143	$\lambda_{1,3}$	180	$\lambda_{18,6}$	.0449
Q_6	1340	$\lambda_{1,18}$	20.6	$\lambda_{14,7}$	.0449
Q_7	267	$\lambda_{4,3}$	264	$\lambda_{15,8}$	.0449
Q_8	1070	$\lambda_{5,3}$	456	$\lambda_{18,9}$	.0449
Q_9	2670	$\lambda_{4,18}$	264	$\lambda_{17,14}$	6.3
Q_{10}	1280	$\lambda_{5,18}$	456	$\lambda_{14,17}$	.0309
Q_{11}	257	$\lambda_{5,4}$	.337	$\lambda_{16,15}$	1.39
Q_{12}	1030	$\lambda_{6,4}$	.841	$\lambda_{15,16}$	.397
Q_{13}	2570	$\lambda_{7,4}$	.561	$\lambda_{20,14}$	.140
Q_{14}	1.82	$\lambda_{7,5}$	.561	$\lambda_{20,15}$	.140
Q_{15}	119	$\lambda_{8,5}$	.337	$\lambda_{1,17}$	.522
Q_{16}	358	$\lambda_{9,5}$	..841	$\lambda_{1,14}$	.522
Q_{17}	20.7	$\lambda_{10,6}$	.0224	$\lambda_{1,15}$	.0654
Q_{18}	.243	$\lambda_{6,10}$	.0234	$\lambda_{1,16}$	.0654
		$\lambda_{11,7}$	.0224		
		$\lambda_{7,11}$	.0234		

Q_i *Quantity of iodine in compartment i.*

$\lambda_{j,i}$ *Fractional rate of transfer from i to j.*

compartment 18 per day, the daily spill of iodide would represent $\simeq$ 26 per cent of the endogenous iodide.

b) It is estimated that 20 per cent (15, 17, 25) of newly trapped iodide is lost by backward diffusion. If the two iodide compartments have the same turnover rates, it may be logical to assume for the iodide spill a similar fraction of endogenous iodide, i.e., 36 µg per day.

c) Derive a value corresponding to the best visual fit of computed curves to the experimental data. In the present case, the best fit is obtained when $\rho_{1,18}$ is taken as 5 to 10 µg per day. The reasons for retaining this value (5 µg) are analyzed in the discussion.

5) Intraglandular recycling and iodide trapping: The difference between the total daily input into compartment 18 and the iodide spill represents iodide recycled. At equilibrium, the iodide accumulated each day is the amount necessary to equal the sum $(\rho_{14,7} + \rho_{15,8} + \rho_{1,18})$.

6) Iodide compartments: Using the binding rate proposed by Berson and Yalow (4) (.50 per min.) and the total daily turnover of iodide (240 µg), the size of the total thyroidal iodide pool was estimated by the relation: daily turnover in µg per fractional rate in days=compartment size in µg. The relative amounts of iodide in compartments 3 and 18 are proportional to the relative fluxes through these compartments since we assumed that binding proceeds at the same rate from both compartments.

7) Equilibrium between coupling and storage compartments: The input from binding to coupling compartments is separated into fractions: one is directly secreted and the other is stored. The fraction transferred to storage compartment has been set equal to the fraction of total thyroidal stable iodine present in the storage compartment.

8) Backward diffusion of trapped iodide ($\rho_{1,3}$): It has been estimated at 20 per cent of $\rho_{3,1}$ diffuses back to the plasma iodide compartment.

B. Extrathyroidal Iodide Compartments

A total iodide distribution space of 38 per cent (5) of BW (26.6 liters for a man of 70 kg) multiplied by the plasma stable concentration determines a total quantity of 75 µg of iodide. This quantity was divided into intravascular and extravascular compartments. A 4.8 liter intravascular iodide space was used (68.5 cc per kg BW); a half-time of equilibrium of iodide from compartment 1 to 2 of 60 minutes was used, on the basis of our experimental data of iodide disappearance from blood.

C. Extrathyroidal Organic Iodine Compartments

In steady state, the daily degradation of hormonal iodine equals the daily secretion. With rates of disappearance of ^{131}I-T_3 (.53 per day) (10) and ^{131}I-T_4 (.10 per day) (16) from plasma, the total size of T_3 and T_4 compartments could be calculated on the basis of the formula: daily degradation/fractional degradation per day = compartment size. These compartments

are also separated into intravascular and extravascular spaces with total distribution spaces of 31 liters for T_3 (10) and 10 liters for T_4 (4, 5, 16, 17). The total excretion of T_3 and T_4 in the feces (17 μg per day) (24) was divided by assuming that their fractional rates of fecal excretion are similar. The remainder of metabolized T_3 and T_4 was considered to be degraded by peripheral deiodination. The half-times of equilibration of T_3 and T_4 from blood to extravascular compartment were taken as around 2.5 hours and 12 hours respectively.

Computation and Simulation of Iodine Kinetics

The model, characterized by these parameters, has been tested by means of a digital computer (CDC 6400 or IBM 360/65) solving a system of simultaneous linear differential equations of the form:

$$dQ_i/dt = \sum_{\substack{j=i \\ i \neq j}}^{n} \lambda_{i,j} \times \underline{Q_j} - \sum_{\substack{j=i \\ i \neq j}}^{n} \lambda_{j,i} \times \underline{Q_i}$$

where: Q_i and Q_j are the quantities of iodine in compartments i and j respectively.

$\lambda_{i,j}$ is the rate constant of transfer to i from j.

t is the time in days.

Studies of the distribution of a tracer dose of iodide were made by taking as initial conditions $Q_1 = 1.00$, while the other 19 compartments were set to zero.

Conditions simulated:

The following conditions were tested:

1) Evaluation of the steady state for a 40-day period.

2) Distribution of a tracer dose of iodine (introduced in the plasma iodide compartment 1) for a period of 40 days (with a spill of 5 μg/day, a coupling to storage pool ratio of .50/.48, and similar direct secretion to storage ratio).

3) Tracer studies of 10 days with various iodide spills, various coupling to storage pool ratios (C/S), and various proportions of direct secretion and storage from the coupling pool (Se/St).

4) Simulation of the action of methimazole by taking $\lambda_{4,3} = \lambda_{5,3} = \lambda_{5,3} = \lambda_{4,18} = \lambda_{5,18} = 0$ after a ten-day tracer study.

5) Simulation of the action of $KClO_4$, after a ten-day tracer study, by taking $\lambda_{3,1} = 0$.

Analysis of the Data on the Basis of the Three-Compartment Model

The computed curves were analyzed by methods based on the three compartment model with formulas developed by Riggs (18), Berson and Yalow (3) and Ermans et al. (7, 8). The computations were as described by DeGroot (5).

In order to ensure a reliable comparison, data were computed at different times corresponding to the usual counting and sampling periods observed in patient studies.

RESULTS AND DISCUSSION

1. Distribution of Iodide Introduced as a Tracer in Compartment 1

After computation had indicated that the model remained in a steady state during a simulated run of 40 days with a constant stable iodide intake, the distribution of a single dose of a tracer was followed in the different compartments. Figure 2 shows the curves obtained during simulation of 40 days. The thyroid takes up 30 per cent of the dose at 24 hours and a maximum of 32.5 per cent of the dose is reached after about 48 to 72 hours. The urinary and plasma iodide curves are parallel and show a very small dip around the fourth day. At that time, the level is .1 per cent of the dose per day for urinary iodide excretion and .002 per cent of the dose per liter for the plasma iodide. The PBI increases slowly and reaches a level of .02 per cent of the dose per liter at 24 hours and .05 per cent of the dose per liter at 48 hours. After 10 days, the curve approaches a plateau in the range of .17 per cent of the dose per liter of plasma. The SA of urinary iodide and plasma PBI are parallel after 10 days, and the urinary iodide SA is always lower than the PBI SA (Figure 3). A very early drop in the thyroid clearance is evidenced during the first five minutes after the introduction of the tracer. All these figures agree well with observed patient data. The uptake and the PBI are in the high normal range for average individuals in the Boston area.

2. Influence of Varying Inorganic Iodide Spill

Three levels were studied for $\rho_{1,18}$, and are shown in Figure 4 (the Se/St

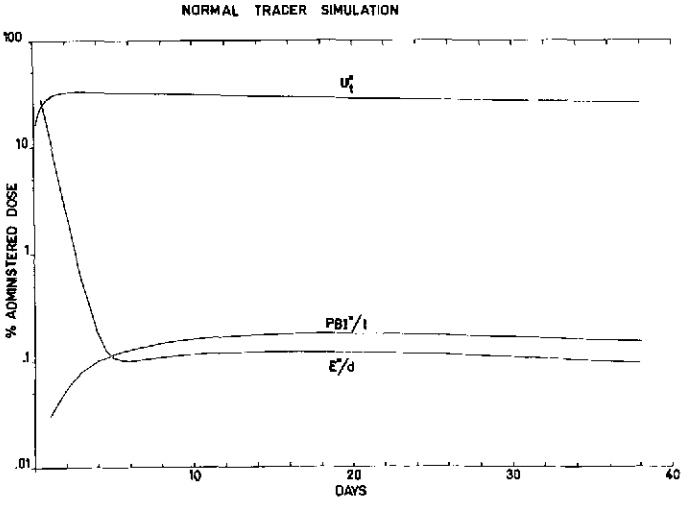

Figure 2. Theoretical distribution of a tracer dose of isotope in the thyroid $U^*_{(t)}$, the serum protein-bound iodine PBI/l and the daily urinary excretion of iodide E*/d.*

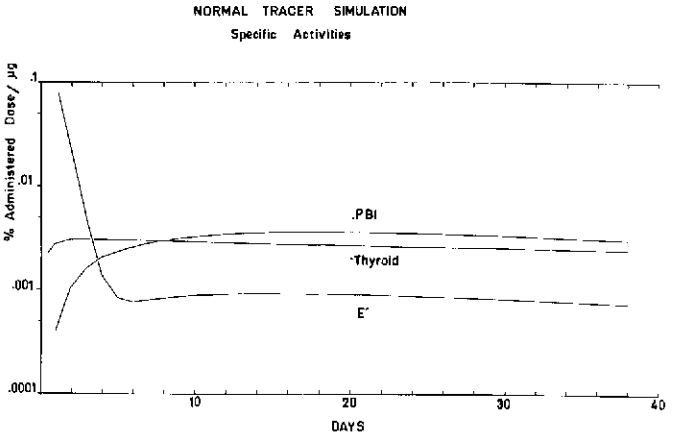

Figure 3. Theoretical evolution of the specific activities in thyroid, serum PBI, and urinary iodide E.

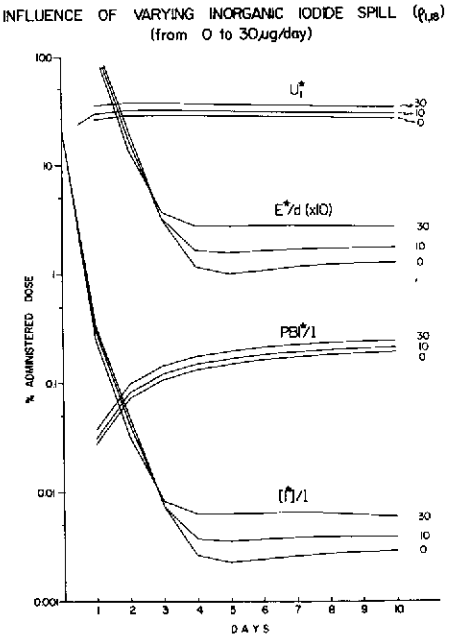

Figure 4. Influence of varying inorganic iodide spill ($\rho_{1,18}$) on thyroid, plasma, and urinary isotope. The small dip, present when the spill is 0 µg/d, disappears progressively by increasing to 10-30 µ.

and C/A ratios were .30/.70). The most striking effects of increasing the spill of endogenous iodide are (a) an elevation of the urinary iodide excretion and of the plasma iodide concentration; (b) the progressive disappearance of the small dip observed in these curves between day 4 and day 8 after administration of the tracer; (c) an elevation of the thyroidal uptake because of the higher amount of iodine necessary to compensate for the total organic and inorganic secretion; (d) the plasma PBI reaches a higher plateau with a similar rate of increase. The higher plateau is probably related to the higher uptake. The modification of the iodide spill thus normalizes the shape of the urinary iodide excretion curve for the period between the fourth and the eighth day but it must be emphasized that no plateau is observed after this period and the curve decreases progressively when a high spill is used. Furthermore, with an organic iodine secretion of 60 μg per day, which agrees with published data, we are limited in increasing the inorganic secretion by the accepted AIU value. A third problem encountered in increasing the spill above a given level is the urinary excretion (above .2 per cent per day) becomes too high in comparison to the patient data.

For most of the following studies, a spill of 5 or 10 μg per day was used because it provided the best visual fit of the computed curves to the patient curves.

3. Influence of Varying the C/S Ratio

The results of this study are given in Figure 5 (the same spill of 10 μg per day was used in each case, with a Se/St ratio of .3/.7).

The following modifications of the curves were related to lowering the C/S ratio: (a) The level of the urinary and plasma iodide curves was higher. There was a progressive disappearance of the dip observed between day 4 and day 8. (b) The rate of ascent of the plasma PBI curve and the level of the PBI plateau were increased. (c) The thyroid secretion rate was increased.. Thus, in choosing the best ratio, we were limited on one hand by the level of the PBI, which fitted the patient data best with a larger coupling pool size, and the dip in the urinary iodide curve which became more prominent with a large coupling pool. Indeed, the dip is maximum with the classic three compartment model, where in effect the coupling pool or secretory pool is maximum.

4. Influence of Varying the Se/St Ratio

Figure 6 shows the very small influence of varying the direct secretion and storage ratio on the various curves. These computations were made with a spill of 10 μg per day and a C/S ratio of .30/.68 for three different Se/St ratios. This factor does not alter to a great extent the kinetics of iodine in the present state of the model.

5. Simulation of Methimazole Action

The results are given in Figure 7. The methimazole action was simulated after 10 days of a normal tracer run by setting the binding rates equal to zero. (10 μg per day of spill, .30/.68 for the C/S ratio, and .30/.70 for the Se/St ratio). This is followed by (a) a large increase of urinary iodide excretion and plasma iodide concentration; (b) an increase in the slope of the thyroid iodine release curve (.015 on day 5 before the block and .036 on the day 15

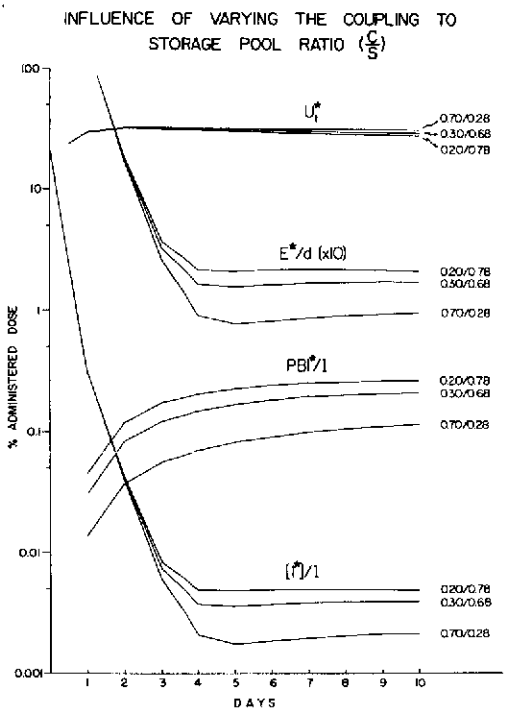

Figure 5. Influence of varying the coupling to storage pool ratio. The smaller the coupling pool the higher the plasma and urinary iodide, the PBI, and the thyroid apparent secretion rate.

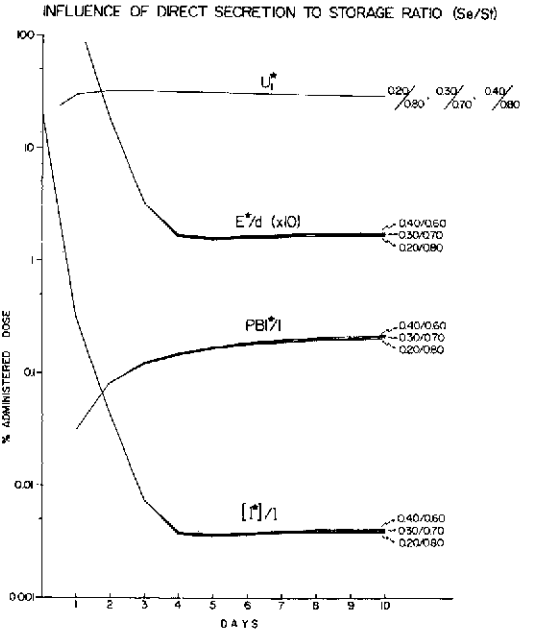

Figure 6. Influence of varying the proportion of newly enhanced iodine directly secreted. A very small change is apparent in urinary and plasma iodide and in plasma PBI.

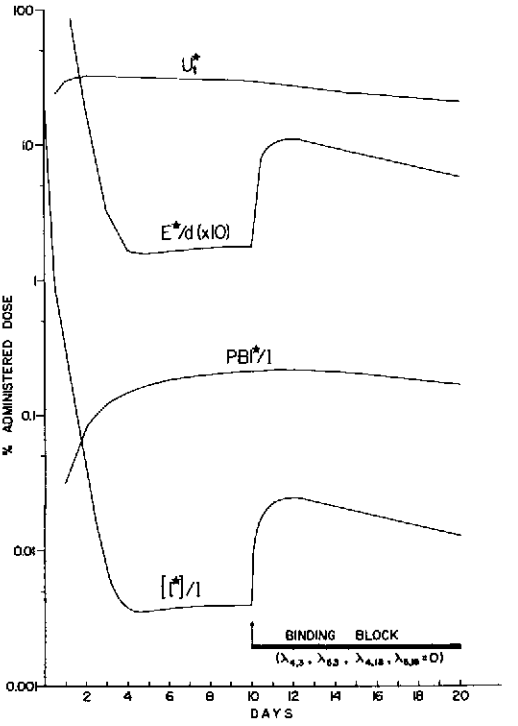

Figure 7. Simulation of methimazole action ($\lambda_{4,3}, \lambda_{5,3}, \lambda_{4,18}, \lambda_{5,18} = 0$). This induces an increased plasma and urinary iodide and a faster release of isotope from the gland.

when binding is blocked). A very small decrease in the plasma PBI level after 10 days. These data are in good agreement with the patient curves except for the iodide levels in urine and plasma, which are too high. This may be related to two different factors in the model: too high recirculation of endogenous iodide (because of a low iodide spill) or an erroneously high iodotyrosine/iodothyronine ratio (1/3) in the model. This problem must be solved by further experiments.

6. Simulation of Perchlorate Action

The action of $KClO_4$ was simulated by setting the trapping coeffient equal to zero ($\lambda_{3,1}$). The curves are given in Figure 8: (a) The change in the thyroidal iodine release rate is not evident on the curve, but when calculated from the formula $K_G = \Delta U^*/U^*$ it shows a small increase after starting perchlorate. (b) A negligible change in the plasma PBI* is seen after a 10-day block. (c) The increase in plasma and urinary iodide levels and the change of the thyroidal slope is due to the absence of iodide recirculated from peripheral degradation of thyroid hormones.

7. Analysis of the Computed Curves on the Basis of the Three-Compartment Model

The results of the calculations are given in Table 3, along with the mean values for four normal patients (5). The most interesting features are as follows:

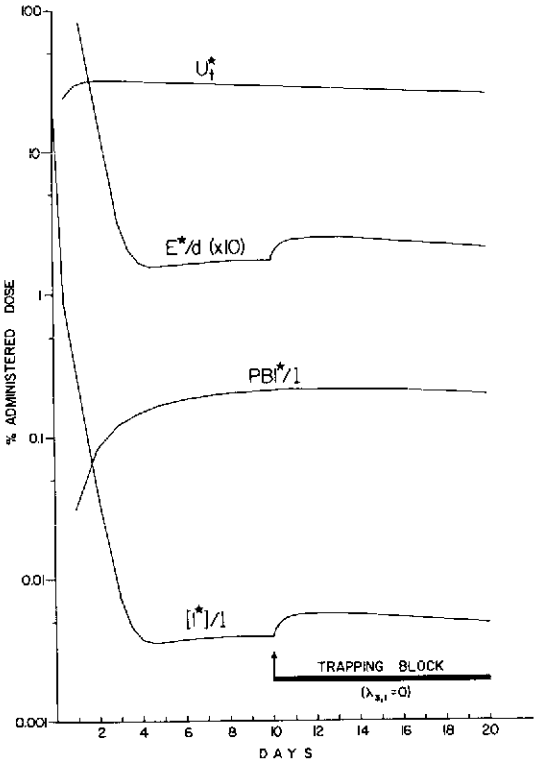

Figure 8. Simulation of perchlorate action ($\lambda_{3,1}$= 0). The evaluation of the plasma and urinary iodide is related to the absence of reutilization of peripheral iodide.

a) The theoretical maximal uptake U*, obtained by extrapolation of the thyroid release curve to time zero, is always larger than the value obtained from the formula $U^* \frac{C_G}{C_G + C_K}$. Indeed, as will be emphasized later, the thyroidal release curve is not a straight line. Its slope varies with time and is much higher in the early period (Table 4).

b) Evaluation of K_{GB}, the thyroidal secretion rate: (1) K_G (the apparent thyroidal secretion rate) obtained from the slope of the thyroid release curve, and K_{GB} 1 calculated by the formula $K = \frac{K_G}{1-U}$ are in good agreement with the patient data.(2) When the secretion rate K_{GB}#2 is evaluated from the rate of approach of the PBI to its asymptotic value, the results are also in good agreement with the patient studies.

With both methods, the value obtained for K_{GB} during the first 15 days is higher than the actual value for the model obtained from the relationship for stable iodine $K_{GB} = \frac{H + Spill}{Q_G}$.

The secretion of isotope differs from the actual behavior of the thyroidal stable iodine because of the "last come, first served" mode of secretion. Both the slope of the thyroidal iodine release curve, and the rate of approach of the PBI to its asymptotic value, are too high early after the maximum uptake. These parameters will not equal the actual rate until complete equilibrium is reached between the various compartments in the gland. Furthermore,

Table 3. Kinetic data of computed curves.

	Units	Normal patient 1/	20 Cpt. Model	
			Tracer simulation	Actual stable iodine value
U^*_{Max}	% dose	29	32	33.7
C_K	ml/min	26.4	31.5	31.5
Initial C_G	ml/min		20	20
Net C_G	ml/min	10.2	15.8	16
K_G	fr/d	.0089	.0081	.0040
K_{GB}#1	fr/d	.0123	.0119	.0060
K_{GB}#2	fr/d	.0106	.0100	.0060
H#1	µg/d	132	114	65
H#2	µg/d	114	95	65
Q_G#1	µg	10,690	9,467	10,700
Q_G#2	µg	10,400	9,481	10,700
AIU#1	µg/d	54	62	65
AIU#2	µg/d	52	64	65

1 *I.J. DeGroot, 1966.*

Table 4. Evolution of thyroid release slope with time.

		K_G Fract. U^*_t/d	$K_{GB} = K_G/1-U^*$
Tracer simulation	6 Days	.0091	.0137
	12	.0075	.0113
	18	.0066	.0099
	24	.0059	.0089
	30	.0054	.0081
	36	.0050	.0075
Thyroid stable iodine		.0041	.0061

the asymptotic value of the PBI curve, used in the second method, is lower than the theoretical maximum. Indeed, the PBI is being degraded as soon as its secretion begins and the curve never reaches the theoretical plateau. Figure 9 shows the influence of the plateau level on the rate ($K_{GB} + K_{BG}$). This last factor may become very important when the T_3/T_4 ratio increases in the secretion.

c) Evaluation of the total thyroid iodine pool:

When the formula $Q_G + Q_B = \frac{PBI^{127}\ \mu g/l}{PBI^{131}\ fr\ \ I^{131}\ ret/l}$, and Q_B =PBI x BW x .14 are used, the results are lower than the actual thyroid iodine content of 10,700 µg (Q_G#1).

Very similar results are obtained from the formula $Q_G\ 2 = \frac{U_t^*}{PBI^{131}\ SA}$ in plasma.

Both of these calculations are restricted by the absence of isotopic equilibrium. Because of the "last come, first served" mode of secretion, the SA in the plasma PBI is higher than the thyroidal SA, and for this reason the stable iodine compartments cannot be perfectly evaluated on the basis of plasma isotope specific activity. In our model, around the eighth day the specific activities are equal in plasma PBI and thyroid and this may explain the relatively good agreement between the actual value of Q and the computed value during the 10- to 15 day-period. It must be emphasized that, in the present model, the PBI plateau does not correspond to the isotopic equilibrium. Furthermore, the equilibrium, after a single dose of tracer, is a very transient state because of the continuous dilution by newly ingested stable iodine.

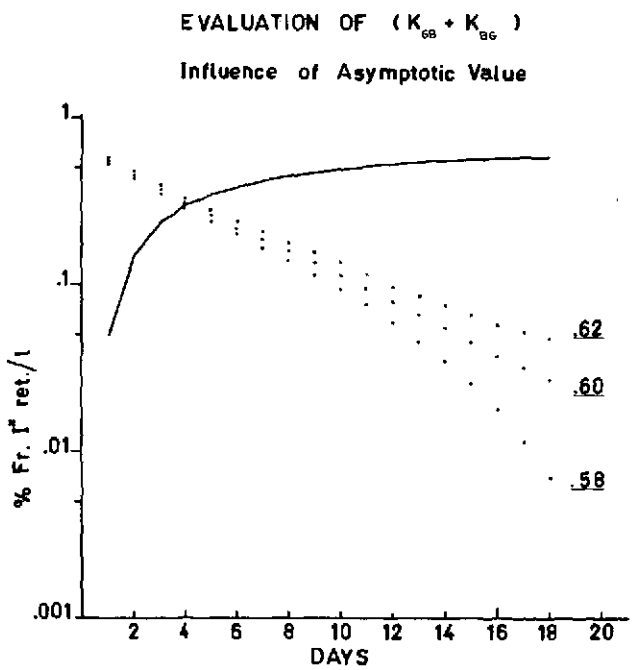

Figure 9. Influence of the level of the PBI plateau on the evaluation of (K_{GB}+K_{BG}). The slope is different with the three levels tested. The apparent level of the plasma is .58 per cent fr. I ret/l.*

d) The evaluation of the thyroidal secretion from the relation $K_{GB} \times Q_G$ gives too high a value when compared with the actual parameter of the model (H + Spill), but is in very good agreement with the patient data.

e) The absolute iodine uptake values obtained from the relations $AIU^{\#1}=\frac{EU}{1-U}$ and $AIU^{\#2}=C_G \times (1) \times 1.44$ are very close to both the patient and model results.

f) The predicted urinary excretion in % dose per day from the relation (BW x 114 x PBI^{131}% dose/l x K_{BI} x (1-U)) is lower than the observed urinary excretion but the ratio is close to one after 10 days. This finding does not agree with the patient data. The reason for this discrepancy is not yet clear.

SUMMARY

A thyroidal iodine kinetic model of 20 compartments has been studied by means of a digital computer. The intrathyroidal sequence includes iodide trapping (first iodide pool), binding, coupling, storage, intrathyroidal recirculation (second iodide pool), and hormone release. The extrathyroidal iodide, T_3 and T_4 compartments, with their respective distribution spaces, are also included along with the urinary and fecal excretion. The 24-hour uptake is around 30 per cent of the dose and the $PB^{131}I$ at 48 hours is in the range of .06 per cent of the dose per liter of plasma. The maximum specific activity (SA) is reached in the binding pool after .25 days, in the coupling pool fater about three days, and in the storage pool after more than 30 days. The PBI SA plateau is reached at 10 to 12 days.

The proposed model accurately reproduces physiological aspects of iodine kinetics in normal man and simulates the "last come, first served" secretion. It appropriately represents the action of $KCLO_4$ and methimazole on thyroidal, plasma, and urinary radioactivity.

Our evaluation of the present model indicates that new interpretations must be given to some of the parameters which are usually calculated on the basis of a standard three-compartment model.

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CHAPTER 5

OBSERVER VARIATION IN GRADING AND MEASURING THE THYROID IN EPIDEMIOLOGICAL SURVEYS

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A major problem in comparing the results of surveys of endemic goiter has been a lack of consistency among investigators in the recording of thyroid size. Many systems of grading have been used in the past, such as those of Kimball and Marine (4), Olesen (7), Ryle (9), Choufoer (1), and Kilpatrick (3). The system perhaps most frequently used today is that of Perez et al. (8).

Grading is inherently subjective. The process involves a decision as to size. Many factors may influence this decision. The criteria of assignment may vary in the same observer at different times or among different observers at the same time. There are few problems with nonpalpable or very large thyroids. Most of the difficulties occur when there is a slight to moderate degree of enlargement and a decision must be made not only as to grade, but as to goiter or not-goiter. Most glands are only slightly to moderately enlarged; few are very large. Whether the criterion is size on palpation or visibility, the difficulty remains, and the decisions may greatly affect the reported prevalence of goiter. In grading by palpable size, the thyroid is compared with a standard, which may be the observer's past experience (standardized in some way), some object of known size such as the terminal phalanx of the subject's thumb (8), or perhaps plasticine models of the thyroid (12).

The problem of observer variation has been recognized in many field studies. In their surveys in Akron, Ohio, Kimball and Marine (4) made a major effort to ensure uniformity of methods and classification. Stocks (10) discussed the large observer variation in a survey of England and Wales by school medical officers. Visible enlargement was the criterion used to define goiter. McCullagh (6) examined 428 persons twice within 2 to 3 days and found 33 per cent goiter on the first examination and 42 per cent on the second. Hennessy (2), using the grading system of Perez et al., examined 200 persons twice and found that the visible goiter rate was less variable then the total goiter rate. Both McCullagh and Hennessy reported variation in a single observer.

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We have sought other methods of recording thyroid size which might be less subjective and variable and which would minimize the need for decisions during field surveys. At first, in addition to grading, we constructed a plasticine model of every palpable gland and later weighed and measured the models. We have also used another method which involves inspection and palpation of the thyroid. A ballpoint pen is used on the skin to outline palpable glands, and the outline is transferred to thin paper for subsequent measurement.

In order to compare grading with plasticine models and surface outlines, a series of trials was conducted in which both grading and an additional method were used by several observers. The objectives of these trials were:

1. To test for consistency within and among observers for each of the parameters as an indication of reliability of the systems of assessing thyroid size.

2. To assess the dependence or independence of each of the measuurements observed: grade, palpability, area, height, width.

We present here the results of these trials.

METHODS

The observers did not know the results of their previous examinations, nor the results of other observers. There was clerical and other assistance so that each observer spent from two to three minutes with each subject. The smallest group examined had 30 subjects. From 12 to 30 days elapsed between repeated examinations. We do not believe that observers remembered their previous findings. They were not permitted to see their results until the conclusion of a trial. The two trials were in the towns of Candelaria and Rozo. All were in schoolchildren aged 10 to 14 years. Candelaria had a prevalence of goiter of about 20 per cent, whereas in Rozo children with easily palpable thyroids were preselected by another observer, and the prevalence of goiter was about 50 per cent.

RESULTS

A. Candelaria

One hundred and one girls were examined by three observers on 18 and 30 May 1966. Palpability and grade were recorded. Children who were judged palpable by all observers were selected by an assistant, re-examined, and models of each gland were constructed from plasticine. These were carefully packed and later measured. The measurements were weight, surface area, greatest transverse width, and midline height of isthmus. The statistical design was that of a two-factor randomized complete block. The two factors were date of examination and observer. The subjects functioned as the blocks in this study. The analyses of variance of these data appear in Table 1.

The analyses indicate that grade and palpability are different from the measurements of area, height, and width and that these measurements are in turn different from the measurement of weight. These differences primarily involve the interactions of the design variables. Not as many of the

*Table 1. Analyses of variance, Candelaria, May 1966.**

Source of variation	d.f.	Grade		Palpability		d.f.	Width		Area		Height		Weight	
		F	P	F	P		F	P	F	P	F	P	F	P
Subject	98	11.28	<.01	9.67	<.01	20	2.12	<.05	4.37	<.01	6.70	<.01	12.36	<.01
Observer	2	15.18	<.01	29.14	<.01	2	15.10	<.01	3.30	<.05	5.86	<.01	92.96	<.01
Date	1	2.49	ns	2.39	ns	1	5.39	<.05	30.53	<.01	44.46	<.01	75.45	<.01
Subject by observer	196	1.02	ns	1.97	<.01	40	<1	ns	1.23	ns	1.17	ns	2.50	<.01
Subject by date	98	1.17	ns	1.27	ns	20	1.16	ns	1.95	<.05	2.01	<.05	2.69	<.01
Observer by date	2	14.24	<.01	11.38	<.01	2	<1	ns	<1	ns	3.86	ns	8.52	<.01
Residual	196					40								

** Mean squares are not included but are available on request.*

interactions are significant for grade and palpability as for the other variables. This may be due to the fact that both grade and palpability were reported as ranks and as such are not normally distributed. Hence, the analysis of variance is not truly appropriate for this kind of data, although some feeling for the forces at work in the system is probably attainable through this analytic approach. In spite of this breach in the assumptions required for the validity of the analysis of variance, an attempt will be made to summarize these analyses.

As usual in these cases, simple tests of hypotheses concerning the significance of the main effects are invalid in the presence of interactions. For example, if the differences between observers were different on different dates we would not know how to compare observers. This is particularly true for the variables grade, palpability, and weight. The existence of interaction of subject and date indicates that the subjects were not behaving similarly on the two dates of examination. The reason for this variability is not understood.

Where the observer by date interaction is not significant the tests of significance for the observers are appropriate. Observers were found significantly different for the variables area, height, and width. Due to the existence of observer by date interaction, tests of significance for the simple effects of observers and date as measured by grade, palpability, and weight are not valid. These interactions suggest that observers changed their criteria during the study. This is not surprising since the method being tested was a new one and dexterity undoubtedly improved with practice.

In view of the interactions detected in the previous analysis, it was decided to examine individual observer reliability using the McNemar tests for the significance of changes. This test is particularly applicable to those "before and after" designs in which each person is used as his own control and in which measurement is made on either a nominal or an ordinal scale. The test focuses on those individuals who on one occasion give one response and on another a second response.

Observers 1 and 2 were consistent in their reporting of grade and palpability on the two dates. Observer 3 was not as consistent, and significant differences were found in grade and palpability on the two dates. Table 2 is representative of comparisons in which McNemar tests were used.

The among observer comparisons of grade on 18 May revealed that all observers were significantly different from one another, whereas on 30 May observers 1 and 3 and 2 and 3 were similar and 1 and 2 significantly different ($p < .05$). With palpability, observers 1 and 2, and 1 and 3 were significantly different ($p < .01$) on 18 May and 2 and 3 were similar, whereas on 30 May observers 1 and 2, and 2 and 3 were significantly different ($p < .01$) and 1 and 3 were similar.

B. Rozo

In this study 67 children aged from 9 to 14 years were examined. All except two had enlarged thyroids. We were interested in testing the effects of daily treatment with thyroid hormones over time. A second objective was to investigate the correlation among various measures of thyroid size among

Table 2. Comparisons of grade within observers, by date.

Observer 1, 30 May

Grade	0	1	2	Total
0	57	6		63
1	14	15	1	30
2	4	2		6
Total	75	23	1	99

Observer 1
18 May

M = 3.20
not significant

Observer 2, 30 May

Grade	0	1	2	Total
0	74	7		81
1	5	9	1	15
2	1	2		3
Total	80	18	1	99

Observer 2
18 May

M = 0.33
ns

Observer 3, 30 May

Grade	0	1	2	Total
0	74	14	3	91
1	0	4	2	6
2			2	2
Total	74	18	7	99

Observer 3
18 May

M = 14.00
$p < .01$

observers. The children were examined on 17 April, 8 May, 13 June, and 5 July 1967. Observers 1 and 3 examined on all dates, but observer 2 in April and May only. Children were grouped by sex and size of thyroid and divided into treated and control groups. Observers were "blind" throughout the entire study.

The statistical design utilized in the analysis was that of a partially nested four-factor factorial experiment. The four factors were the observers, 1 and 3; the time of observation, dates 1, 2, 3, and 4; the treatment, l-triiodothyronine and control; and the subject. Subjects failing to attend school for treatment or those not examined on all dates were excluded from the analysis. Thus observers, treatment, and date of examination are all crossed, while the subjects are nested within treatments. Thyroids were graded and outlines drawn on the skin by each observer. Assistants cleaned the skin after each examination before the subjects were seen by the next observer.

To initiate the analysis a correlation matrix was developed for the variables grade, area, height, and width over all subjects examined by all observers on all dates. That correlation matrix along with its sample sizes and 95 per cent confidence limits is presented in Table 3. All of the correlations are significantly different from zero. From the confidence limits certain additional observations are available. These are:

1. That the correlation between grade and area is significantly greater than that between grade and height of isthmus or width.
2. That the correlation between area and width is greater than that between area and height of isthmus.

*Table 3. Correlation matrix for grade, area, height, and width over all subjects examined by all observers on all dates, Rozo.**

Variable	Grade	Area	Height	Width
Grade	1.00	0.72 (.66 - .75)	0.61 (.55 - .67)	0.62 (.56 - .68)
Area		1.00	0.81 (.77 - .83)	0.87 (.86 - .90)
Height			1.00	0.59 (.54 - .66)
Width				1.00
Sample size	670	678	578	578

* *Sample sizes and confidence limits (95%) of coefficients in parentheses.*

3. That the correlation between height and width is lower than any of the correlations among the measurements and is consistent with the findings of our other investigations.

To further investigate the inter- and intra-observer variability of these measurements a second correlation matrix was developed. It is presented in Table 4. One is able to discern:

1. That observers are relatively consistent in their measurements. Most of the intra-correlations for observers 1, 2, and 3 are in the high 80s.

2. That the inter-correlations between observers 1 and 3 are higher than those between observers 1 and 2 or 3 and 2. In fact in many instances it appears that the correlations between the measurements area, height, and width as recorded by observer 2 and those recorded by observers 1 and 3 are not significantly different from zero. Observer 2 palpated from in front whereas observers 1 and 3 had previously examined other children together and palpated from behind. Using the McNemar test previously mentioned, grades were compared among observers on each date. In April all three observers were significantly different from each other ($p < .05$). In May, observer 2 differed significantly from 1 and 3 ($p < .05$), but 1 and 3 were similar. In June and July, 1 and 3 were similar. Comparisons within observers showed that observers 1 and 3 were similar in April and May, whereas observer 2 differed significantly ($p < .05$).

The second phase of the study consisted of several analyses of variance. Only data from observers 1 and 3 were analyzed for the variables grade, area, height of isthmus, and greatest transverse width. The analyses are summarized in Table 5 and indicate:

1. That subjects are significantly different.

2. That there is a linear time trend.

3. That the date by subject within-treatment interaction is significantly larger than the observer by date by subject within-treatment interaction.

4. That the observers are significantly different when judged by grade and area, but are similar for width and height.

The remainder of the sources of variation for which analyses were made in this study were found to be non-significant. These sources of variation were: treatment, quadratic and cubic effects over time, date by treatment interaction, observer by treatment interaction, observer by subject within-treatment interaction, observer by date of examination interaction, and observer by date by treatment interaction.

DISCUSSION

More experience is needed for adequate evaluation of grading and other methods of recording thyroid size. We abandoned plasticine models in favor

Table 4. Correlation matrix for area, height, and width, all observers, two dates, Rozo (confidence intervals of coefficients in parentheses).

OBSERVER		ONE			TWO			THREE			Sample
	Variable	Area	Height	Width	Area	Height	Width	Area	Height	Width	Size
ONE	Area	1.00	0.84 (.78, .88)	0.90 (.86, .93)	0.23 (.30, .41)	0.20 (.00, .38)	0.24 (.05, .42)	0.46 (.30, .61)	0.42 (.24, .57)	0.40 (.22, .55)	118
	Height		1.00	0.67 (.55, .77)	0.16 (-.04, .35)	0.19 (-.02, .37)	0.18 (-.02, .37)	0.41 (.22, .55)	0.30 (.10, .46)	0.34 (.15, .50)	118
	Width			1.00	0.26 (.06, .43)	0.19 (-.01, .38)	0.30 (.10, .46)	0.43 (.24, .57)	0.40 (.22, .55)	0.44 (.27, .58)	118
TWO	Area				1.00	0.83 (.75, .87)	0.88 (.83, .91)	0.26 (.06, .43)	0.15 (-.05, .34)	0.45 (.28, .59)	120
	Height					1.00	0.61 (.45, .71)	0.24 (.05, .42)	0.16 (-.04, .35)	0.40 (.22, .55)	120
	Width						1.00	0.28 (.09, .45)	0.17 (-.03, .36)	0.50 (.34, .63)	120
THREE	Area							1.00	0.86 (.80, .90)	0.85 (.79, .89)	120
	Height								1.00	0.64 (.50, .73)	120
	Width									1.00	120

*Table 5. Analyses of variance, Rozo, 1967.**

Source of variation	d.f.	Grade		Area		Height		Width	
		F	P	F	P	F	P	F	P
Treatment	1	2.18	ns	< 1	ns	1.29	ns	1.14	ns
Subject within treatment	36	5.83	.01	11.06	.01	5.52	.01	9.51	.01
Date	3	1.68	ns	2.27	ns	1.57	ns	2.77	.05
Linear	1	4.31	.05	6.58	.05	4.27	.05	5.87	.05
Quadratic	1	< 1	ns	< 1	ns	< 1	ns	< 1	ns
Cubic	1	< 1	ns	< 1	ns	< 1	ns	2.29	ns
Date X treatment	3	< 1	ns	< 1	ns	< 1	ns	< 1	ns
Date X subject within treatment	108	2.51	.01	4.81	.01	2.01	.01	4.75	.01
Observer	1	10.97	.01	7.14	.01	1.31	ns	< 1	ns
Observer X treatment	1	< 1	ns	< 1	ns	< 1	ns	3.70	ns
Observer X subject within treatment	36	< 1	ns	< 1	ns	1.06	ns	< 1	ns
Observer X date	3	1.22	ns	< 1	ns	< 1	ns	< 1	ns
Observer X date X treatment	3	< 1	ns	1.78	ns	< 1	ns	2.21	ns
Observer X date X subject within treatment	108								

* *Mean squares are available on request.*

of skin outlines because of difficulties in estimating thyroid depth and the time needed to construct models. Marking the skin and transfer to paper is a more direct method of recording permanently what is felt on palpation. It requires fewer decisions as to size at the time of examination, and uniform criteria can be applied later to all subjects. It provides a check on grading, or perhaps a substitute for it. The method is more sensitive than grading. Changes occurring in the course of a treatment program should be more readily detected.

Our subjects had slight to moderate enlargements of the thyroid, and the lobes were difficult to palpate accurately. Experienced observers were more consistent than non-experienced, especially if they had had experience together. Nevertheless, when grades plus outlines were used, experienced observers were less variable for transverse width and height of isthmus than for grade and surface area. Surface area requires a planimeter for measurement, whereas width and height are measured with a ruler. Stocks et al. (11) advocated the use of transverse width rather than area. McCarrison and Madhava (5) recommended width expressed relative to body size. We believe that outlines have advantages over the calipers used by Stocks for measurement.

Skin outlines do not abolish variability. The skin may stretch during marking or with subsequent movement of the head. The paper may be crinkled or too stiff. Nevertheless, when the outlines are done in a standard manner there seems to be less variability. The method may be recommended for use in other areas, particularly where the effects of preventive or therapeutic programs are to be evaluated. Comparisons among programs from different parts of the world might be facilitated. A film defining the operations involved might be a useful standardizing device.

SUMMARY

Observers were compared in two trials using grading and another method to record thyroid size. Although experienced observers were consistent in grading the same subjects on two or more occasions, they differed significantly from one another. They were more consistent when using a new method of measurement employing surface outlines of the thyroid than with conventional grading techniques.

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CHAPTER 6

PREVENTION OF ENDEMIC GOITER IN LATIN AMERICA

John P. Kevany, M.D., M.P.H.[1]

INTRODUCTION

One of the most extensive problems of malnutrition occurring in Latin America today is endemic goiter resulting from a dietary deficiency of iodine, and its sequelae of cretinism, deaf-mutism, and other serious neurological defects. This problem is of long standing in this region and without question has a major impact on the health, education, and productivity of the affected populations.

Though endemic goiter is widespread throughout Latin America, it tends to be concentrated in the western mountain range extending from the highlands of Mexico and Central America down through the Andean cordillera as far south as Chile and Argentina. As an approximate estimate, it can be said that some 13 million people are affected by this condition. No precise figure can be obtained inasmuch as morbidity statistics are incomplete or unavailable and special surveys have been limited in geographic area or by age group. It is evident, nevertheless, that an important percentage of the population is at risk from a deficiency disease whose severe forms present a serious threat to health and whose milder forms may well have an unfavorable effect on physical development and intellectual capacity. Because of the many other adverse factors in the ecology of the populations affected by endemic goiter, however, it is not possible at this stage to define with precision all the possible consequences of this disease in its milder forms. It is found in its highest prevalence and severest form in small communities located in isolated mountain areas, where the ethnic composition of the population varies from pure Amerindian stock to the more common Amerindian-European mixture. These communities usually have little communication with urban areas, transportation facilities are very limited, and migration levels are low. Little interchange of products occurs and practically all of the foodstuffs that compose the basic diet are produced locally.

Of the 25 Member Governments of the Pan American Health Organization (PAHO), 17 have a declared problem of endemic goiter. Endemic cretinism is known to exist in several of these countries, though it is not always defined as a specific health priority. The definition of the problem is usually based on general observations of health conditions as reported through local health services, backed by specific surveys for endemic goiter carried out in representative populations. These surveys usually involve simple clinical examination of the thyroid gland and classification of abnormality on the basis of size and texture (nodular or diffuse). They are carried out in stratified

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population samples or in selected groups such as schoolchildren, which can be considered as a sensitive indicator of the problem in older groups. Occasionally these studies are supported by biochemical determinations of iodine in blood and urine samples and analysis of ^{127}I content in foods, soil, and water. In all endemics studied in this region, deficient dietary intake of iodine has been demonstrated to be the basic cause.

PREVENTIVE ACTIVITIES

Though the problem of endemic goiter and cretinism has been the subject of extensive clinical and epidemiological study, the approach to the control and prevention of this condition has not been so progressive. In an attempt to define the existing situation regarding the prevention of goiter in the Americas, the Pan American Health Organization conducted a survey in mid-1968 by means of a mail questionnaire sent to its country staff, and completed in consultation with the competent national authorities. Information was obtained regarding the existence of legislation, the year of its approval, and the coverage required. The type of iodine compound used and the recommended levels of iodine concentration in the final product were also reported. Information on the agency responsible for program development, on the source of equipment and supplies, and on the effectiveness achieved in applying approved legislation was also obtained. The preventive measures referred to in this study were confined to the iodization of salt in its various forms. The response to this questionnaire was prompt and complete and a simple analysis of the information obtained is presented in Table 1.

Of the 25 Governments surveyed, 17 had defined problems of endemic goiter using the criteria established by the World Health Organization (WHO),[2] at least in specific areas of the country. Of these 17 countries, 15 had enacted legislation requiring iodization of salt for goiter prevention. In the other two countries, preparatory steps were being taken to present proposals for approval.* It is interesting to note that the great majority of the legislation in the 15 countries has been enacted within the last 15 years. In four countries the legislation refers only to salt destined for human consumption, leaving that for animal and industrial use uniodized. In eight countries total available salt utilized for all purposes is required to be iodized, and in three countries only that salt destined for use in the endemic goiter area is covered by the law. In 13 countries potassium iodate is used as the additive, whereas in three countries potassium iodide is used. In one country iodate and iodide are used in different areas of the country according to the choice of individual state governments. The concentration of iodine, expressed per 1,000 parts of salt, varied from 1:100,000 to as high as 1:10,000. The majority of these figures refer to iodine concentration at the time of processing. Losses incurred in distribution and storage were not indicated as these are variable according to environmental conditions and forms of packaging. In all but one country the national health service was responsible for the planning, execution, and surveillance of the program. In Colombia the National Institute of Nutrition, a semiautonomous dependency of the Ministry of Health, is solely responsible for the administration of the program. The equipment and supplies

2/ Clements, F.W. et al. Endemic Goitre. World Health Organization Monograph Series No. 44. Geneva, Switzerland, 1960.

* Since the survey was conducted, both countries have approved legislation requiring the iodization of salt for human consumption.

Table 1. Status of goiter prevention programs in Latin America (June 1968).

Countries with public health problem	Year of legislation	Coverage (1)	Iodine compound (2)	Concentration (3)	Responsible institution (4)	Source of equipment and supplies (5)	Level of program activity (6)
Argentina	1967	G	A + I	30	HS	P	F
Bolivia	1968	T	A	20	HS	I	I
Brazil	1956	T	A	50	HS	G + P	P
Chile	1966	T	A	10	HS	P	I
Colombia	1955 + 60	T	I	15	IN	G	F
Costa Rica	1941	H	I	100	HS	-	P
Ecuador	-	-	-	-	-	-	P
El Salvador	1961	T	A	15	HS	G + P	F
Guatemala*	1954	T	A	15	HS	P	F
Honduras	1960	H	A	15	HS	-	P
Mexico	1963	T	A	50	HS	P	F
Nicaragua	-	-	-	-	-	-	P
Panama	1955 + 1966	H	A	15	HS	-	P
Paraguay	1958	H	A	20	HS	I	P
Peru	1940 + 61	G	A	10	HS	G	P
Uruguay	1963	G	A	30	HS	P	F
Venezuela	1966 + 68	T	A	50	HS	P	I
	15 + 2-	T 8 H 4 G 3	A 13 I 3	< 50-1 25-50-5 > 25-9	HS 14 IN 1	P 8 G 4 I 2	F 6 I 3 P 8

* *Problem now reduced below 10 per cent level.*

(1) T Total coverage (all salt produced and imported is iodized).
H Only salt for human consumption is iodized.
G Only salt in areas of endemic goiter is iodized.

(2) I Potassium iodide.
A Potassium iodate.

(3) Thousands of parts of salt to one part of iodine.
Figures given represent minimum levels established by law.

(4) HS Health Service.
IN Institute of Nutrition.

(5) G Government.
I International assistance.
P Private sector.

(6) F Fully active.
I Being implemented.
P Problems encountered.

for iodization were purchased by the private sector in eight programs, and by the Government in five. In two of the latter programs, costs were shared by the private and the public sectors. In two cases, the basic equipment and the initial supply of iodate were donated by the United Nations Children's Fund (UNICEF) through its international assistance program. Five countries have not yet established facilities.

Finally, a broad judgment was requested regarding the application of the law where it existed. In six countries it was felt to be effective whereas in eight substantial problems had been encountered. In three countries progressive implementation is being undertaken to meet a specific deadline.

DISCUSSION

As stated previously, the greater part of the legislation for the prevention of endemic goiter by salt iodization has been approved in the past 15 years. On first impression it may seem surprising that it is only in recent years that official action has been taken to prevent this long-standing problem of public health, especially when it is recalled that salt iodization was first utilized successfully in 1917 by Marine and Kimball, in Ohio, U.S.A., and has since been regarded as a safe and effective approach to prevention. Historical perspective, however, makes this apparent lack of attention more understandable. Endemic goiter has existed in many parts of the Americas for more than two centuries and, to a great extent, the evolving health agencies of the countries affected have grown accustomed psychologically to its presence. It is not a reportable disease and death resulting directly from enlargement of the thyroid gland is comparatively rare. In consequence morbidity and mortality statistics have never justified the assignment of a high priority to it in health planning, whereas the overwhelming importance of infectious and parasitic diseases demanded the full attention and resources of health agencies. The lack of a clearly established causal relationship between maternal iodine deficiency and cretinism has further reduced the demand for priority in action. Even the prevalence of cretinism cannot justify priority on an economic basis because the greater part of these cases continue to live at home, without any special care or services. Thus the cost of institutionalization is largely theoretical. In terms of medical services, the potential costs of treating disease of the thyroid gland and disorders of metabolism arising from endemic goiter could be extremely high. In fact, however, few facilities for diagnosis and treatment are available to the major part of the populations at risk.

Of considerable importance is the fact that goiter and cretinism tend to occur to a greater extent in isolated rural areas. The affected populations exist at a very low level of social development and thus have very limited socio-political importance, at least by comparison with the more highly organized and vocal groups of urban communities. In consequence there is little social pressure on government agencies to seek an early solution to this problem. Given these concurrent circumstances, it is less surprising that legislation on salt iodization has been relatively recent, or that in some countries it had not been enacted. This also explains, to some extent, why existing legislation is ineffective in nearly 50 per cent of the cases.

A final factor of some influence is the rationalization of some public authorities that with improved communication there is an increase in geographic

mobility of isolated populations and, coincidentally, an increase in the interchange of foodstuffs between one area and another and a resultant tendency for endemic goiter to disappear or diminish without any specific action for prevention. Though this concept is valid technically, it in no way indicates the time that will elapse before such a phenomenon occurs, especially in view of the slow evolution of communication in many developing areas of this region.

With regard to the application of existing legislation, the process of salt iodization presents certain specific problems of both an administrative and a technical nature. In many countries iodization implies a close collaboration between the public and the private sector. Often the private sector has considerable political power to resist the enactment and application of the law, while at the same time the authority and resources of the health agency for its enforcement are frequently minimal. When legislation is not planned in close cooperation with the salt producers, they are poorly motivated to resolve social problems of this kind. In some countries the production of salt is highly fragmented between many small producers who in turn sell their product directly to a local market or to an intermediary responsible for its packaging and distribution. Under these circumstances, the most realistic approach to salt iodization is the formation of a cooperative that will pool salt production for processing. Clearly, this requires the acceptance and cooperation of many producers who are often perfectly satisfied with the status quo.

Another major administrative problem is the absorption of the costs of iodization, in terms of both permanent equipment and the iodine compound utilized. For any program of iodization to be effective, there should be no significant difference in the price of iodized salt over that of common salt. Salt is one of the basic dietary elements that cannot be produced domestically by the subsistence farmers that compose a large segment of the rural population of Latin America. It is therefore one of the few cash commodities that is universally required. Any increase in the retail price of salt for purposes of financing an iodization program thus has considerable political implications and may meet with resistance from the legislature. In other situations where only part of the salt is iodized (e.g., that destined for human consumption or for a specific area where goiter is prevalent), a price differential may be established. In these cases the large segment of the population with low purchasing power will contrive, often successfully, to obtain uniodized salt on account of its lower price, regardless of the advertised benefits of the iodized variety. By the nature of the disease, it is the lowest economic strata that suffer most from endemic goiter and thus the cost of iodization must be absorbed either by the Government or by the private sector, so that the retail price of the product will not increase.

It is understandable, if not laudable, that the private sector is unwilling to surrender part of its long-established profit margin for a health problem that is often both socially and geographically far removed from the producer's immediate environment. On the other hand many Governments are unwilling to assume the ongoing cost of iodization, especially when this must be charged to a health budget which is often severely restricted. UNICEF has attempted to avoid this impasse by offering iodization equipment and a year's supply of iodine compound in order to get programs started. In some countries this approach has met with success and the Government or the private sector has assumed the continuing costs after the first year. In other countries

this offer has failed to produce the expected reaction for a variety of political, administrative, and technical reasons. Once legislation is enacted, a major problem in law enforcement often arises in terms of surveillance of iodization procedures and analysis of samples to assure conformity. Such a responsibility is usually assigned to the health agency and in some countries the necessary laboratory facilities and technicians may not be available. In addition, local health staff may be so dispersed or overloaded with other duties that the necessary sampling and analysis for compliance with specifications may be difficult to undertake. It is apparent from the foregoing that numerous administrative problems may prevent the passage of appropriate legislation and its effective application.

Finally, purely technical problems can present obstacles to the introduction of salt iodization. Poor quality salt, with a high level of impurities, is often unsuitable for routine iodization procedures. High levels of humidity also present problems affecting the flow and adequate mixing of the iodizing compound. In the past the instability of potassium iodide under conditions of high environmental temperature and humidity caused serious problems in maintaining adequate iodine levels in the product at the retail level. The use of potassium iodate, tested by the Institute of Nutrition of Central America and Panama (INCAP) in 1952, has done much to overcome these difficulties in recent years.

The levels of iodine recommended for treatment of salt vary widely between the countries of the region. More conservative health administrations have commenced with extremely low levels (e.g., 1:200,000) in order to avoid any supposed risk of Jod-Basedow phenomenon. In most cases countries using these levels have found that no measurable reduction in goiter has been achieved and have later increased the concentration. Other countries which experience severe endemics have decided on much higher levels as they regard the risk of Jod-Basedow phenomenon to be negligible and wish to achieve a rapid reduction in prevalence and severity in the shortest possible time. Higher levels are also chosen to allow for losses in storage and distribution, this being especially true where potassium iodide is used. It is difficult to recommend any universal level of iodization without taking into account many variables, such as natural iodine levels, other sources of iodine intake, degree of severity of the endemic, expected losses in distribution, and the presence of potential goitrogenic elements in the common diet. The PAHO/WHO seminar on salt iodization held in Salta, Argentina, in 1965,[3] recommended levels of between 1:10,000 and 1:50,000 but added that the final decision should be taken only after assessing local conditions.

The foregoing discussion illustrates some of the multiple factors that can influence legislation on salt iodization and its effective application in this Hemisphere. It is clear that for some countries these problems have been insuperable, at least up to the present. In view of this fact, PAHO decided to look for alternative methods for the prevention of endemic goiter and cretinism. This decision in no way conflicted with the priority accorded to salt iodization but rather was directed to finding an interim alternative until existing obstacles could be overcome. It was also recognized, however, that

3/ Pan American Health Organization. "Report of the Seminar on Salt Iodization in Salta, Argentina." Washington, D.C., 1965.

the isolation of some of the worst-affected population is such that even if salt iodization exists on a national scale those groups may be so far removed from normal commercial channels that they will continue to use primitive sources of uniodized salt.

In 1966, pilot studies on the use of intramuscularly administered iodized oil were initiated under the auspices of PAHO in Ecuador and Peru. The details of these studies are described in Section VII, Chapters 26 and 27, and in Section IX, Chapter 34, of this volume. Recent results have demonstrated that this is an effective method of preventing endemic goiter and cretinism and for reducing the prevalence of existing goiter, especially in younger age groups. The side effects of this treatment are minimal, easily controllable, and insignificant in comparison to the potential effects of severe goiter and its sequaelae.

SUMMARY

Slow but steady progress has been made in this Hemisphere toward the elimination of endemic goiter as a public health problem. The great majority of countries with a goiter problem have adopted legislation for its prevention, but only a limited number have achieved successful programs on a national scale. The majority have encountered various obstacles of a political, administrative, or technical nature, but considerable efforts are being made by both national health services and international technical assistance agencies to overcome these difficulties. The treatment of salt with potassium iodate remains the method of choice for goiter prevention; however, the intramuscular administration of iodized oil to selected populations may prove a useful alternative until iodization programs can be implemented.

CHAPTER 7

ENDEMIC CRETINISM: A SEARCH FOR A TENABLE DEFINITION

A. Querido[1]

Endemic cretinism must be considered as the most serious consequence of a severe goiter endemia, because the affected persons have irreversible damage to the central nervous system. The frequency of permanently defective persons in a severely affected area may be as high as 10 per cent (4, 6). Deafmutism, partial or complete, might be observed in 70 per cent or more of the defective persons (4, 6). The term "endemic" indicates the strict geographic localization of the syndrome, in contrast to sporadic cretinism.

Many reviews have been written on the definition and the syndrome of endemic cretinism, such as those by DeQuervain (9), Trotter (18), and Costa (7). In the past decade interest in this disease has been renewed, because investigators found that in areas where for centuries endemic cretinism has been known, defective people were still highly prevalent. Modern studies have been reported from the Congo (2), Brazil (12), Peru (15), Ecuador (11), Italy (8), India (17), Eastern New Guinea (14), and Western New Guinea (6).

In these recent reports and in personal discussions it has appeared that the applied terminology has not always been identical. It is now clear that several factors have contributed to these differences. Most of the recent investigators were clinicians interested in thyroid disease and therefore familiar with sporadic cretinism. This syndrome is always caused by and accompanied by hypothyroidism and its sequelae such as stunted growth. Only a small fraction of the defectives in an area with endemic cretinism have signs of hypothyroidism, and therefore most of the defectives attain heights identical with the nondefective people of the area. The description in the old literature of extreme cases of endemic cretinism (which were dwarfs) made investigators look mainly for "true" cretins, i.e., short-statured defective people, and pay less attention to the deaf, deafmutes, and oligophrenics of different severity and normal stature. Also, it was sometimes difficult for these investigators to accept that damage to the central nervous system leading to deafness or deafmutism is part of the epidemiological entity of endemic cretinism, because sporadic cretinism is never accompanied by deafmutism. Pendred's syndrome is an exception, and in those patients the functional destruction of the eighth nerve is attributed to a genetic defect (4). Adding to the confusion were the early statements of McCarrison (3) and of the World Health Organization Study Group on Endemic Goiter (20). They wrote, "(a) Cretinism occurs in areas where goiter has been endemic for long periods. (b) Feeble-mindedness, apart from cretinism, rises distinctly in incidence in areas of endemic goiter. Educability is also lowered, with resultant economic loss. (c) Reports have suggested a significant correlation between the incidence of

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deafmutism and that of endemic goiter. Factors other than goiter may cause feeble-mindedness or deafmutism, and much more work will be required for critical assessment of such factors in populations." These conclusions were drawn at a time when few people had personal experience with endemic cretinism in areas without iodine prophylaxis, and they remained unchallenged until a few years ago, when a number of investigators were confronted with the problem.

It was soon understood that a description of the defects which are present in a region with severe endemic goiter, and which are connected with the goiter or its cause, cannot be complete when deduced from studies of cretins living in institutions, since they constitute a preselected group of patients. To establish all the variants of the defects, epidemiological studies were required, in which all the inhabitants of the region were examined and the observed defects listed. It had to be kept in mind that each population, and especially isolated groups with high frequency of intermarriage, have a certain number of defective people. The proof that the observed defects were indeed related to goiter or its cause is very difficult and complicated. The logical approach would be to look for a control group under identical socioeconomic conditions and with the same genetic characteristics, but without goiter. Indeed, Choufoer et al. (6) came close to such an observation. An alternative is to observe what happens when preventive measures against the goiter endemia are taken. It is clear, however, that these measures never lead to the introduction of one single factor, such as iodine prophylaxis. They will always be accompanied by more medical attention, change of nutritional habits, etc., which impair sound conclusions.

In the case of deafmutism a careful follow-up study was made by Wespi (19) after administration of iodized salt in different cantons in Switzerland. There was a clear correlation in time of prophylactic measures and a decline in deafmutism, and what is even more convincing, iodine prophylaxis did not affect the acquired forms of deafmutism. The percentage of deafmutism existing since birth was only diminished to the level known for countries without iodine deficiency.

Identification of defects as part of a goiter endemic is supported by identical descriptions of defective people from epidemiological studies in different parts of the world. When comparing those reports the local genetic abnormalities are neutralized. It appears that the recent independent epidemiological descriptions of the defectives by at least three groups of investigators from Eastern New Guinea (6, 14), Ecuador (11), and Western New Guinea (6) are in full agreement.

For detailed descriptions of the defective people the original publications should be consulted, together with the authoritative monograph of DeQuervain and Wegelin (9). The major symptomatology can be summarized as:

a) Damage and retardation of the central nervous system, manifested by variable degree of oligophrenia, deafness and deafmutism, spastic diplegia (so-called nervous cretinism of McCarrison), and squinting;

b) Abnormal development of bones (delayed enchondral ossification and delayed appearance of centers leading to short stature, abnormal gait (coxa vara and coxarthrosis), and changes in the skull.

c) Hypothyroidism through atrophy and connective tissue infiltration of the thyroid.

It appears that most of these signs and symptoms can be present in different degree and in various combinations, but with some restrictions. The severest form of nervous cretinism with diplegia is always accompanied by oligophrenia and deafmutism. Whether deafmutism exists without mental retardation is unlikely.

Extensive experimental studies of Campbell et al. (5) on the behavioral development and brain biochemistry of neonatally thyroidectomized rats have shown the dependence on thyroid hormone for normal development of the central nervous system. Few data are available on the histology of the central nervous system in cretins. The brain weight seems to be normal if corrected for body size (9). It is clear that defectives with severe neurological complications have little chance of survival in communities with low socioeconomic development. This affects the percental distribution of the defects in different populations.

In an excellent review Trotter (18) discusses the relation of deafness and thyroid function. There seems to be only one exception to the rule that endemic deafmutism occurs only in regions with severe endemic goiter. Sekretan (16) studied a Swiss village, Ayent, where of 2,200 inhabitants 50 persons were suffering from hereditary deafmutism. He specifically states that there was no indication of endemic goiter and cretinism. Recently Srinivasan (17) made a detailed study of Himalayan "endemic" deafmutism and deafness and found bilateral loss of hearing of the perceptive and conductive type. There was no difference in thyroid function between the deafmutes and the other inhabitants who were affected by endemic goiter only. The pathology of the middle ear is characterized by thickening of the tympanic cavity lining, leading to narrowing of the windows. The ossicles are also abnormal. The inner ear sometimes shows a hyaline edge near the membrane of Corti. Bargman et al. (1) recently produced lesions in the embryonic ear of the chick embryo by injecting the embryo with an antithyroid drug. Specific consistant morphologic alterations were observed in the sensory hair cells of the acoustic papilla and cells of the spiral ganglia of the cochlea.

As stated before, investigators used the term "true cretin" only for dwarfed defective people. The recent epidemiological report of Choufoer et al. (6) from New Guinea specifically mentioned that dwarfism was hardly seen. It should be taken into account that the assessment and evaluation of retarded growth in an area with bad socioeconomic conditions is extremely difficult, and that the definition of dwarfism is arbitrary.

DeQuervain and Wegelin (9) indicated that in their material only 7 per cent of the defectives were real dwarfs (below 140 cm when adult), and that these dwarfs were predominantly without goiter or with a small goiter. They related size and function of the thyroid with height, and applied the term "Frühatrophie" (early atrophy) to patients with retarded growth. As early as 1908 McCarrison (13) described atrophy and sclerosis of the thyroid in a myxedematous cretin. DeQuervain et al. described this pathology in detail, and in the Congo (10) a relation of ^{131}I thyroid uptake (being low in cretins with short stature) and height has been observed. All these observations lead to the conclusion that the major factor for retarded growth in defectives is

thyroid insufficiency, and that an etiologically unexplained process may lead to destruction of thyroid tissue in a severe goiter endemic.

Thus far we have been mainly concerned with a description of the clinical picture of defective people in a severe goiter endemic. The question arises whether a common denominator can be found or a definition can be given which includes all defective people who are victimized by the goiter endemic. A definition in medicine can be based on etiology, on a basic morphological or physiological lesion common to all defects, or it can be purely of a descriptive nature.

The basic lesion in the cells of the affected organs in cretinism is unknown, and therefore the two other possibilities are open. Endemic cretinism is geographically limited to areas of severe endemic goiter, but endemic goiter is not necessarily accompanied by endemic cretinism. The relation between the two diseases may therefore be a common cause, or the result of two factors one leading to endemic goiter, while the factor causing endemic cretinism is only effective in the presence of severe endemic goiter. Until now the only convincing evidence for the cause of endemic goiter points to iodine deficiency. In areas with sufficient iodine supply, endemic goiter is absent, and many reports have shown the effectiveness of iodine prophylaxis. This does not exclude the possibility of more than one factor being present in endemic goiter. It is, for example, possible that with a borderline supply of iodine other factors are provocative.

We are inclined to relate the mechanisms which lead to endemic cretinism to thyroid hormone deficiency during fetal life or postnatally, because a number of the features (not all) of endemic cretinism are also present in sporadic cretinism. Sporadic cretinism is always accompanied by hypothyroidism. Endemic cretins may be euthyroid, and this limits the analogy of the two diseases. Description of the signs and symptoms presents difficulties because of the polymorphism of the abnormalities in the affected individuals. This is certainly not surprising; other diseases, such as those of an infectious nature, also show a large variation of signs and symptoms.

Even a sharp description of the signs and symptoms of the defects related to the goiter endemic is only possible to a limited degree, because of a number of complicating factors.

1) Not all mothers in the endemic goiter region give birth to cretins, and those that have cretinous children also may have normal children. Apparently conditions for the development of a normal child are borderline. This could explain the polymorphous expression of the disease. The borderline condition might be disturbed in different degree, and at different and critical periods of intrauterine or postnatal development.

2) As far as is known, endemic cretinism occurs only in poor, socially backward societies. The inhabitants of those areas are always poorly nourished qualitatively or quantitatively, or both. The interpretation of signs such as retarded growth or mental retardation in their relation to mechanisms leading to endemic cretinism becomes very difficult. Poorly nourished populations show retarded growth. The performance of children in socially backward areas is

low. It is conceivable that undernutrition is a factor in the development of cretinism, and therefore that improvement of nutrition without change of iodine supply could reduce cretinism.

3) Adequate thyroid hormone medication in sporadic cretins improves the mental condition in a number of cases. It is not known which part of the syndrome of endemic cretinism depends on the intrauterine environment, which part arises postnatally, and which lesions are reversible if treatment starts early.

4) Certain forms of mental deficiency also are characterized by retarded growth. Does this relation also exist in endemic cretinism and therefore is it a contributing factor to growth retardation?

5) The poor general conditions (nutrition, social development, absence of secular trend, illiteracy, etc.) of populations affected by endemic cretinism make it practically impossible to draw a sharp line between normal and abnormal somatic and mental development. It is therefore not known whether endemic cretinism is sharply delineated from the "normal" population, or whether the existing defects are part of a continuum stretching from abnormal to normal.

We therefore feel that a descriptive definition of defective persons in severe endemic goiter also has its limits. It is for these reasons that we maintain the definition given earlier (6):

> "Endemic cretinism is the collective term for a number of developmental abnormalities, which geographically coincide with severe endemic goiter and are caused by lesions acquired before or shortly after birth. More precisely, it may be defined as the excess of these abnormalities, which is found in a goitrous population, as compared with a similar population without goiter, and, in due time, is abolished by adequate goiter prophylaxis."

This conclusion shows the importance of concentrating investigational activities in those areas of severe endemic goiter which have defective people. Adequate prophylaxis in those areas may lead to a gratifying change of the somatic phenotype and the mental performance of the whole community.

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CHAPTER 8

ENDEMIC CRETINISM

J.E. Dumont, F. Delange, and A.M. Ermans[1]

Numerous defective persons are found in populations affected by severe endemic goiter (18). A relation between the excess of defects and endemic goiter is shown by the geographic coincidence and, most convincingly, by concurrent eradication of these defects and of goiter by iodide prophylaxis (6, 18, 19, 20, 33). Defects which have been related to endemic goiter are:

1) Mental deficiency.

2) Deafness and deafmutism.

3) Neuromuscular disorders including signs of upper neurone defects, squinting, and dysarthria.

4) Abnormal somatic development with disproportion of the body and characteristic facies.

5) Growth retardation and dwarfism.

6) Hypothyroidism.

These defects occur more often combined than singly; various combinations have been called endemic cretinism and sometimes endemic deafmutism. Most often the definition of endemic cretinism has included defect No. 1 and sometimes defects Nos. 2, 3, or 4 (23, 24, 30) or the definition has grouped defects Nos. 1, 4, 5, and sometimes 6 (3, 6, 21, 31). Restrictive definitions have selected homogeneous groups which were very different from each other (3, 14, 20, 23, 24, 27, 30), while wide definitions provided, except in New Guinea (5), heterogeneous groups of patients (7, 8, 13). In order to provide a definition which would include all the defectives classified by various authors as endemic cretins, the Pan American Health Organization Scientific Group on Research in Endemic Goiter (28) described the endemic cretin as an individual with irreversible changes in mental development, born in an endemic goiter area, and exhibiting a combination of some of the following characteristics not explained by other causes:

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* Contribution No. 423 of the Biology Department, Euratom.

1) Irreversible neuromuscular disorders.

2) Irreversible abnormalities in hearing and speech leading in certain cases to deafmutism.

3) Impairment of somatic development.

4) Hypothyroidism.

The study of endemic cretinism has been complicated by two facts:

1) Endemic cretins were often investigated at a time when the conditions for the appearance of the disease no longer existed (32).

2) Defects occurring in areas where endemic goiter does not exist can also be found in endemic regions; it is therefore probable that any series of mental defectives in an endemic goiter area will include cases of sporadic cretinism and cases in which the other defects have no relation to endemic goiter (6, 20, 33).

In the last few years, two studies on endemic cretinism have been carried out in areas where conditions for the appearance of the disease still exist: Congo and New Guinea (5, 10, 11, 14, 15). In both places it was shown that endemic cretinism was present only in the endemic goiter area. In New Guinea the high incidence of mental deficiency made insignificant any possible contribution to the series of non-cretin mental defectives; the series did not include "sporadic hypothyroid cretins" since the cretins were no more hypothyroid than the controls (5). In the Congo, all the patients who were studied were hypothyroid. This excluded mental defectives who were without thyroid disease; persons with metabolic defects of the thyroid or cryptothyroidism were not detected by extensive studies (except possibly in one case) (14, 15). We propose to show that in these two areas, where "native endemic cretinism" may still be studied, this term was applied to two very different entities, each probably having a pathogenesis of its own.

In New Guinea a systematic survey of the population of Mulia failed to reveal any case either of hypothyroidism or of marked growth retardation and dwarfism (5). On the contrary, deafmutism and mental deficiency were common and most often associated (5.8 per cent of the population). Severe neuromuscular disorders (squinting, lack of expression and, in the young children, motor retardation; in the adults, dysarthria and motor disturbances) sometimes accompanied deafmutism and mental deficiency. Thyroid size and function were similar in these patients and in non-defectives of the same area (1, 5). Similar findings have been obtained in Brazilian cretins of Goias (23) and in Himalayan deafmutes (30). The pathogenesis of endemic cretinism in New Guinea is unknown, but Choufoer et al. (5) hypothesized that it could have been caused by a reduction of the supply of maternal thyroid hormone to the fetus at critical stages of pregnancy. Mental deficiency and deafmutism sometimes occurred separately, and there was no strict correlation between the severity of mental deficiency, of deafmutism, and of the motor syndrome. These discrepancies suggest differences in pathogenesis.

In the Uele endemic area (Congo), a systematic survey of the population demonstrated a high incidence of hypothyroid dwarfism (0.32 per cent of the

population). Deafmutism was less prevalent (0.095 per cent of the population) (4, 14).[2] In a more recent survey of the goiter endemia of the island of Idjwi (Congo), all defects were tabulated (9, 10). In 9,000 people examined, 89 hypothyroid dwarfs and 11 euthyroid mental defectives (nine of them deaf mutes, seven showing neurological signs) were found. The results of the Idjwi survey, therefore, agree with those obtained in the Uele. The Uele Congolese dwarfs were hypothyroid by all criteria: clinical examination, electrocardiogram, bone X-ray examination (bone maturation retardation, and epiphyseal dysgenesis of the trochanter), and blood chemistry (PBI, cholesterol) (14). The uptake and the clearance rates of iodide by the thyroid were low when compared to the values obtained in non-defectives. It could be calculated that with the low amount of iodide available, the thyroids of the dwarfs were able to synthesize only small amounts of hormone. The thyroid insufficiency was therefore caused by failure of the thyroid to adapt to extreme iodine deficiency by an increase of its iodide clearance. In agreement with this conclusion, the smallest cretins had the lowest radioiodide uptake (15). Similarly, in the Idjwi hypothyroid dwarfs radioiodide uptake in the thyroid and growth retardation were inversely correlated (Figure 1) (9).

In the Uele as in Idjwi the thyroids were much smaller in dwarfs than in the non-defectives; they were often not palpable in the worst cretins. Furthermore, extensive studies of iodine metabolism in the dwarfs demonstrated a small but rapidly turning over exchangeable pool of organic iodine in the thyroid (15). These facts suggested that the low iodide uptake by the thyroid was a consequence of an extreme reduction of functioning thyroid tissue. Directional counting of the thyroid in the Uele, and thyroid scanning in Idjwi showing a normal position of the thyroid, excluded cryptothyroidism as a cause of the thyroid failure (22). The scan showed evidence of irregular atrophy of the gland. These data are compatible with the pathological data of DeQuervain and Wegelin (13, 14) from the hypothyroid endemic cretins of Switzerland. The description of Wegelin (34) of thyroid degeneration with atrophy and sclerosis can therefore presumably apply to Congolese hypothyroid cretins. The chronology as well as the origin of the pathological process is unknown.

In Uele hypothyroid dwarfs, the severity of all the signs of hypothyroidism and of mental deficiency was correlated with the severity of dwarfism. The correlations of mental ability and bone maturation with standing height were similar in non-deafmute cretins (14). The presence of deafmutism and neurological signs in some dwarfs was not correlated with the severity of dwarfism.[3] Hypothyroidism itself therefore accounts for the mental deficiency of the dwarfs of the endemic goiter area, as it does for this defect in sporadic cretins (2, 16, 25, 29). The defects observed in the Congolese endemic cretins have therefore been ascribed to hypothyroidism beginning in early infancy or in the fetus (14).

2/ The remark by Choufoer et al. (5) that in the Uele "the available survey data do not convincingly show that dwarfism is the predominant form of endemic cretinism in that country" was ill-founded. Indeed, these data were the result of a survey of the whole population of the Uele area by a medical team of the FOREAMI; in this survey communicable diseases, as well as goiter, deafmutism, and hypothyroid dwarfism were looked for (4).

3/ In the deafmute dwarfs, mental deficiency was much worse than the height would have indicated.

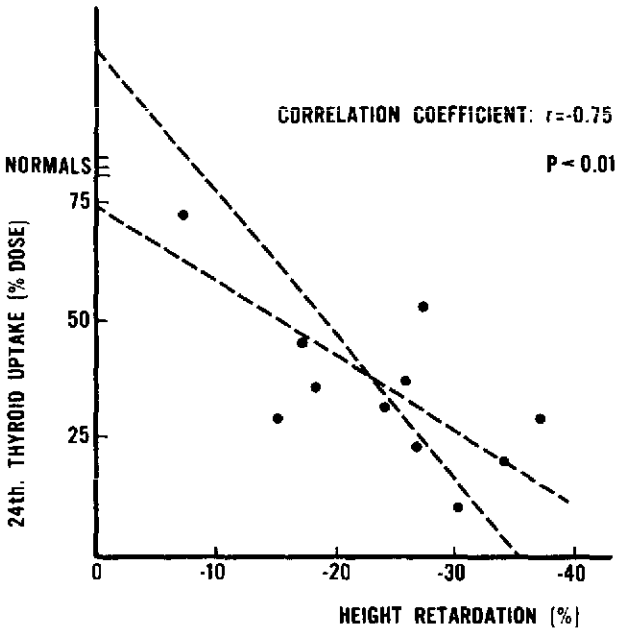

Figure 1. Relation between the 24-hour radioiodide uptake in the thyroid and height retardation in myxedematous Idjwi endemic cretins (9). Height retardation was calculated with reference to the mean height of 50 euthyroid subjects of the same age.

Two very different syndromes are therefore described as endemic cretinism (Table 1): The first is characterized by mental deficiency or deafmutism or both, and in the severest cases by both defects and by neuromuscular disorders; the second is a form of hypothyroidism, which begins early in infancy or in fetal life, caused by thyroid hypoplasia. Using the terminology of McCarrison (26) in a broader sense, we propose to call the former syndrome nervous endemic cretinism and the latter, myxedematous endemic cretinism. We know that both syndromes are related to endemic goiter, but we do not know the pathogenesis of the defects in nervous cretinism or of thyroid atrophy in myxedematous cretinism.

The heterogeneity of endemic cretinism and the probability that different pathogenetic mechanisms are involved have been suggested or implied by several authors (5, 12, 13, 17, 20, 26, 33). Those authors who have studied one type of cretinism have sometimes applied their conclusions of pathogenesis to the pathogenesis of the other syndromes, and in particular have postulated, without adequate grounds, a single pathogenesis for mental deficiency in both syndromes (5, 14, 20).

Both types of cretinism have been observed in several endemic goiter areas and sometimes both syndromes can be recognized in the same patients. Of course in areas where iodine prophylaxis has been introduced, "myxedematous cretins" may no longer be hypothyroid; they should still be characterized by a smaller thyroid, a lower radioiodide uptake, and a faster turnover of

Table 1. Comparison between "nervous endemic cretinism" as observed in New Guinea by Choufoer at al. (5) and "myxedematous endemic cretinism" described by Bastenie et al. (3) and Dumont et al. (14) in the Congo.

	Nervous	Myxedematous
Incidence in New Guinea	5.8%	0 %
Incidence in the Uele (Congo)	0.095%*	0.32%
Incidence in Idjwi (Congo)	0.1%	1 %
Mental deficiency	+++	+++
Deafmutism	+++	Occasional
Motor defects	+++	Occasional
Malformation of dentition	0	+++
Growth retardation	0	+++
Bone maturation retardation	0	+++
Fragmentation or deformity of femoral epiphysis	0	+++
Clinical hypothyroidism	0	+++
PBI	Normal	Very low
Radioiodide uptake by the thyroid	Slightly decreased	Very much decreased
Incidence of goiter	Normal	Much decreased
Thyroid size	Normal	Much decreased
Dimensions of the sella turcica	Normal	Increased

All characteristics are evaluated in comparison with non-defectives of the same area.

** In fact deafmutes; the real figures for nervous endemic cretins could therefore, according to data of Choufoer et al. (5), be slightly higher.*

thyroidal iodine than in the non-defectives, but these differences may tend to disappear with increasing supply of iodide in the diet. Thyroid regeneration may also erase these characteristics. Depending on the duration of hypothyroidism, dwarfness may be more or less evident in the myxedematous cretins. Since nervous endemic cretinism does not fit the description of congenital hypothyroidism, they have often not been classified as "cretins." The data of Choufoer (5) and Lobo (23) suggest that most of them will appear under the heading "deafmutes" in such surveys. Taking these limitations into account, we think that both types of cretinism can still be recognized in the descriptions of the endemias of the Congo (10, 14), India (26, 27), Brazil (23), Switzerland (13, 20, 21), Italy (7, 8), and perhaps Yugoslavia (19) and Argentina (31). The fact that only nervous endemic cretins have been found in some endemias (5, 20), such as in New Guinea, may reflect differences in pathogenesis, but it may also be the consequence of the very low survival rate of hypothyroid children in areas where infant mortality is high. Moreover, parents may tend to hide cretins. Survey data on the relative incidence of both forms of cretinism should therefore not be taken, as they have been (5), as representative of the true incidence of both forms in an area. Only systematic and periodic examination of all newborn infants and children in an area could provide such information.

Even though the heading "endemic cretinism" covers two different syndromes, there is still a good rationale for grouping these syndromes. The

relation of the two is proved by their common relation to endemic goiter and by the fact that nervous cretinism (in the form of mental deficiency, deafmutism, and sometimes neurological signs) was more frequent in the hypothyroid cretins than in the general population in the Uele (14 per cent versus 0.095 per cent), in India (26), and perhaps in Brazil (23). That this relationship is not direct is suggested by the separate occurrence of the two forms, by the differences of incidence in different regions, and by the absence of correlation between the severity of hypothyroidism and the existence of deafmutism in the Uele cretins.

The delineation of at least two syndromes in endemic cretinism has the merit that it helps to define future lines of investigation. In myxedematous endemic cretinism the most important question is the cause and pathogenetic mechanism of thyroid atrophy. Various hypotheses have been or could be proposed: exhaustion atrophy (15), autoimmune mechanisms (3), fibrosis as a consequence of hemorrhages at birth in hyperplastic tissue, and insensitivity of the mechanism of adaptation to iodine deficiency. Nothing is known about the pathogenesis of deafmutism, mental deficiency, or neuromuscular disorders in nervous endemic cretinism. The relative independence of these defects may suggest different pathogenetic mechanisms or similar mechanisms operating at different periods of fetal life. Deafmutism and the motor syndrome could be caused by a lack of maternal thyroid hormone supply during early pregnancy, while hypothyroidism due to this fact and to fetal deprivation of iodide could account for mental deficiency (5). In both forms of cretinism, the etiological role of genetic factors (6), iodine deficiency, goitrogens, and perhaps other environmental factors (20) should be considered.

SUMMARY

The endemic cretin as defined by the Pan American Health Organization is an individual with irreversible changes in mental development, born in an endemic goiter area, and exhibiting a combination of some of the following characteristics not explained by other causes: irreversible neuromuscular disorders, irreversible abnormalities in hearing and speech, impairment of somatic development, and hypothyroidism. Critical examination of the results of the investigations on endemic cretinism carried out in the Congo and in New Guinea suggests that the broad definition of the PAHO includes two different syndromes, both related to endemic goiter: a first syndrome which is called nervous endemic cretinism is characterized by mental deficiency or deafmutism or both, and in the most severe cases by both defects and by neuromuscular disorders; a second syndrome, which is called myxedematous endemic cretinism, is a form of hypothyroidism which begins early in infancy or in fetal life and is caused by thyroid hypoplasia.

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SECTION II

ENDEMIC GOITER IN THE CONGO AND NEW GUINEA

CHAPTER 9

PERMISSIVE NATURE OF IODINE DEFICIENCY IN THE DEVELOPMENT OF ENDEMIC GOITER[1]

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(With the technical assistance of C. Walschaerts)

Iodine deficiency constitutes the main etiological factor of endemic goiter (43). The significance of its role is underlined in numerous studies (3, 7, 13, 22, 24, 27, 29, 36, 37, 39), and formal proof is supplied by the considerable drop in the prevalence of goiter as soon as additional iodine is introduced into the diet of affected regions (6, 26).

Nevertheless a series of observations has suggested that iodine deficiency is not the only etiological factor. Indeed, endemic goiter has been described in regions with no iodine deficiency (8, 9, 33) and sometimes even in the presence of an excess of iodine (44). Other authors have described conditions of extremely severe iodine deficiency where the population shows no abnormal thyroidal hyperplasia (7, 38). The influence of hereditary (28) or immunological factors (42) and the action of some goitrogenic factors (8, 19, 33) have been suggested.

The purpose of the present study has been to try to define the role of iodine deficiency and that of other factors, if any, in the development of endemic goiter on the island of Idjwi (Lake Kivu, Democratic Republic of the Congo) (11). The epidemiological and metabolic conditions of this endemic seem particularly suited for this type of study, since the existence of a very severe iodine deficiency has been described throughout the island, and yet only a part of the population, clearly limited from the geographic point of view, is affected by endemic goiter (12).

The investigations carried out in Idjwi covered six separate aspects:

1) Epidemiological survey of endemic goiter and cretinism.

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2) Study of iodine metabolism.

3) Assessment of diet conditions.

4) Geological study.

5) Estimation of thiocyanate in the blood and urine. (The presence of abnormal quantities of thiocyanate in the blood and urine has been considered as an indication of the presence of some natural goitrogenic compounds in the food (41).)

6) Treatment with iodized oil.

MATERIALS AND METHODS

Description of the Endemic Area

The island of Idjwi is situated in the middle of Lake Kivu in the east of the Republic of the Congo. Its area is about 300 square kilometers and its population is estimated at 30,000 inhabitants. These are Negroes of the Havu ethnic group. A chain of mountains rising to more than 3,000 meters in height cuts the island into two distinct regions: north Idjwi and south Idjwi. Contact between the populations of these two regions is infrequent. Furthermore, exchange between the island and the surrounding countryside is fairly limited because of the width of the lake and climatic conditions. Conditions of existence are still very primitive and the people live at subsistence level.

Epidemiological Survey

The prevalence of goiter was investigated according to the recommendations of Perez et al. (34): a thyroid gland with lateral lobes of a volume greater than the terminal phalanx of the thumb of the person being examined was considered goitrous.

Cases of cretinism were determined according to the criteria defined for the Uele goiter endemic by Bastenie et al. (4) and Dumont et al. (14, 15).

The survey covered 24,157 inhabitants, corresponding to the whole population of 57 communities throughout the island.

Study of Iodine Metabolism

The investigations entailed the measurement of the thyroidal uptake of ^{131}I, the plasma $PB^{127}I$ level, the ratio between iodine and creatinine in the urine, and the iodine concentration in the drinking water.

a) ^{131}I thyroidal uptake was measured by a transistorized portable scaler (Philips, Belgium) powered from a 12-volt battery. The 1-inch thallium-activated sodium iodide crystal, shielded with a conical collimater measuring 88 mm in length and 78 mm in diameter, and weighing 3.750 kg, was placed at 40 cm from the neck. In these conditions, the area seen by the counter has a diameter of 12 cm. The experimentally-calculated correction factor for back-scattering and autoabsorption was 0.97.

b) The levels of plasma PB^{127}I and stable iodine in the urine were measured by means of a modification (18) of the Baker et al. method (2) using an Autoanalyzer Technicon (Chauncey, New York).

c) The urinary iodine/creatinine ratios were calculated according to the method of Jolin and Escobar del Rey (23).

d) The concentration of iodine in the drinking water was measured by neutron activation according to a method described previously (18). Samples of spring water, river water, and lake water were taken from the usual sources of supply of the local population.

The subjects investigated were selected from the lists of names established during the medical census. They were clinically euthyroid patients of both sexes and ages between 15 and 30 years. Fifteen to 20 subjects were chosen at random from each village. At the time of selection no account was taken of the presence or absence of goiter.

Thyroidal uptake was determined 24 hours after the oral administration of 10 μc of ^{131}I to 602 patients belonging to 29 villages (from a total of 12,709 inhabitants). Urine samples were taken at about the same time (between 2 and 4 P.M.) from 605 subjects in these villages. Iodine concentration was measured in 213 samples, each being constituted by a mixture of identical volumes of urine from three patients of the same village. Out of these samples, 56 taken in the north and 25 taken in the southwest were used to determine the urinary iodine/creatinine ratio. The daily renal excretion of ^{127}I was measured from the 24-hour urine collections of 33 subjects aged from 10 to 18. Seventy-one collections were taken in the north (21 subjects) and 48 in the southwest (12 subjects). The concentration of iodine in the drinking water was measured in 36 samples, 26 collected in the north of the island and ten in the southwest.

Assessment of Diet Conditions

Food surveys were conducted over 18 months in two villages: one in the north (Shugi) and one in the southwest (Mpene). Each village was studied for 14-day periods by the same three investigators, eight times a year. Each of them visited five families selected for the survey two or three times per day. They noted the number, age, sex, and family relationships of the people sharing the food; they weighed the food, measured the drinks, and noted the way the meals were prepared (cooking times, seasonings).

The theoretical dietary needs of the populations were expressed in Kcal and in reference proteins, on the basis of the recommendations of the FAO Committee (21) and Joint FAO/WHO Committee (45). The nutritional value of the food was established either from tables issued by the FAO and Medical Research Council (30) or from direct analysis of the food (Vis et al., unpublished data).

Geological Studies

The data concerning the geological characteristics of the island of Idjwi collected in 1939 by Boutakoff (5) were completed by geological surface studies carried out in 1967 by G. Bonnet and P. Guibert (Geophysics Department, IRSAC Institute, Lwiro).

Estimation of Thiocyanate in the Blood and Urine

Thiocyanate was estimated according to the method of Aldridge (1), modified by Michajlovskij and Langer (31).

RESULTS

Epidemiological Study

The results of the goiter surveys carried out in 1965 and 1966 are given in Figure 1. Three areas can be distinguished in which the average prevalence of goiter attains the following values:

North	54.4 per cent	(range: 31.7 to 71.4 per cent)
Southwest	5.3 per cent	(range: 3.7 to 8.8 per cent)
Southeast	17.5 per cent	(range: 9.2 to 35.4 per cent)

The evolution of goiter frequency as a function of age and sex for the high endemic (north) and low endemic (southwest) areas is given in Figure 2. A high frequency of goiter is observed in the north, regardless of age, including in very young children. Goiter occurrence is far more frequent among the female sex, and 92 per cent of girls aged between 14 and 15 show abnormal thyroid hyperplasia.

One hundred cases of cretinism were recorded in the northern area, which corresponds to a prevalence of 1.1. per cent. In 89 of the subjects, clinical and biological signs of hypothyroidism were observed)plasma $PB^{127}I$: 1.8 ± 0.2* µg per 100 ml; 24-hour thyroidal uptake: 42.5 ± 5.7* per cent of the dose): 25 per cent of these patients were goitrous. The other 11 cretins were clinically euthyroid; six of them had goiters, nine were deaf-mutes, and seven suffered from spastic paralysis of the legs. The clinical characteristics of the two types of cretins encountered in northern Idjwi are shown in Figure 3. No cases of cretinism were recorded in the southwest of the island.

Study of Iodine Metabolism

The results of the comparative study of iodine metabolism in the populations of the high and low goitrous endemic regions of the island are given in Table 1. Thyroidal uptake of radioiodine is very high in the two areas, but slightly more so in the goitrous area ($P < 0.01$). Urinary excretion of iodine is very reduced in both parts of the island ($P > 0.05$). The urinary ratio iodine/creatinine is also reduced but more so in the northern area ($P < 0.005$). A significant reduction of the plasma $PB^{127}I$ level was also observed ($P < 0.001$). The iodine content in the drinking water is comparably low in the two regions ($P > 0.1$) (10).

The frequency distribution of the values obtained for the 24-hour ^{131}I thyroidal uptakes, for the levels of plasma $PB^{127}I$, and for the urinary excretion of ^{127} iodine in each of the two regions are given in Figures 4, 5, and 6.

* Standard error of the mean.

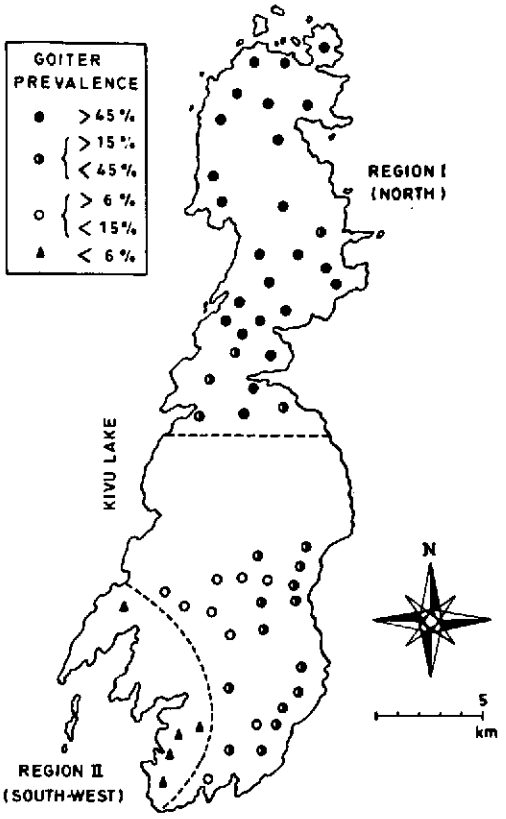


Figure 1. Distribution of goiter prevalence in 57 communities of Idjwi Island. Comparative studies of iodine metabolism were made in the goitrous (Region I) and nongoitrous (Region II) areas.

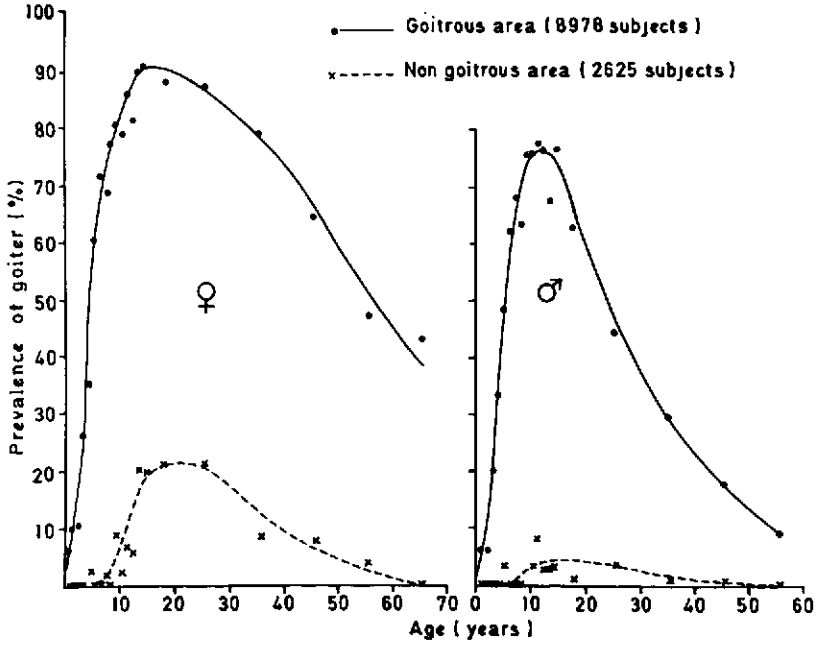


Figure 2. Evolution of goiter frequency as a function of age and sex in the high (north) and low (southwest) endemic areas.

Figure 3. Clinical appearance of two types of endemic cretins observed in northern Idjwi. On the left, 19-year-old male, clinically euthyroid, height 154.5 cm, deafmutism, severe mental deficiency, spastic diplegia (PBI^{127}: 2.0 µg per 100 ml; 24-hour ^{131}I thyroidal uptake: 68.4 per cent). On the right, 20-year-old female, clinically severe hypothyroidism, 109.0 cms, mental deficiency (PBI^{127}: 1.3 µg per 100 ml; 24-hour ^{131}I thyroidal uptake: 23.0 per cent).

Table 1. Comparison of various parameters of iodine metabolism in the high (Region I) and low (Region II) goitrous endemic regions of Idjwi Island.

	REGION I : NORTH	REGION II : SOUTHWEST	t TEST (p. values)
Goiter prevalence (in %)	54.4 (9000)*	5.3 (3600)	
Cretinism prevalence (in %)	1.1 (9000)	0.0 (3600)	
^{131}I 24-hr thyroidal uptake (% dose)	79.5 ± 0.7** (376)	70.8 ± 1.2 (104)	<0.010
Daily urinary iodine output (ug)	12.6 ± 0.8 (71)	19.4 ± 2.0 (48)	>0.050
Urinary ratio iodine (ug)/ creatinine (g)	22.2 ± 1.3 (56)	31.2 ± 3.6 (25)	<0.005
Plasma PB^{127}I (ug/100 ml)	3.7 ± 0.1 (301)	4.6 ± 0.2 (59)	<0.001
Iodine content of water (ug/1)	4.2 ± 0.5 (26)	5.7 ± 1.2 (10)	>0.100

* *Number of cases or samples.*
** *Standard error of the mean.*

Figures 4 and 5 show that, in spite of considerable overlapping of the individual values, the frequency curves of the thyroidal uptakes and of the plasma $PB^{127}I$ are distinct in the two regions.

By contrast, the frequency curves for the urinary excretion of the ^{127}I are superimposable, except for a few much higher values observed in subjects from the nongoitrous regions.

Diet Conditions

The overall results concerning the supplies in energy, proteins, and lipids calculated over a period of 18 months in the two regions are summarized in Table 2. For the two regions of the island, the total caloric supply is from 10 to 15 per cent less than the optimum level recommended by the FAO Committee (2). Protein needs seem to be covered satisfactorily. Lipid supplies are, however, extremely reduced, since they represent only about 4 per cent of the theoretical caloric supply. Seasonal variations in food supplies are given in Figure 7 for caloric supplies and in Figure 8 for protein supplies. These figures show that throughout the year for the food supply the population relies mainly on four products: beans, bananas, sweet potatoes, and cassava roots. During the gap in the farming year between September and December there is a drop in the protein supply.

The average daily food intakes of the two investigated regions are compared in Table 3. Shown are the relatively higher consumption rates for cassava roots, peanuts, bananas, fresh pumpkins, and pumpkin seeds in the goitrous area. These differences depend on the season, except where cassava is concerned. Local cassava (Manihot utilissima) is a sweet variety and is consumed, without preliminary steeping, fresh or in the form of flour. This product is prepared in the same way in the two areas. The quantity of cassava eaten per inhabitant corresponds on average to 94.3 gm of flour in the goitrous area and to 61.9 gm in the nongoitrous area.

Geological Study

Boutakoff (5) showed in 1939 that the type of soil existing in the north and southwest of the island of Idjwi was made up of granite and ruzizi, whereas

Table 2. Energy, lipid (in kcal) and protein (in gm of reference proteins) intakes in Shugi (goitrous area) and Mpene (nongoitrous area). The figures in brackets indicate the percentage in relation to the theoretical figures defined for calories by the FAO Committee (1957) (21) and for proteins by the FAO/WHO Joint Expert Group (1965) (45).

	SHUGI	MPENE
Theoretical energy requirements (in kcal)	2 090.0 (100.0)	1 947.0 (100.0)
Real caloric intake	1 876.8 (89.8)	1 709.4 (87.8)
Real lipid intake	91.9	81.7
Theoretical protein requirements (in gm of ref. prot.)	32.7 (100.0)	33.1 (100.0)
Real protein intake	35.3 (107.9)	36.6 (110.7)

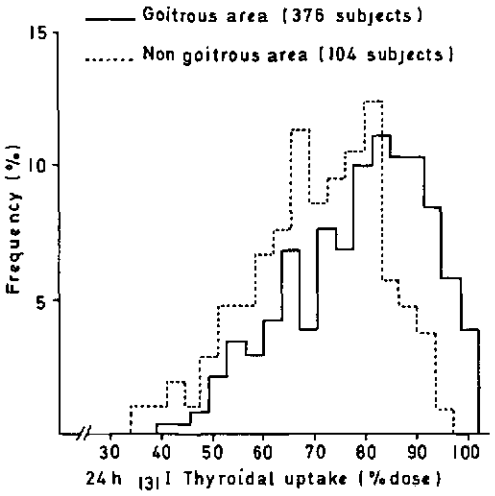


Figure 4. Frequency distribution for 24-hour ^{131}I uptake in the goitrous and nongoitrous areas.

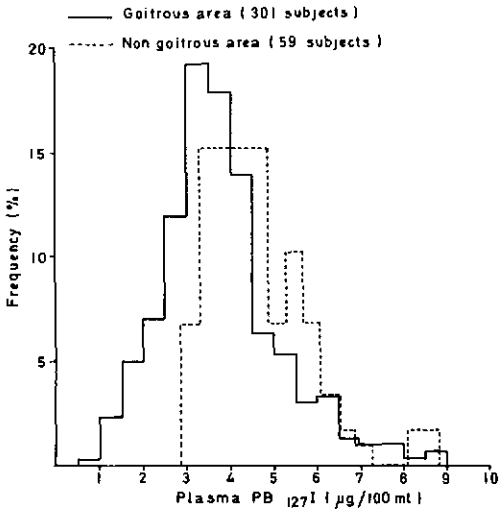


Figure 5. Frequency distribution for PBI concentrations in the goitrous and nongoitrous areas.

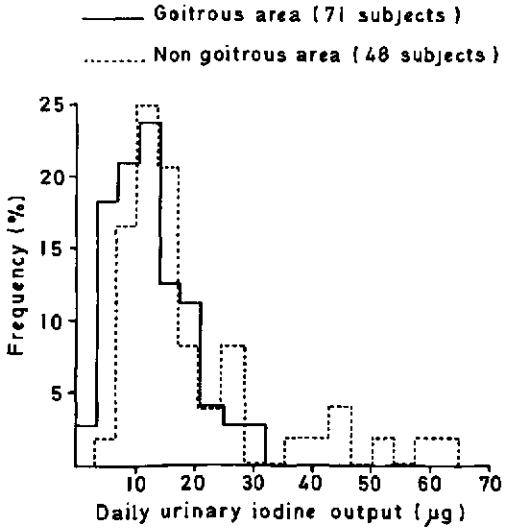
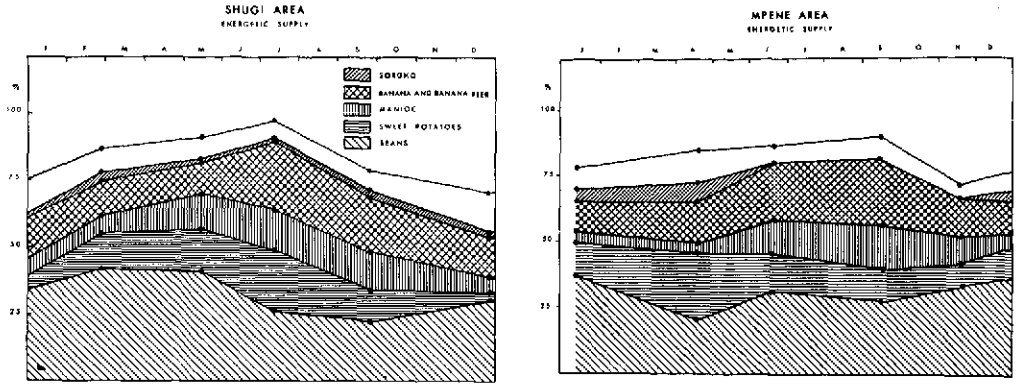
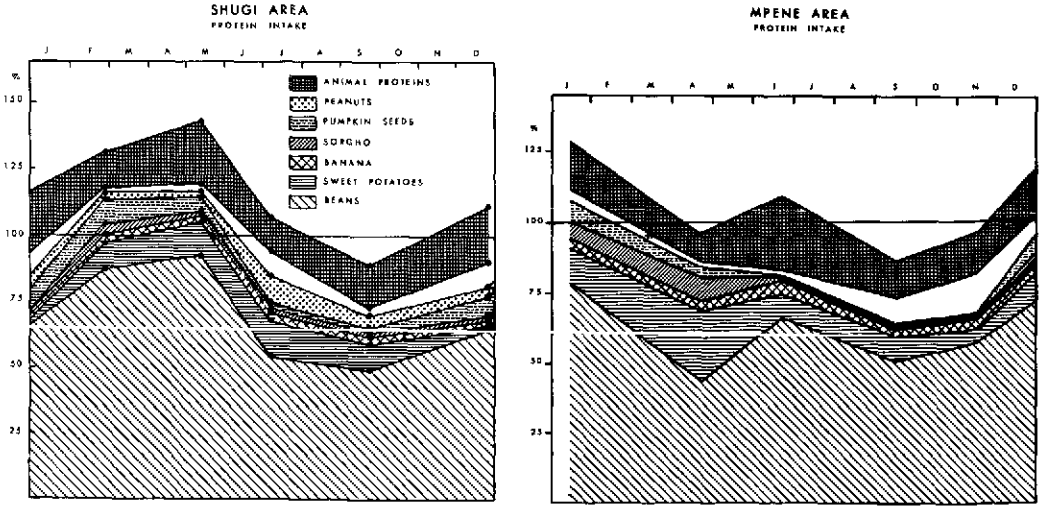


Figure 6. Frequency distribution for daily urinary excretion of iodine in the goitrous and nongoitrous areas.



(a) (b)

Figure 7. Annual evolution of caloric supplies in the regions of Shugi (goitrous area) (a) and Mpene (nongoitrous area) (b). One hundred per cent represents the quantity of calories recommended by FAO (21). The white space between the upper line of the sorghum and the line representing total supplies concerns the food products consumed in small quantities in the region (green leaves, meat, and fish).



(a) (b)

Figure 8. Annual evolution of protein supplies in the regions of Shugi (a) and Mpene (b). One hundred per cent represents the quantity of reference protein recommended by the Joint FAO/WHO Committee (45). The white space between the supplies of sorghum and those of animal proteins represents the food products consumed in small quantities but rich in proteins (cassava, leaves, milk, etc.).

Table 3. Average daily food consumption (in gm) per capita in the goitrous area (Shugi) and the nongoitrous area (Mpene).

	SHUGI	MPENE
Sweet potatoes	213.3	245.9
Cassava roots	94.3	61.9
Taro	1.6	-
Yams	2.1	traces
Potatoes	-	6.8
Sorghum	2.7	9.4
Maize	18.8	7.3
Rice	-	0.2
Wheat	-	-
Beans (Phaseolus vulgaris)	252.7	237.8
Peas	0.6	0.4
Peanuts	22.4	15.0
Pumpkins (seeds)	5.3	1.4
Cassava leaves	11.7	16.1
Green leaves	20.2	12.9
Vegetables (tomatoes, onions)	-	3.9
Pumpkins (fresh)	48.0	8.4
Meat	12.8	15.0
Fish	25.6	18.5
Milk	1.4	0.9
Bananas	219.5	295.3
Banana beer	555.0	375.3
Palm oil	1.2	1.8

the soil in a localized area of the southwest was made up solely of basalt. These results are reported in Figure 9 in which the geological map established by this author and the present epidemiological data are compared. The geographic distribution of the nongoitrous area coincides exactly with that of the basalt area of the island. Preliminary data collected by G. Bonnet and P. Guibert (Geophysics Department, IRSAC Institute) have confirmed the geological data of Boutakoff (personal communication, unpublished data)

Thiocyanate Levels

The results of the analyses for thiocyanate in the serum and urine samples taken from Idjwi (north and southwest) and from Belgian controls are shown in Table 4.

The serum levels recorded on Idjwi were the same for the goitrous and nongoitrous regions; the levels were three times as high as those recorded in Belgium. The quantities of thiocyanate excreted in urine per 24-hours were significantly higher in the goitrous regions ($P < 0.01$).

Treatment by Iodized Oil

The results obtained during these investigations are given in detail in an accompanying paper. Single injections of iodized oil (0.05 to 1 gm iodine) were administered to nine-tenths of the population of two villages in

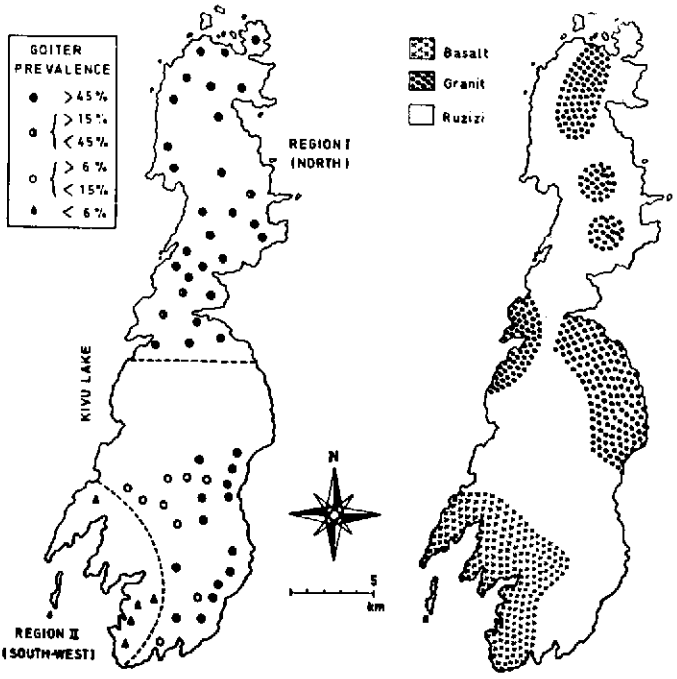


Figure 9. Comparison between the geological and epidemiological maps of the island. Contour of the island according to Rahme et al. (35), geology according to Boutakoff (5).

Table 4. Thiocyanate levels in plasma and urine in Idjwi inhabitants and in Belgian controls.

	Plasma thiocyanate concentration (mg/100 ml)	Renal thiocyante excretion (mg/24 hours)
Idjwi Island		
Goitrous area	1.10 ± .09* (56)**	14.3 ± 1.5 (30)
Nongoitrous area	1.07 ± .11 (56)	10.0 ± 0.9 (47)
Belgian controls	.24 ± .03 (30)	-

* *Standard error of the mean.*
** *Number of samples.*

the north of the island (911 subjects). After one year goiter prevalence fell in these subjects from 49.0 to 16.3 per cent, and in 80 per cent of the cases there was an important reduction in the size of the gland, whatever the initial type of goiter.

DISCUSSION

Role of Iodine Deficiency

After the investigations carried out on Idjwi in the high and low endemic areas, it is possible to envisage the relation between iodine deficiency and goiter prevalence in the light of three principal findings (cf. also Chapter 10).

1) The island of Idjwi as a whole has an extremely severe iodine deficiency, in both the north and the southwest. The thyroidal uptake and the urinary iodine/creatinine ratio are slightly different in the two regions, but there is still considerable overlapping of the values recorded in each area.

2) On the other hand, striking differences in the prevalence of goiter in a ratio of ten to one are observed between the two regions. Furthermore, the existence of a high level of endemic goiter in the north of the island coincides with a very high frequency of endemic cretinism.

3) The correction of the iodine deficiency by iodized oil injections has led to a marked drop in the incidence of goiter.

The question arises whether a more marked iodine deficiency in the north of Idjwi could alone account for the epidemiological differences observed. This explanation seems difficult to accept for various reasons. First, whereas the differences in epidemiological data are clearcut, the figures for uptake and urinary excretion are quite comparable for the greater part of the two populations. Moreover, the iodine deficiency observed in the

nongoitrous region is far more severe even than that recorded in other goitrous endemic areas (29, 37).

Finally, a relative increase in ^{131}I uptake observed in the goitrous region might to some extent be linked with the presence of goiter. Indeed, endemic goiter is associated with the setting-in of a series of metabolic anomalies which slow down intrathyroidal hormonogenesis (16) and accelerate iodide leakage (17). This factor could suffice to explain the slight differences in iodine metabolism between the goitrous and nongoitrous populations. This hypothesis has been verified by Thilly et al. in a recent study covering 605 subjects from 29 communities spread throughout Idjwi (unpublished data). These authors found no statistical relation whatsoever in the villages studied between, on the one hand, the six-hour ^{131}I thyroidal uptake and the prevalence of goiter and, on the other hand, the urinary concentration in iodine, which is incidentally remarkably stable from one community to another. However, there is a highly significant correlation between the prevalence of goiter and the thyroidal uptake.

It seems, therefore, that iodine deficiency cannot account alone for the difference in the epidemiological situation between the north and southwest of the island and that the intervention of an additional goitrogenic factor, acting specifically in the north, should be considered.

Nevertheless, correction of iodine deficiency produced a rapid reduction of the disease. This suggests that iodine deficiency is a permissive factor rather than the sole causal factor of endemic goiter on Idjwi. This observation agrees with the data collected by Roche (38) among Venezuelan Indians and by Choufoer et al. (7) in New Guinea.

Goitrogenic Factor

The goitrogenic factor responsible for the epidemiological differences observed on Idjwi has not been identified. Our observations have, however, revealed a series of characteristics differentiating the goitrous area from the nongoitrous one. The differences are related to:

A. Food conditions: Although protein and caloric needs are covered to a more or less comparable extent in the two regions, certain foods are consumed in much higher quantities in the goitrous region (cassava roots, beans, bananas, fresh pumpkins, pumpkin seeds, and peanuts).

The goitrogenic character of cassava has been demonstrated experimentally by Ekpechi (19), who suspects the presence of a substance from the thionamide group in this food. This author attributes the cause of endemic goiter in eastern Nigeria to cassava consumption and an iodine deficiency (20).

B. Geological characteristics: The fact that isolated nongoitrous areas in an endemic goitrous region can correspond geographically with areas of basalt-based soil has already been noted by Wilson (46) in Nigeria. It is conceivable that the structure of the soil can play a role in the concentration of certain goitrogenic substances in plants. Observations made by Sedlak et al. (40) lend support to this theory.

C. Thiocyanate levels: Blood thiocyanate levels recorded on Idjwi in both regions are markedly higher than those observed in European controls and are comparable to the values obtained by Langer (25) in an endemic goiter area of Slovakia. However, renal excretion is higher in the goitrous part of the island. An increased level of thiocyanate could reflect the absorption of goitrogenic food, as suggested by Michajlovskij and Langer (32).

All these points deserve further careful investigation. Indeed it is probable that, after iodized oil treatment, a goitrogenic factor of some kind might nevertheless set to work in some particular conditions; this would account for the residual prevalence of goiter usually observed in regions submitted to adequate prophylaxis.

SUMMARY

Insufficient iodine supply is usually considered as the main factor causing endemic goiter, but the part played by other factors has often been underlined. This question of relative roles arises once again in view of the particular epidemiological characteristics observed on the island of Idjwi, Lake Kivu (Congo).

In the greater part of the island, the population shows a very high prevalence of goiter (54.4 per cent); this value drops to 5.3 per cent in a well-defined area in the southwest. And yet iodine deficiency is shown to be of about equal severity in the two regions. This observation suggests the intervention of a goitrogenic factor distinct from the inadequate iodine supply. However, the administration of iodized oil reduced the incidence of goiter by two-thirds after one year. While it is not the causative factor, iodine deficiency acts as a permissive factor in the development of goiter on the island.

Parallel systematic studies of the diet conditions of the two populations over a full year have revealed that larger quantities of cassava are consumed in the goitrous region. Furthermore, the level of thiocyanate is very high in the serum and urine of the two populations, whereas urinary excretion is greater in the goitrous region.

The geographic locations of the goitrous and nongoitrous areas correspond to two geological areas of the island. In the first the soil has a granite base, and in the second, basalt.

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CHAPTER 10

TREATMENT OF IDJWI ISLAND ENDEMIC GOITER BY IODIZED OIL[1]

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(With the technical assistance of C. Walschaerts)

The administration of iodized salt has proved to be an effective method for correcting an iodine-deficient diet and has been widely used in the prevention of endemic goiter (2, 7, 14, 16, 19, 21, 22, 24). This method is difficult to apply in places where health services are unable to ensure the systematic distribution of iodized salt to the whole population (14, 15).

These difficulties led McCullagh (17) to replace the distribution of iodized salt by single intramuscular injections of high doses of iodine in the form of iodized oil. The first administrations were carried out in 1957 in a population group of New Guinea where the iodine deficiency was severe; the doses of iodine injected ranged between 0.4 gm and 2 gm (17). Subjects over 50 years of age or with visible goiters were not injected. Out of the 7,881 subjects selected, half received injections of iodized oil and the other half physiological serum. The effect of the treatment was then studied by McCullagh (17), Hennessy (13), and Buttfield et al. (3, 4, 5). The data collected by these authors are summarized in Table 1. They show an effective correction of the iodine deficiency which lasted for more than four and a half years after the administration of the iodized oil. In 61 goitrous patients, Buttfield et al. noted that the goiter disappeared in all but one of the subjects after a lapse of three months following the injection of iodized oil (5). Finally, none of the authors reported any immediate secondary effects or any subsequent setting-in of hyper- or hypothyroidism.

The present study describes the results obtained after the administration of iodized oil to inhabitants of the island of Idjwi, in the Kivu Lake (Democratic Republic of Congo), where endemic goiter is also associated with a deficient iodine supply (8, 9).

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Table 1. Results of prophylaxis of iodine deficiency by iodized oil in New Guinea. (Doses: 0.4 to 2.0 g iodine)

Time	Prevalence of goiter (stages II and III (% subjects)	24h ^{131}I thyroidal uptake (% dose)	Plasma PB^{127}I (µg/100ml)		^{127}I daily renal excretion (µg)	Reference
0	(5.5 ?)*	70	1.8	4.1	11	A+
3 months		6		44.7	258	A
1 1/2 year		31		8.2	119	A
3 years		37		7.8	35	A
4 1/2 years		44		6.4	23	A
5 years	2.7		4.8			Bx

* *Percentage obtained in untreated patients five years after the injection.*
\+ *Ref. A: Buttfield et al. (5).*
x *Ref. B: Hennessy (13).*

For the Idjwi study, three major modifications were made to the system of treatment adopted by the Australian authors:

1) The whole population was treated, including patients with visible goiters and those over 50 years of age.

2) The doses proposed by McCullagh (17) were reduced by one-half to one-tenth.

3) In one random-selected patient out of every ten, the injection of iodized oil was replaced by that of a placebo.

Furthermore, in a separate series of investigations, the influence of high single doses of potassium iodide on thyroidal uptake of ^{131}I was reassessed by a method used previously by Stanbury et al. (23).

MATERIAL AND METHODS

Endemic Goiter on Idjwi Island

The epidemiological and metabolic characteristics of endemic goiter on this island have been reported in previous publications (8, 9, and Chapter 9). The population of the island consists of Negroes of the Havu ethnic group, whose living conditions are very primitive. Their diet is made up exclusively of local crops, mainly beans, sweet potatoes, cassava, and bananas. Consumption of salt is irregular (27). The mean prevalence of goiter in the goitrous

region reaches 54.4 per cent, and that of cretinism 1 per cent. Thyroidal uptake of radioiodine at the 24th hour is 79.5 per cent of the dose, the plasma $PB^{127}I$ is 3.4 µg per cent, and urinary excretion of iodine per 24 hours is 13.1 µg (9).

Doses of Iodine Administered and Choice of Patients

The iodized oil used was Lipiodol* constituted by ethyl esters of the fatty acids of poppy-seed oil iodized to 40 per cent. The injections were given in January 1966 in the villages of Bugarula and Bukenge situated in the north of Idjwi Island. All the inhabitants of the two villages, including babies and pregnant women, were treated with single injections, except one out of every ten subjects chosen at random depending on the time of attendance for treatment. The randomly-selected exceptions were given vitamin injections with no iodine (placebo). The iodine doses administered are shown in Table 2. They varied between 50 mg and 1 gm of iodine according to age, the volume of the thyroid, and the presence of nodules if any; patients showing large (stage III) or nodular goiters received half doses.

Table 2. Doses of iodized oil used in the island of Idjwi (mg of iodine).

Age	No goiter Diffuse goiter (stages I and II)	Diffuse goiter (stage III) Nodular goiter
0 - 11 months	125	50
1 - 4 years	250	125
5 - 9 years	750	375
10 years and more	1,000	500

The size of the thyroid glands of all the inhabitants of the two villages was measured one year before the treatment (1,207 subjects) and at the time of the treatment (1,379 subjects). One year later 1,026 of them were re-examined. They were identified with certainty at that time by means of lists of names made out at the time of the injections. Nine hundred and eleven had received injections of iodine and 115 a placebo.

Epidemiological Study

Goiter stages were defined according to adapted WHO criteria established by Perez et al. (20).

Any thyroid with lateral lobes larger than the terminal phalanx of the thumb of the person examined was considered as goitrous.

* Lafay, Paris.

Stage 0 : No goiter.

Stage I : Goiter can be felt.

Ia:: Goiter can be felt but not seen, even when neck is stretched.

Ib : Goiter can be felt but not seen when neck is in normal position, becomes visible when neck is stretched.

Stage II : Goiter easily visible with neck in normal position.

Stage III: Very large goiter.

The absence or the presence of palpable nodules was noted.

Studies of Iodine Metabolism

Thyroidal uptake was measured after oral administration of 10 μc of carrier-free ^{131}I* by means of a scintillation detector connected to a pulse analyzer and a rate meter (Philips, Belgium). The one-inch thallium-activated sodium-iodide crystal fitted with a large open angle lead collimator conforming to IAEA recommendations (12) was placed at 40 cm from the center of the gland. At this distance, the surface seen by the crystal measures 12 cm diameter. The experimentally-calculated correction factor for back-scattering and auto-absorption was 0.97.

The plasma $PB^{127}I$ and the amounts of iodine in the urine were determined according to a modification (11) of the method of Barker et al. (1) using an Autoanalyzer Technicon (Chauncey, New York).

The influence of the treatment on the main parameters of iodine metabolism was studied in 102 clinically euthyroid patients. This group comprised 53 children between 8 and 14 years of age and 49 adults selected at random independently of goiter size. Eighty-one of them were treated with iodized oil according to the program described in Table 2, and the 21 others with a placebo. It was possible for 42 of the patients treated with iodized oil to be re-examined 6, 9, and 12 months after the injection.

Thyroidal uptake at the 24th hour was measured before treatment in 92 of these subjects. It was possible to take control measurements a year later in 64 of them. The treatment of these patients had comprised:

1,000 mg	38 subjects
750 mg	1 subject
500 mg	11 subjects
375 mg	1 subject
0 mg	13 subjects

The concentration of iodine in the urine was measured before treatment and 6, 9, and 12 months after treatment, by means of urine samples all taken at the same time of day (between 2 and 4 P.M.). The level of the plasma $PB^{127}I$ was estimated before the injection and 6 months after.

* From the "Centre d'Etudes nucléaires", Mol, Belgium.

Influence of a Single Dose of Iodide on Thyroidal Uptake

The blocking effect on ^{131}I uptake induced by the simultaneous administration of iodide doses of 2 or 4 mg was studied in 16 Idjwi patients living in a village near the two villages treated. This group comprised 14 goitrous subjects and two nongoitrous subjects aged between 10 and 15 years. The patients were submitted to two successive radioiodine tests at ten days' interval. For each test, thyroidal uptakes were measured hourly until the 7th hour after the administration of ^{131}I and at the 24th hour. The first test was performed after intravenous injection of a sterile solution of 10 μc of carrier-free ^{131}I. For the second one, ^{131}I was administered per os with a capsule containing 2 or 4 mg potassium iodide.

During these tests the error due to cervical extra-thyroidal radioactivity was corrected by subtracting from the neck radioactivity, the product of the radioactivity measured at the same time over the thigh by a factor estimated for every subject. This factor is the quotient of the neck and thigh radioactivities measured during the first six minutes after the intravenous injection of radioiodine.

RESULTS

Influence of the Treatment

The prevalence of goiter

The results of the surveys performed in the villages of Bugarula and Bukenge in 1965, 1966, and 1967 are summarized in Figure 1. The prevalence of goiter one year before and at the time of the treatment was very similar--51.4 and 48.7 per cent of the total population. One year later, it fell from 49.0 to 16.3 per cent in the group of 911 patients submitted to the iodized oil treatment.

A perceptible decline in the goiter incidence was also observed in the 115 patients living in the same villages but who received a placebo and not iodized oil; prevalence of goiter in these subjects fell from 57.4 to 33.9 per cent after one year. In two other neighboring villages used as controls, goiter prevalence did not change during the same period of time (43.0 to 39.0 per cent and 31.4 to 32.8 per cent).

Figure 2 shows the distribution of the various types of goiter within the group treated with iodized oil, before and after the injection. A decline in frequency is observed for all categories of goiter, including stages II and III. A similar observation appears in Table 3, in which the trend of goiter size after treatment is considered as a function of thyroid size at the time of the injection. A decrease of the thyroid size was observed in more than 80 per cent of the cases, whatever the initial size.

It was possible to follow the evolution of goiter in 42 patients at intervals of 6, 9, and 12 months after the administration of the iodine. The results are shown in Figure 3. A progressive decline is observed for all types of goiter during the periods considered. The results seem to indicate that for goiters of stage II, the decline takes place chiefly between the 9th and 12th month.

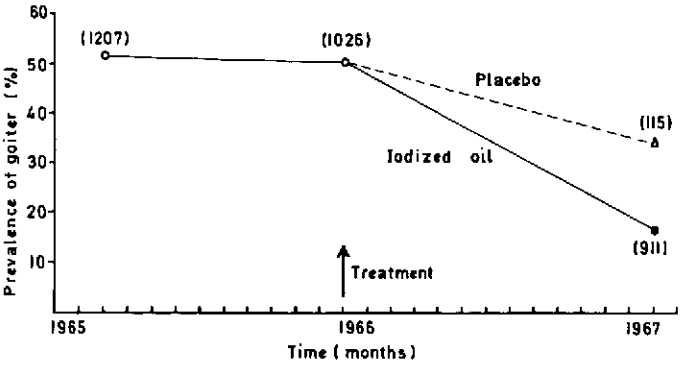

Figure 1. Evolution of goiter prevalence among the total population of the two villages investigated one year before the treatment, at the time of the injection (1966), and one year later.

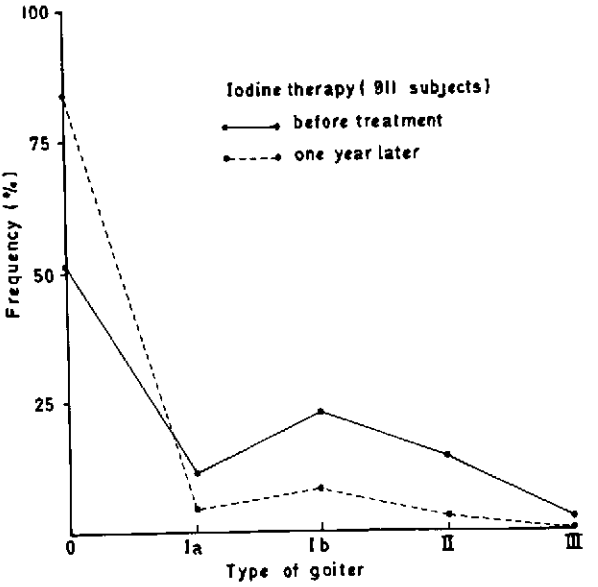

Figure 2. Frequency of the various types of goiter within the group of 911 subjects treated with iodized oil at the time of the treatment and one year later.

Table 3. Modification of thyroid size one year after the administration of iodized oil.

Type of goiter	Number of subjects	Thyroid size		
		Unmodified (%)	Decreased (%)	Increased (%)
0	462	97.4	-	2.6
Ia	95	9.5	87.3	3.2
Ib	204	14.2	81.4	4.4
II	133	13.5	86.5	0.0
III	17	17.6	82.4	-

No secondary effects were observed in the week following the 911 injections of iodized oil and in particular no cutaneous reaction suggestive of iodism. Localized temporary induration of a more or less painful nature was observed in a great number of patients but their normal activity was not hampered. No flagrant clinical evidence could be obtained on the possible development of delayed hyper- or hypothyroidism in the patients treated with iodine. The precarious conditions of this field study may not have enabled minor disturbances to be revealed.

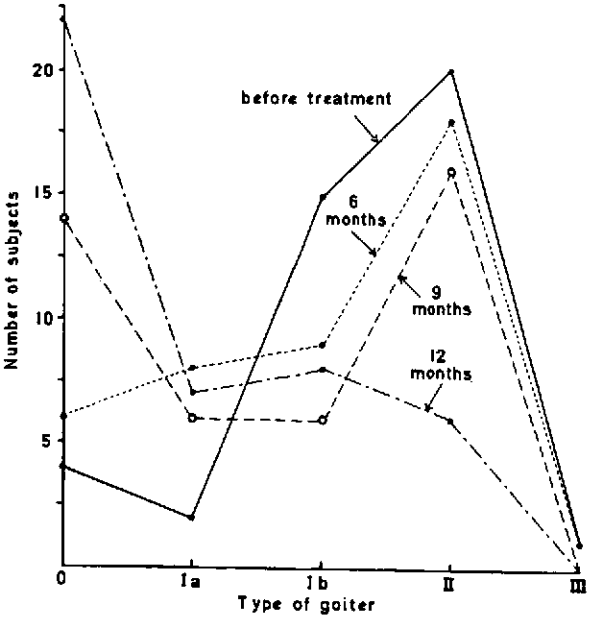

Figure 3. Evolution of thyroid size in 42 patients at the time of the treatment by iodized oil and 6, 9, and 12 months later.

Iodine metabolism

A. Thyroid uptake of ^{131}I at the 24th hour. Uptake dropped from 79.6±1.8 to 16.2±1.1 ($P < 0.001$) in 51 patients one year after treatment with iodized oil. The values obtained for patients who had received 1 gm and 0.5 gm of iodine are considered separately in Table 4. The drop in uptake is significantly more pronounced in patients of the first group ($P < 0.025$). In the 13 patients who had received the placebo, uptake was not modified ($P > 0.2$).

Table 4. Modification of the thyroidal ^{131}I uptake (24 hr, o/o dose) one year after the administration of iodized oil.

Doses (mg iodine)		Before treatment	One year after treatment	t Test
1,000	(38)*	77.7 ± 2.0+	14.0 ± 0.7	P <0.001
500	(11)	81.5 ± 3.9	21.8 ± 3.9	P <0.001
0	(13)	81.1 ± 4.7	87.5 ± 2.1	P >0.2

* *Number of subjects.*
\+ *Standard error of the mean.*

B. Urinary excretion of stable iodine. The evolution in time of urinary concentration of iodine as a function of the dose of iodine administered is shown in Table 5. The initial level of about 2 µg/100 ml is the same for all the groups considered ($P > 0.1$).

Six months later, it reached 215 µg/100 ml in the patients who had received 1 gm of iodine and 134 µg/100 ml in those treated with 0.5 gm. These levels varied greatly from one individual to the next. In 18.2 per cent of the cases the concentration exceeded 250 µg/100 ml, which would correspond to an iodine excretion rate of about 2 mg per 24 hours for average urine volume of 800 ml per day. Urinary concentration in iodine decreased progressively in time and after one year attained levels that were almost identical whatever the quantity of iodine injected (Table 5). In the patients treated with the placebo, urinary excretion remained practically unchanged. In four urine samples with large amounts of iodine, 100 per cent of the iodine was found to be fixed after five minutes on an Irosorb resin (Technicon). This suggests that it was constituted exclusively of inorganic iodine.

C. Level of the plasma protein bound iodine. The results of the measurements of the plasma $PB^{127}I$ levels before treatment and six months afterwards are given in Table 6. In the subjects treated with 0.5 and 1 gm of iodine, the values increased from 3.7 to 28.4 µg/100 ml and from 3.9 to 31.7 µg per 100 ml respectively. In the patients injected with a placebo, the plasma $PB^{127}I$ levels remained unchanged ($P > 0.5$).

Out of six serum samples, of which the average level of $PB^{127}I$ reached 26.4±3.4* µg per 100 ml, after being passed three times over Irosorb resin, the extraction by butanol carried out according to a technique described previously (11) gave an average recovery rate of 37.0±3.8* per cent.

* Standard error of the mean.

Table 5. Modifications of urinary iodine concentration in respect to the time and the doses of iodized oil.

Doses (mg iodine)	Before treatment		6 months*		9 months*		12 months*	
	Number of subjects	^{127}I concentration (µg/100ml)	Number of subjects	^{127}I concentration (µg/100ml)	Number of subjects	^{127}I concentration (µg/100ml)	Number of subjects	^{127}I concentration (µg/100ml)
1,000	55	2.0 ± 0.3[+] (0.6 - 4.6)[x]	49	215.5 ± 23.6 (40.5 - 760.0)	45	82.3 ± 6.6 (19.0 - 185.0)	37	54.7 ± 5.9 (12.0 - 158.0)
500	19	1.9 ± 0.2 (0.3 - 3.0)	17	134.2 ± 44.7 (10.7 - 700.0)	17	63.0 ± 8.3 (13.0 - 135.0)	10	51.4 ± 17.8 (2.2 - 177.0)
0	19	1.7 ± 0.2 (0.4 - 4.1)	14	2.3 ± 0.2 (1.1 - 3.5	14	2.0 ± 0.2 (1.2 - 3.0)	12	2.4 ± 0.2 (1.0 - 4.0)

* *Time elapsed from the administration of iodized oil.*

\+ *Standard error of the mean.*

x Range.

Table 6. Modifications of the plasma $PB^{127}I$ six months after the administration of iodized oil.

Doses (mg iodine)	Before treatment		Six months after treatment	
	Number of subjects	$PB^{127}I$ (µg/100ml)	Number of subjects	$PB^{127}I$ (µg/100ml)
1,000	51	3.7 ± 0.2* (0.9 - 7.4)+	51	28.4 ± 1.2 (14.5 - 54.0)
500	15	3.9 ± 0.5 (1.2 - 8.5)	18	31.7 ± 4.2 (12.0 - 87.0)
0	21	5.3 ± 0.3 (3.0 - 8.0)	15	5.0 ± 0.3 (2.3 - 7.7)

* *Standard error of the mean.*
\+ *Range.*

Effect of an Oral Dose of 2 or 4 mg of Potassium Iodide on Thyroidal Uptake of Radioiodine

Maximum uptakes of ^{131}I observed in the 16 patients during the control investigations were 78.9±2.9 per cent of the dose. After simultaneous oral administration of ^{131}I and 2 mg of KI (nine subjects) thyroid uptake dropped to 48.2±7.6 and after 4 mg of KI (seven subjects), to 31.6±6.3 per cent of the dose. The analysis of the uptake curves reveals, in six of the nine subjects treated with a dose of 4 mg and in four of the subjects treated with 2 mg, a characteristic biphasic aspect. This is illustrated in Figure 4; after a phase of rapid accumulation of the tracer during the first hours, a large fraction of the accumulated radioiodine left the gland and then reaccumulated once again in the following hours.

DISCUSSION

The socioeconomic, epidemiological, and metabolic conditions of endemic goiter in the north of Idjwi are comparable to those described in the Peninsula of Huon in New Guinea (5, 9, 17). In both cases a population lives in very primitive conditions in a closed economy. In the two regions severe iodine deficiency prevails and the occurrence of visible goiter (stages II and III) attains fairly comparable rates: 18 per cent in Huon (5) and 25 per cent on Idjwi (9).

The gravity of the problem raised by the Idjwi endemic is above all linked with the very high frequency of cretinism. The latter has been proved to be directly dependent on iodine deficiency (10). It was therefore essential to treat all the inhabitants of the island, including the goitrous subjects.

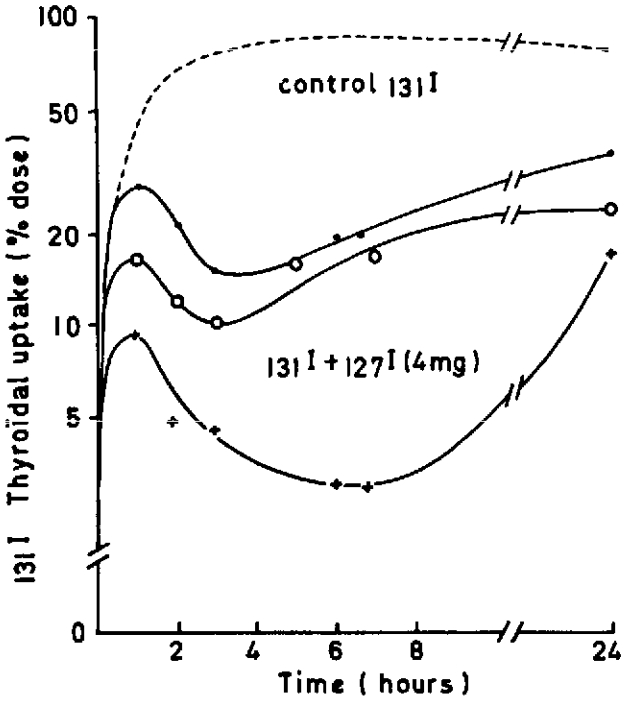

Figure 4. Retention curves of radioiodine in the thyroid gland after the administration of ^{131}I and 4 mg IK in three patients. Dotted line shows the corresponding curve obtained with carrier free ^{131}I in one of these patients.

Furthermore, in view of the evidence that an excessive supply of iodine in the diet has a clear goitrogenic effect, as shown by Suzuki et al. (26) in Japan, the doses proposed by the Australian authors were reduced perceptibly. The results obtained on Idjwi provide formal confirmation of the effectiveness of the method advocated by McCullagh (17). A year after the injection of iodized oil, urinary concentration still attained 50 µg per 100 ml, which approximately corresponds to a daily excretion of about 400 µg of ^{127}I. Moreover, the treatment produced a drastic reduction in the prevalence of goiter; one year after, it fell to a third of its initial level and a notable decline was observed in more than 80 per cent of the goiters, whatever their initial volume.

The harmlessness of the method is also confirmed. In the 911 iodine-treated patients, no cases were encountered of immediate intolerance nor were there any delayed complications of the jod-Basedow type or hypothyroidism linked with the treatment. Since these investigations were carried out in the field, minor anomalies may not have been noticed.

The question arises as to whether on the basis of present findings treatment with iodized oil can be applied unreservedly on a large scale. The crucial point seems to be the question of dosage, since the method entails the introduction into the organism over a period of months and years of iodine quantities that are much higher than normal physiological needs. It is thus that in a high percentage of patients the amount of iodide excreted in the urine per 24 hours is sufficient to induce a typical blockage of organification (6, 18, 28, 29).

The research carried out on Idjwi also shows a perceptible decline in the prevalence of goiter within a group of patients who did not receive iodized oil but who were living in the villages submitted to this treatment. The absence of similar modifications in the neighboring villages seems to indicate that these patients also benefited from the iodine treatment, which suggests a recirculation of the iodine within the community. At all events, the iodine excretion in the urine and the uptake of these patients remained unchanged. This implies that a much smaller quantity of iodine is capable of modifying the occurrence of goiter to a considerable degree.

It is concluded that the administration of iodized oil among populations where endemic goiter is linked with an iodine deficiency constitutes a prophylactic and therapeutic method of great value, which is so far devoid of unfavorable effects (3, 4, 5, 13, 17). However, in view of the huge overdosage of iodine that this method entails over prolonged periods of time, it seems that initially the method should be generalized only by using greatly reduced doses of iodized oil. On the basis of these conclusions, the prophylactic program that will be applied for the whole of Idjwi island comprises the administration of doses as low as one-fifth those described for the present study.

SUMMARY

Correction of the iodine deficiency prevailing in the island of Idjwi (Lake Kivu, Republic of Congo) was attempted by means of intramuscular injections of single doses (50 to 1,000 mg of iodine) of iodized oil (Lipiodol) among the inhabitants of two villages where the prevalence of goiter reaches 49 per cent. These doses represent reductions of between one-half and one-tenth by comparison with those proposed by McCullagh (17). The treatment was given to 90 per cent of the 1,379 inhabitants of the two villages; one in ten random-selected controls was not treated.

One year after the injection, the prevalence of goiter had fallen to the third of its initial level. In 80 per cent of the cases a marked improvement was noted, whatever the original type of goiter. No secondary reactions were observed.

A systematic study of iodine metabolism was carried out on 102 subjects, before and during the year following the administration of Lipiodol. After six months the urinary concentration of iodine exceeded 200 µg per 100 ml in patients treated with 1 g of ^{127}I, and after one year it dropped to 50 µg per 100 ml in all the subjects treated. Plasma $PB^{127}I$ attained 30 µg per 100 ml after six months. After one year uptake was reduced to 16 per cent, against a value of 79 per cent in the beginning.

The prevalence of goiter had diminished considerably in the tenth of the population that had not received the injection of iodized oil. No change was observed in the iodine metabolism of these patients.

The inhibiting action of a single dose of 2 or 4 mg of potassium iodide was studied in a group of 16 patients. The uptake curves of ^{131}I suggest a blockage of organification of the Wolff-Chaikoff type.

These results confirm the effectiveness and harmlessness of the method proposed by McCullagh. They suggest, however, that the doses used are probably still too high and that similar results could be obtained by injecting perceptibly smaller quantities of iodine.

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CHAPTER 11

ENDEMIC GOITER IN NEW GUINEA AND THE PROPHYLACTIC PROGRAM WITH IODINATED POPPYSEED OIL

I. H. Buttfield[1] and B. S. Hetzel[2]

The use of iodinated poppyseed oil in the prevention of endemic goiter was commenced by Clarke et al. (3) in 1960. They first showed that injection of 2 ml of iodinated poppyseed oil had demonstrable effects on thyroid funtion for as long as two years. Shortly thereafter, McCullagh (6) and later Hennessy (4) reported the results of controlled trials of iodinated oil in doses of 4 ml (= 1,600 mg of iodine) given intramuscularly to nongoitrous subjects. This dose was found to reduce the incidence of goiter developing in previously non-goitrous people.

Since only clinical studies had been performed, we undertook further observations in this endemic in order to investigate the precise etiology, to study the role of the goiter in adaptation to iodine deficiency, and to document more accurately with laboratory data the duration of action of depot iodine. This study was carried out in the Huon Peninsula region of the Australian-administered Territory of Papua and New Guinea, some 30 miles from the coastal town of Lae (1). Figure 1 shows the known goitrous areas of new Guinea and the area studied in dotted outline.

The native people of New Guinea are a culturally mixed group who are subsistence farmers with a high-carbohydrate, low-protein diet consisting largely of sweet potato, with very occasional meat, either fresh or, more recently, of the tinned variety. The people themselves are preliterate. They usually live in small village units, or in diffusely scattered family or clan units, often having no central point except an area selected by the European patrol officers who carry on government business at these rest houses once or twice a year.

It is of interest that the area studied lies in rugged mountains which rise to a peak of 13,000 feet. The people may walk to gardens several thousand feet above or below their villages in temperatures which range from very hot in the day to frigid at night. Anyone who develops a serious ailment would be unable to survive in the area, and this almost surely would occur should myxedema supervene.

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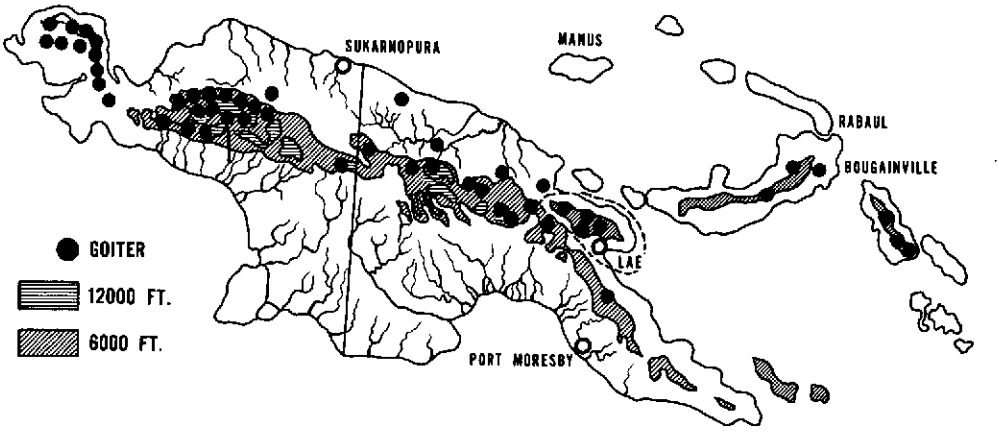

Figure 1. Known areas of endemic goiter in New Guinea.

In the initial study 60 villages at heights from 2,000 to 6,000 feet above sea level and with populations between 80 and 5,000 people were examined for goiter. The results were expressed as visible goiter rate, and log VGR (using the classification of Perez et al. (7)) was plotted against altitude (Figure 2).

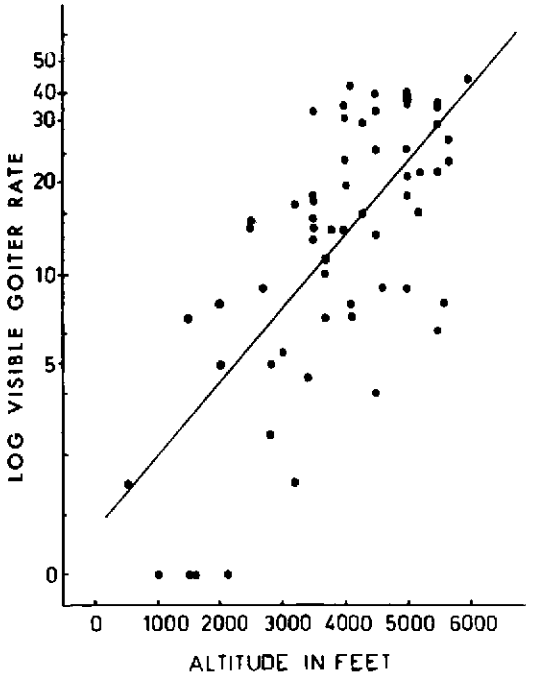

Figure 2. Relation between visible goiter rate and altitude, Huon Peninsula, New Guinea.

The regression line is highly significant and demonstrates statistically what has been well known clinically for many years, namely, that goiter rate increases with altitude.

Because of the great difficulty of contact with the people (which could only be made by foot), and because of the opportunity offered by people living in small units at various heights, it was desirable to perform studies within the villages rather than by separating patients from their environment. Blood and urine samples were collected and dispatched by runners to the nearest strip (up to 1-1/2 days' walk away), and then on to the laboratory in Adelaide, South Australia. Radioactive iodine studies were performed in the villages using an entirely portable, battery-operated scaler with a collimated scintillation probe.

Control specimens and ^{131}I uptake date were collected from medical orderlies in the coastal town of Lae (where the indigenous people were also somewhat iodine deficient) and from normal European persons in Adelaide. In all the following data a goiter is defined as thyroid, size 2 or 3.

The urinary iodine values in untreated natives are compared with the control groups in Lae and in Adelaide in Figure 3. The mean value of untreated indegenous inhabitants was 13 µg per 24 hours. The coastal control group had a mean of 49 µg per day, while the normal range for Adelaide was 70-140 µg per 24 hours.

Urinary iodine values expressed per gram of creatinine in various areas appear in Figure 4. The goitrous areas of New Guinea lie within group 5 of Follis, while the so-called normal coastal control group was in Group 4, and Adelaide in Group 1. Tasmania and Venezuela (Group 3) are added for comparison.

The ^{131}I uptake values in untreated natives appear in Figure 5. The 3 and 24-hour figures in the goitrous persons (shown in black) were both significantly higher than in the nongoitrous (in white). The mean 3-hours uptake was 64 per cent for goitrous and 42 per cent for nongoitrous persons. The 24 hours figures were: goitrous, 77 per cent, and nongoitrous, 67 per cent.

The PBI values are shown in Figure 6. The goitrous group had a mean PBI of 2.9 µg per 100 ml, while the mean of the nongoitrous was 4.9 µg, the former being subnormal, although none of these persons was clinically hypothyroid.

Perchlorate discharge tests were all normal. The serum TSH was also elevated. It is therefore suggested that severe iodine deficiency was present in this area and is the most likely major etiological factor for the goiter in this endemia.

Because of the significantly higher ^{131}I uptake and the significantly lower PBI in the goitrous groups it may be suggested that goiter is a failure of adaptation to severe iodine deficiency, since nongoitrous persons living in the same area had high ^{131}I uptakes (although lower than in goitrous persons) but normal PBI levels.

Further support for this idea is the fact that persons with large or size 3 goiters had a mean PBI of 1.9 µg per 100 ml, which is significantly

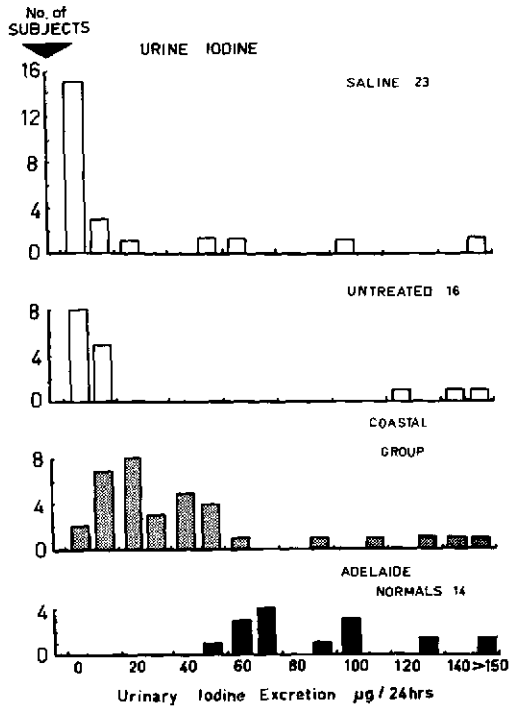

Figure 3. Urinary iodine data in untreated highland New Guineans with control groups.

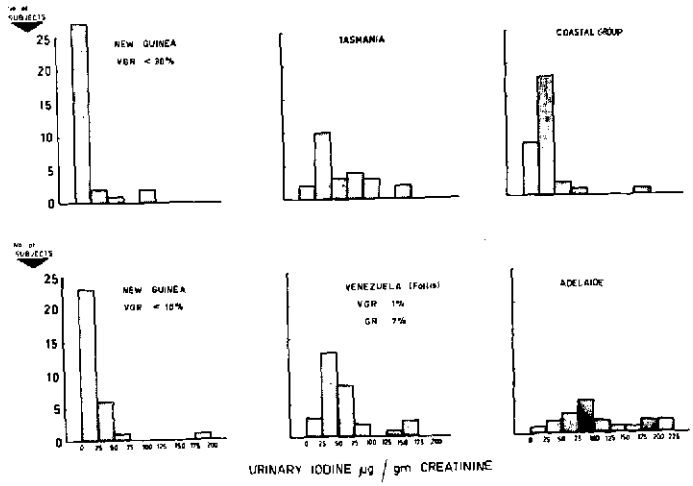

Figure 4. Urine iodine determinations in the various New Guinea groups compared with Australia and Venezuela.

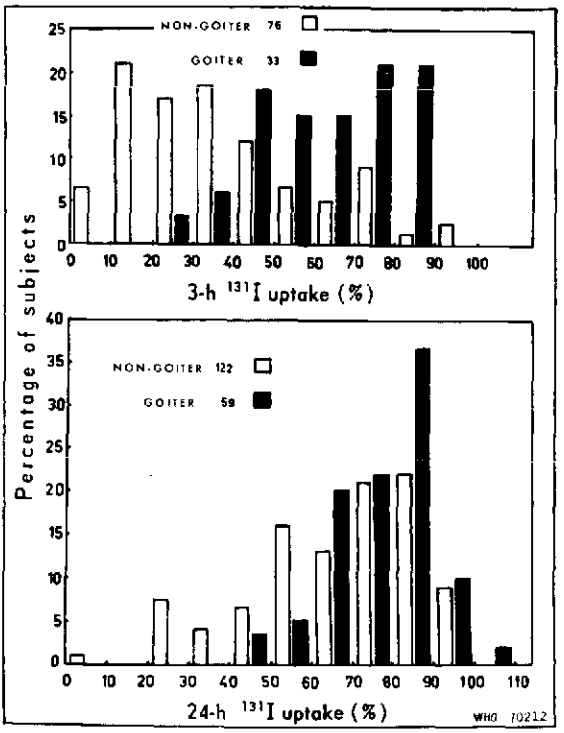

Figure 5. Three- and 24-hour ^{131}I uptake studies in goitrous and nongoitrous New Guineans.

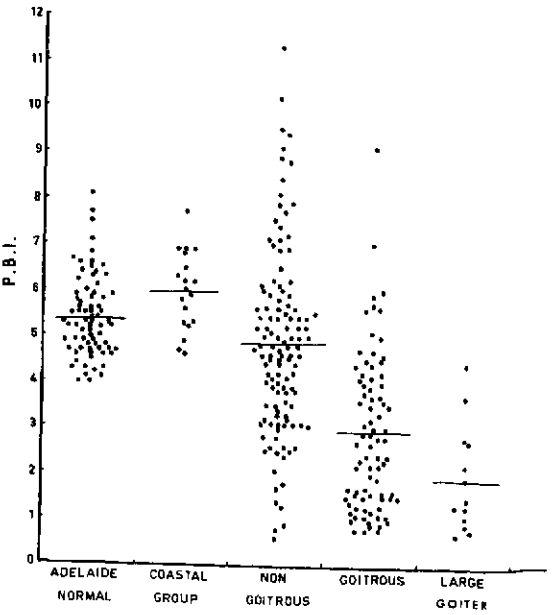

Figure 6. Serum PBI in goitrous and nongoitrous New Guineans.

lower than those of the nongoitrous (mean = 4.9 µg), and size 2 goitrous groups (mean = 2.9 µg).

The reason for the absence of clinical hypothyroidism with low PBI values is puzzling. The T_3 resin appeared to be a more reliable, if less sensitive guide to the clinical thyroid status of the patient.

Treatment with iodized poppyseed oil in doses of 4 ml was given and the groups were followed up at three months, 18 months, three, and four and a half years after injection. The results were compared with the untreated population. Administration of iodized oil in such preliterate and remote populations has many virtues when compared with other forms of prophylaxis.

Apart from the relative economy and ease of administration, there appeared to be few side effects. Thyrotoxicosis is very uncommon in New Guinea, and in 5,000 persons injected in the first trials no thyrotoxicosis was observed. No abcess was seen at the site of injection. X-ray studies showed that the iodized oil is rapidly distributed throughout the muscular septa and is absorbed within a few weeks (Figure 7).

The urinary excretion of iodide per 24 hours by untreated and treated groups after 18 months and three years is shown in Figure 8. At three months after injection the value per 24 hours was greater than 250 µg per 24 hours and at four and a half years it was 23 µg per 24 hours.

Figure 9 displays the 24-hours iodine uptake of all treated and untreated groups; the means were 70 per cent (untreated), 31 per cent (at 18 months), 37 per cent (at three years), and 44 per cent (at four and a half years), respectively.

The PBI values for the various groups are shown in Figure 10. The mean PBI for the group treated four and a half years earlier was 6.4 µg per 100 ml, which is above the normal mean of 5.3 µg. This probably indicates that some iodized oil is still bound to plasma protein and is acting as a contaminant and a reservoir.

The T_3 resin uptake was within the normal range in both treated and untreated groups, and the serum BEI and free thyroxine estimations in eight treated persons were within normal limits.

From the observations of urinary iodine and PBI which are raised above the levels in the untreated population, and from the ^{131}I uptakes which fell below normal, but were slowly rising, it may be concluded that an injection of 4 ml of iodized poppyseed oil will prevent iodine deficiency for up to four and a half years. The exact mechanism of binding is as yet unknown, but the oil probably forms a loose lipoprotein complex which first permits a rapid release of iodine over three to six months, and a slow release of the remainder over several years.

The values for serum TSH measured before and three months after treatment appear in Figure 11. There was a significant fall to normal (These estimations were performed by Dr. W. Odell of California, who used a radio-immunoassay technique.) These data correlated well with the clinical observation that 60 out of 61 persons with large goiters had a reduction in size within

Figure 7. X-ray studies of iodized poppyseed oil showing relatively rapid absorption (pictures three weeks apart).

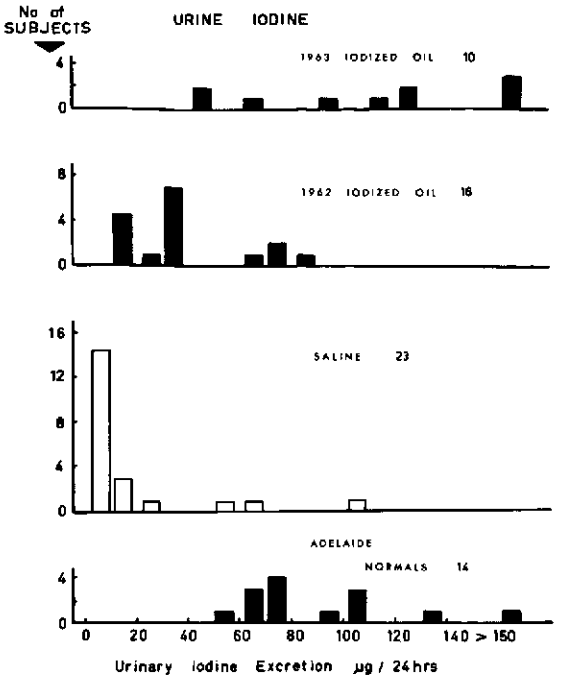

Figure 8. Urinary iodine excretion in untreated and treated subjects.

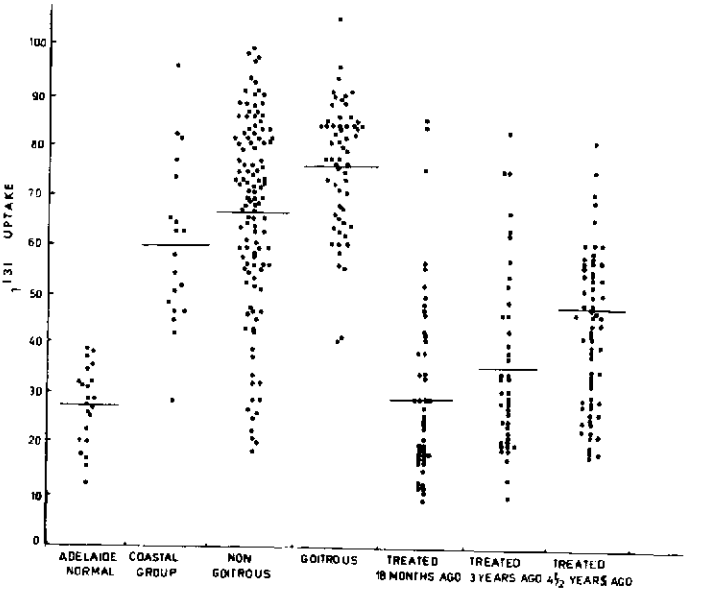

Figure 9. ^{131}I uptake figures at 24 hours in untreated and treated groups.

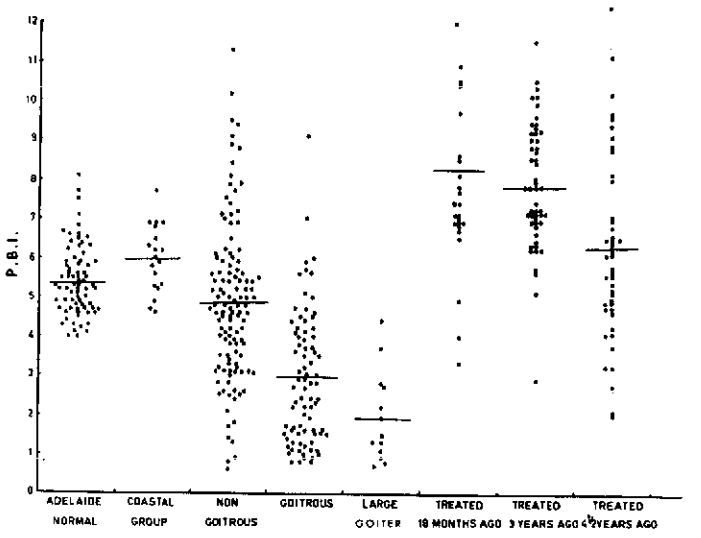

Figure 10. Serum PBI in treated and untreated groups in New Guinea and Australia.

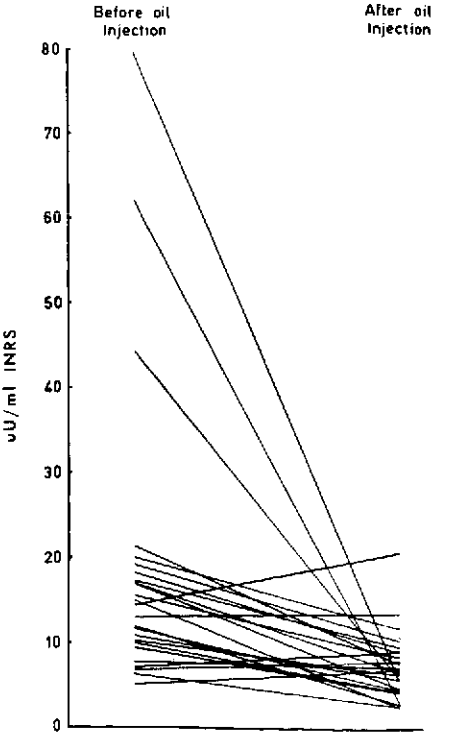

Figure 11. Serum TSH estimations before and three months after iodine treatment.

three months after injection. Of these, 30 changed from size 2 to size 1 or 0.

A photomicrograph of a thyroid removed at operation showed a densely hyperplastic gland which should be capable of returning rapidly to normal (Figure 12). Any remaining goiter would probably be scar tissue plus normal quantities of functioning thyroid gland.

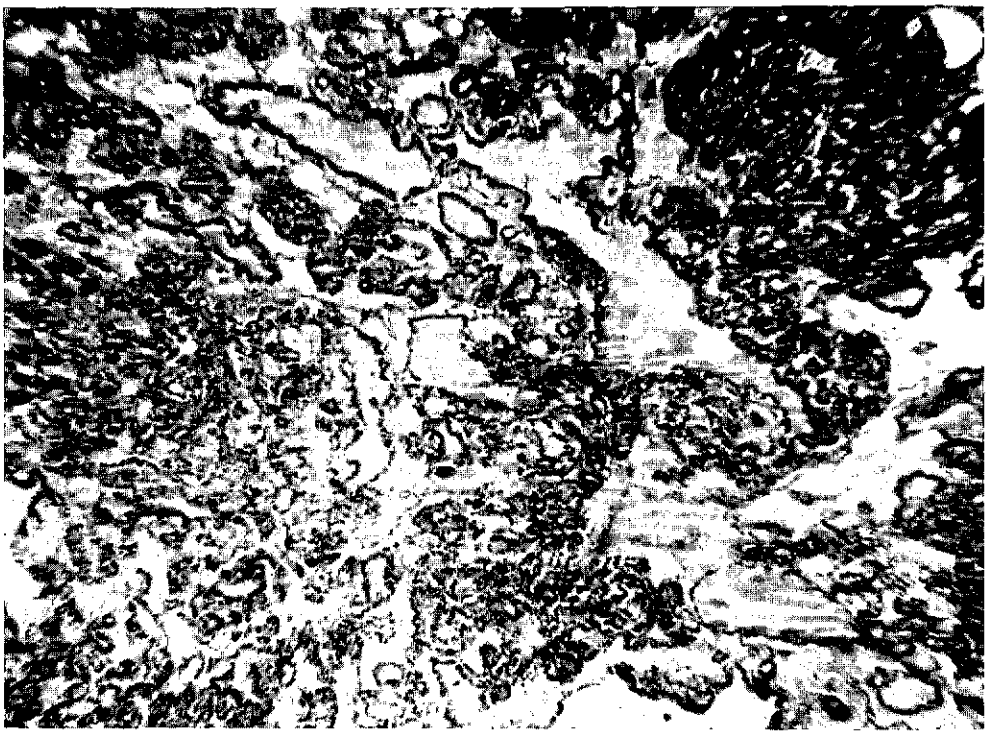

Figure 12. Histological section of thyroid from an untreated New Guinean.

Marine and Lenhardt (5) showed that dogs and other animals could be made iodine-deficient and would develop hyperplastic goiter. When physiologic doses of iodine were given a colloid goiter formed. When large doses of iodine were given the thyroid gland rapidly returned to normal size and histology. The same mechanism may well operate in man.

Endemic Cretinism

In the area which we first investigated, there were 254 persons labelled as endemic cretins in a population of 10,000 people (i.e., a 2.5 per cent

incidence). Affected persons were of all ages and all grades of severity, from deafmutism to severe dementia and neuromuscular incoordination. Like Choufoer et al. (2), we felt that all types of the disease should be included in the one syndrome.

Our criteria of diagnosis included:

(i) Obvious deafmutism.

(ii) Mental deficiency, as judged by the villagers themselves together with our observation of the interaction of the patient with his environment, e.g., could he care for himself, or could he run his own garden.

(iii) Obvious neuromuscular incoordination, as shown by walking with legs which did not straighten at the knee. This was always accompanied by mental deficiency.

Other hallmarks were characteristic facies with a depressed bridge of the nose, and often, but not always, dwarfism. No clinical evidence of hypothyroidism was found except one obvious classic cretin aged about 12 months, who died within a week or so of being discovered.

Of the 254 people, 177 (75 per cent) were deaf. On examination of the ear drum the normal light reflex was seen to be diminished or absent and the ear drum appeared to be thickened.

All the cretins, except mild deafmutes, were shorter than the rest of the population, and 25 per cent were classified as dwarfs. No reason for the dwarfism was obvious. A common finding in the endemic cretins was a delayed or partial puberty with atrophic genital organs.

The mild form of disease appeared to be deafmutism. The slightly more severe form than this included deafmutism with extensor plantar responses, and brisk reflexes. In all, 65 per cent of the cretins had brisk reflexes and 48 per cent had extensor plantar responses. Seventy persons, or about 30 per cent of these endemic cretins, were both deafmute, demented, and walked with flexed knees for which no anatomical reason could be found. In view of the other findings, this was assumed to be neurological. Several had severe dementia and were so incoordinated as to be unable to stand and balance unaided. About 25 per cent had this neuromuscular incoordination which was always associated with the flexed knee type of gait. Fifty per cent were demented enough so that it was obvious to the examiners. Unfortunately accurate testing of mental function was impossible because of the difficulties in communication. More recent evidence suggests that the incidence of mental deficiency is greater.

In the most gross form of this disease the patient was unable to stand or feed himself and was entirely dependent on his family, who clothed and fed him. It was of interest that a primitive community, which is required to work so hard to grow the food on which it must live, could take such good care of these "long-longs," as they are called, when life is cheap and food is difficult to obtain.

The relation of endemic cretinism to visible goiter rate is shown in Table 1. There was a high incidence of endemic cretinism in villages with a

Table 1. Endemic cretinism and visible goiter rate.

Goiter rate %	Population seen	No. of cretins	Cretins %
0 - 10	3,523	32	0.9
11 - 30	3,966	108	2.7
30 +	2,620	114	4.4

Use of X^2 test revealed a significant relation between visible goiter rate and incidence of cretinism ($P < 0.001$).

VGR of greater than 30 per cent. Forty-six per cent of the normal adult female population had goiters, while 70 per cent of the mothers of the cretins also had goiters. Size 3 or very large goiters were found in 4 per cent of the normal females in the population, while 16 per cent of the mothers of cretins had size 3 goiters (Table 2).

Table 2. Maternal history of goiter in endemic cretins.

Group	No.	Total no. in group	% age
History of goiter in mother	92	254	36
Visible goiter rate in mothers of endemic cretins	100	131	76
Size 3 goiter in mothers of endemic cretins	21	131	16
Visible goiter rate in adult females	1,131	2,707	42
Size 3 goiter in adult females	104	2,707	4

The serum PBI of various untreated normal members of the population had a mean of 4.1 µg per 100 ml, while that of the untreated endemic cretins had a mean of 2.6 µg per 100 ml. This difference is significant. The ^{131}I uptake at 24 hours in endemic cretins had a mean of 64 per cent and was significantly lower than the mean of 70 per cent for the untreated normals. Goitrous cretins had a mean PBI of 1.8 µg per 100 ml, while the nongoitrous cretins had a mean of 3.3 µg.

The PBI values of these cretins returned to normal after treatment with iodized oil, the mean (after injection) being 5.6 µg per 100 ml. The difference between the treated and untreated groups was significant. Perchlorate discharge studies in deafmutes were normal. This ruled out the Pendred syndrome. ECG, bone age, and hemoglobin in 20 of these persons were normal, except that one subject had delayed bone maturation, but no other sign of bony damage.

Thus it can be seen that in endemic cretins the thyroid gland is less efficient than in normal persons in the population, but these people did not show any clinical evidence of hypothyroidism.

Two possible theories can be offered from the above data.

(i) the goitrous mother (who is more likely to bear a cretinous child) takes up iodine more avidly and has a lower PBI, making both iodine and thyroid hormone less available to the fetus.

(ii) The endemic cretin who in later life has a significantly less efficient thyroid gland than the "normal" population may be unable to produce enough thyroid hormone in utero or in neonatal life for normal brain development.

(iii) Any combination of the above may occur.

It is of interest that in the area treated with iodized oil, 13 cretins were found who had been born since the iodized oil program began. Only one of these may have been born to an iodine-treated mother, and this is in doubt. A further controlled trial of iodized oil in the prevention of endemic cretinism is now under way in an area with a very high incidence of the disease.

SUMMARY

Severe iodine deficiency has been demonstrated as a major etiological factor in endemic goiter in eastern New Guinea.

Goiter, in an endemic goiter area, represents a failure of adaptation to iodine deficiency.

Data determining the useful life of a single injection of iodized oil indicate that it may be as long as four and a half years.

Clinical studies attempting to relate endemic cretinism to iodine deficiency in the mother have been presented, and a controlled study of iodine prophylaxis of endemic cretinism has begun.

ACKNOWLEDGMENTS

This investigation was carried out with the collaboration of the Department of Public Health, Territory of Papua and New Guinea. We wish to thank the Director, Dr. R.F.R. Scragg; the Regional Medical Officer, Dr. J.L. Jamieson; and members and staff of the Angau Memorial Hospital, Lae; Mr. and Mrs. G. Bergmann, Professor H.N. Robson, Dr. F.W. Clements, Dr. M.L. Wellby, and Dr. B.F. Good for their advice; Mr. A. Dewar, Mrs. M. Black, and Miss E. Mason for technical assistance.

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SECTION III

ENDEMIC GOITER IN ARGENTINA AND PARAGUAY

CHAPTER 12

CHARACTERISTICS OF ENDEMIC GOITER IN A MAPUCHE INDIAN TRIBE IN CHIQUILLIHUIN, EL MALLEO, PROVINCE OF NEUQUEN, ARGENTINE REPUBLIC

I. GENERAL ASPECTS AND SOME FUNCTIONAL AND GENETIC STUDIES

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Héctor Forcher, M.D.,[3] Angel A. Zaninovich, M.D.,[4]
Osvaldo M. Mutchinick, M.D.,[5] and Carlos L. Enriori, Chem.D.[6]

Endemic goiter occurs over much of Argentina, particularly in the Andean area (4, 19, 21, 23, 24, 28, 30). Available information is consistent with iodine deficiency as the main etiological factor for this endemia.

An earlier study carried out in an endemic zone of the southwestern Andean region demonstrated the existence of isolated Indian tribes with a high incidence of goiter (26). With the purpose of searching for other factors than iodine deficiency in the pathogenesis of endemic goiter, we have undertaken a study of the isolated Mapuche tribe, which inhabits the region of Chiquillihuin (or Chiuquillihuin) in the Andean Cordillera.

THE REGION AND ITS INHABITANTS

The Chiquillihuin area is located at 39°30' south latitude. The isolated Mapuche tribe, a subgroup of the Araucans, live on 4,000 acres of arid land in a valley at an average altitude of 1,500 meters above sea level. The area is located 43 kilometers northwest of Junín de Los Andes, the nearest town in the region, and is isolated for six months out of the year because of rain and heavy snowfalls in winter. The roads are very primitive.

At the present time the Mapuche Indians of Chiquillihuin comprise about 200 people, who are primarily dedicated to the breeding of goats. They are

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genetically related to the Pewenche Indians studied by Barzelatto et al. (2, 5) in Chile (Cf. also Chapters 20 and 21).

Male adults and children leave the area to work in neighboring ranches. The women are dedicated to housekeeping and farming of cabbage, turnips, and small spring onions. Children help in the care of animals. Their houses comprise one room only and are built with mud, wood, and straw. The kitchen, made of stones, is built outside the house in the open air. Sanitary services are unknown. Water is obtained from wells and small rivers--one of them is the Chiquillihuin--which collect into the main river of the area, the El Malleo.

We examined 146 inhabitants of Chiquillihuin, 74 males and 72 females, ranging from less than one year to 75 years of age. Studies were done in the public school and occasionally in private dwellings. This report refers only to those patients who were examined by the authors. References as to goiter in relatives who were not examined were not taken into consideration. In this paper we present the results obtained from the preliminary investigation conducted in the Chiquillihuin area.

METHODS

Careful attention was paid to kind and degree of relationship among members of the tribe, number of children, and breeding. Qualitative and quantitative aspects of the diet were established. At least two of the authors put special emphasis on the examination of goiter and its degree of enlargement.

Blood and urine samples were obtained for measurement of serum proteins, serum cholesterol, glucose, $PB^{127}I$, binding capacity of thyroxine-binding proteins (TBG and TBPA), serum free thyroxine, urinary iodine, and certain routine tests. Phenylthiourea (PTU) tasting ability, determination of iodine in water, and serum chromatographic studies were also performed (1, 7, 12, 13, 14, 16, 17, 27).

A preliminary field investigation of population genetics was developed. This involved: (a) pedigree charts of the group under study, mainly related to the presence of goiter and other disease; (b) ABO and Rh blood-group studies; (c) dermatoglyphic analysis from a sample of individuals; and (d) odontological studies in a group of 45 subjects. Similar studies were conducted on normal inhabitants of Buenos Aires.

Mean values and standard errors were obtained for thyroid status in every determination. A one-way analysis of variance was performed in each assay to test the significance of group differences, after which paired group comparisons were done with Duncan's multiple range test to maintain the level of significance ($P<0.05$) for the experiment (11, 31).

RESULTS

In this tribe of Mapuche Indians, 92.5 per cent of the inhabitants under examination had an enlarged thyroid gland. No differences in the percentage of goiter between males (93.2 per cent) and females (91.7 per cent) could be detected (Table 1). There was no difference in the prevalence of goiter

Table 1. Number of patients investigated in the tribe of Chiquillihuin.

Cases	Male	Female	Total	
Without goiter	5	6	11	(7.5%)
With goiter	69	66	135	(92.5%)
Total of cases	74 (50.7%)	72 (49.3%)	146	(100.0%)

Total population of Chiquillihuin: 196 Mapuches
Number of patients studied: 146 (74.5 %)

between persons under 17 years old (92.1 per cent) and those above that age (92.8 per cent) (Table 2).

Of the total population with goiter 43.7 per cent had the nodular type; 54.5 per cent of the females had nodular goiter, while 33.3 per cent of the males had nodules. There was a positive correlation between size and appearance of nodular goiter and age (Figure 1). Practically all females over 40 years of age who had goiter presented the nodular form. Of six normal adults without goiter (Table 2), four were Mapuches of another tribe from the Chilean side of the Andean Cordillera who had been living with the Indians under study for at least 30 years.

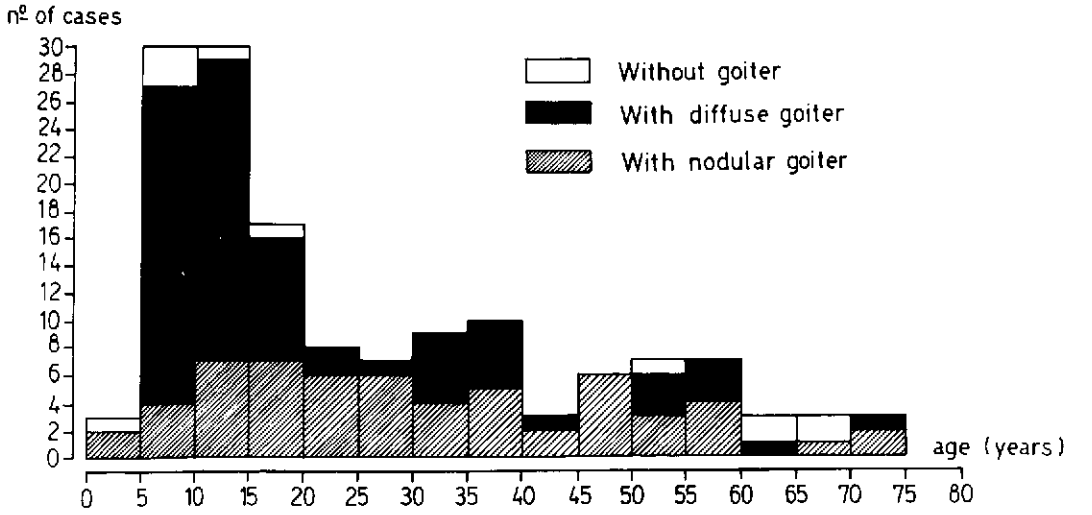

Figure 1. Distribution of goiter according to age.

The average height of adults was 1.69 meters (S.E.±0.04) for males, and 1.45 (±0.03) for females; the average weight was 66.8 kg ±1.7 and 58.9±1.6 respectively (Table 3). This ethnic group has a tendency to short height. This tendency to short stature compared to ideal height was observed in 90.5 per cent of cases under 17 years of age. Deficient weight in relation to

Table 2. Distribution of patients investigated according to age, tribe of Chiquillihuin.

Age	Cases	Male	Female	Total	%
Above 17 years	Without goiter	4	2	6	7.2
	With goiter	29	48	77	92.8
Below 17 years	Without goiter	1	4	5	7.9
	With goiter	40	18	58	92.1

ideal weight referred to ideal height appeared in 85.7 per cent of this population under 17 years old; if weight is referred to real height, only 49.3 per cent of the children were underweight.

The study of the characteristics of the diet disclosed an intake of about 1,800 calories per day in adults. Of these calories, 22 per cent were from protein sources, mostly of vegetable origin, 18 per cent from fats, and the rest from carbohydrates. The main component of the diet is the pine-nut, seed of the fruit of the "araucaria" (a conifer similar to a pine). Ingestion was estimated at 150 to 200 gr per day.

PTU test: The PTU-tasting ability showed 97.3 per cent positive results. No correlation could be made between type of goiter and results of the test.

Cholesterol: Serum cholesterol concentration had a mean of 109 mg per 100 ml: 108 mg (S.E.$\pm$6) in males and 110 mg (S.E.$\pm$7) in females. These measurements were made simultaneously with the controls in Buenos Aires, who had values between 170 and 240 mg per 100 ml (Table 3). Cholesterol concentrations showed a slow rise as the age of the patients of Chiquillihuin increased, but the corelation was poor (r = 0.30).

Table 3. Weight, height, and serum cholesterol values in patients of Chiquillihuin.

Measurements		No. of cases	Male	Female
Weight (kg)	$(\bar{x})$	31	66.8 $\pm$1.7	
	S.E.$\pm$	52		58.9 $\pm$1.6
Height (cm)	$(\bar{x})$	31	160 $\pm$4	
	S.E.$\pm$	52		145 $\pm$3
Serum Choresterol (μg/100 ml)	$(\bar{x})$	17	108 $\pm$6	
	S.E.$\pm$	25		110 $\pm$7

Serum protein and blood glucose: The average serum protein concentration was 8.30 g per 100 ml (Table 4), with an increase in gamma globulin (1.83 g per 100 ml, S.E.±0.08) and an albumin-globulin ratio of 1.17. In 31 controls in Buenos Aires the mean serum protein value was 7.04 g per 100 ml (±0.35) with an albumin-globulin ratio of 1.42. Blood sugar concentration was normal in all cases under study except in one which showed a value of 150 mg per 100 ml (normal values 60 to 100 g). Later studies established a diagnosis of diabetes in this patient.

Table 4. Serum investigation in patients of Chiquillihuin.

Study	No. of cases	$\bar{x}$	S.E. ±
Urinary iodine	25	20 µg/d	1.1
PBI^{127}	30	4.5 µg/100 ml	0.5
Serum total proteins		8.30 g/100 ml	0.16
Albumin		4.46 "	0.11
Globulin		3.84 "	0.40
$\alpha 1$		0.45 "	0.02
$\alpha 2$	18	0.73 "	0.04
β		0.80 "	0.04
γ		1.83 "	0.08
albumin / globulin Ratio		1.17 "	0.41
Resin T-3 uptake (Thyro-Binding-Index)	32	104	1.7

$PB^{127}I$ and urinary iodine: $PB^{127}I$ studies were carried out in 30 adult patients, six normal and 24 with goiter. Of the latter ten were diffuse and 14 nodular. Two determinations of each subject were done. The average value was 4.5 µg per 100 ml (S.E.±0.5). This value is lower than was found in 542 controls of Buenos Aires (mean value 5.6 µg per 100 ml, S.E.±0.2), but is within the normal range (3.7-8.3). Urinary iodine was measured in 25 patients with an average of three determinations on three different days. The mean value was 20 µg per day (S.E.±1.1). Normal subjects in Buenos Aires had an excretion of iodine of 88 µg per day (range 55-170) (Table 4).

Serum thyroxine-binding capacity and free serum thyroxine: The labelled T_3 uptake by granular resin in equilibrium with serum samples (Thyro-Binding-Index) showed normal values with an average index of 104 (S.E.±1.7) (Table 4). Our 35 controls in Buenos Aires had a mean index value of 102±0.9 (range 94-109).

TBG thyroxine-binding capacity in 20 subjects of Chiquillihuin was 33.7 µg per 100 ml (S.E.±1.51), which was slightly higher than our controls in Buenos Aires. TBPA binding capacity was 128.6 µg per 100 ml (±9.80), which is in the normal range (16).

Serum free thyroxine was within normal range for the method (17). Since the $PB^{127}I$ values were also normal, the total serum free T_4 concentration was

in agreement with that observed in our control subjects. The average value for 20 subjects in the Chiquillihuin area was 0.048 per cent (S.E.±0.003).

Chromatographic studies: The methods employed included paper and column (Dowex 1x8, 20-50 mesh resin) chromatography. No abnormal iodinated compounds were detected in serum. Only in one instance, a nodular goiter of regular size, column chromatography showed a small amount of MIT-DIT.

Iodine content of water: Determination of the iodine content of water from wells and small rivers of the Chiquillihuin revealed that the area has iodine deficiency (Table 5).

Table 5. Iodine content of water.

Source		µg per liter
El Malleo River		0.00
Chiquillihuin little river		0.25
Unnamed little rivers	A	0.00
	B	0.00
	C	0.00
Wells	A	0.09
	B	0.11

Population genetics: Ninety-seven of the 146 individuals examined and their relatives are represented in a pedigree in Figure 2. The solid symbols indicate goiter. The symbols with a central dot are subjects without goiter, while the empty symbols are subjects who were not examined. The figure suggests that this is a closed population with minimal gene inflow from outside the group, and with a high degree of inbreeding. The calculations to obtain the inbreeding-coefficient need further and more detailed studies.

The ABO and Rh phenotype distribution of 127 individuals in this population appear in Table 6. Similar data were obtained from a Mapuche group studied by Nagel et al. (22) in Chile and from a "Chippeway" group of American Indians living in Michigan by Matson (20). Our preliminary data suggest that the Mapuche group here presented is in the process of mixing. The data in regard to gene-B point to the presence of some inflow of foreign genes into the population under study, perhaps of European origin.

Digito-palmar prints were obtained from 60 individuals. Ten were discarded because they were faulty. The dermatoglyphic patterns were analyzed, codified, and transferred to punch-cards of the standard IBM 80 column type. Table 7 shows the observed values of figure-type frequencies in 382 analyzed fingers and the ridge-counts from 100 fingers. We wish to stress the low ridge-counts, mainly because of a high frequency of arches, as compared to a general Caucasian population.

Odontological studies showed 100 per cent of double shovel-shaped incisor teeth in the upper jaw and of semishovel-shaped incisor teeth in the lower jaw, according to Hrdlicka's classification (15). This finding suggests

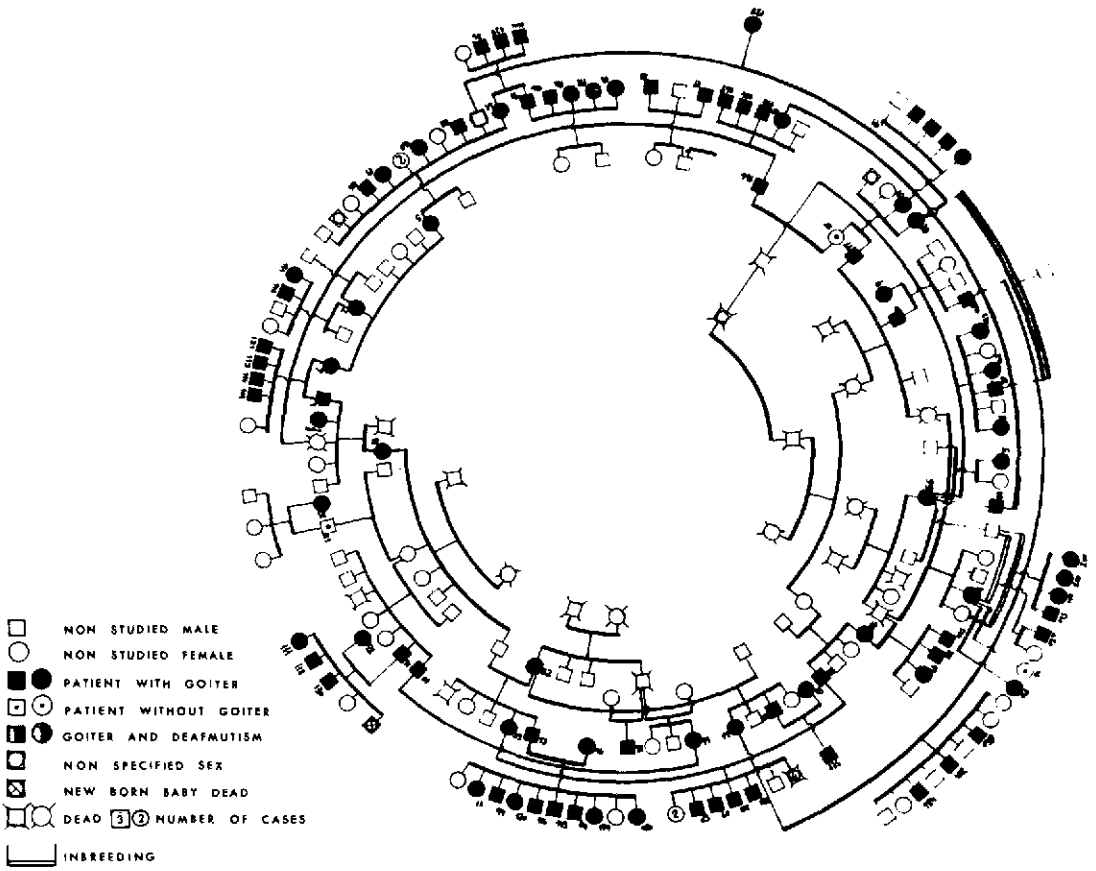

Figure 2. Pedigree of 97 Mapuche Indians of Chiquillihuin.

Table 6. Blood groups in Chiquillihuin.

Population	Number of cases	Blood groups				
		0	A	B	AB	Rh
Mapuches Chiquillihuin tribe	127	99 (78.0%)	17 (13.4%)	8 (6.3%)	3 (2.4%)	(100%)
Mapuches Nagel et al. Chile, 1962	450	358 (79.6%)	50 (11.1%)	42 (9.3%)	0 0	
Chippeways Matson, USA	128	96 (75.0%)	31 (24.2%)	1 (0.8%)	0 0	

Table 7. Observed values of figure-type frequencies in 382 analyzed fingers and ridge-counts from 100 fingers.

Studies	Values
Fingerprints studies	500
Fingerprints utilized	382
Archs	38 (9.9%)
Internal loop	224 (58.9%)
External loop	9 (2.3%)
Vertical	111 (29.0%)
Ridge-counts	100 fingers
Mean finger value in males	95.0 ridges
Mean finger value in females	90.2 ridges

that these subjects have the dental "mongolic complex" described in North and South American population and in eastern Asiatic population (6), and that they may have a similar ethnical origin.

DISCUSSION AND CONCLUSIONS

The Chiquillihuin area of the Argentine Andes has a severe goiter endemia which affects 92.5 per cent of the inhabitants of the Mapuche tribe of Indians dwelling there. The incidence of goiter is equal among children and the adult population. There is a predominance of nodular forms in females as their age increases. Serum protein-bound iodine concentration values were normal, but lower than our control. There are references to high, normal, or low $PB^{127}I$ values in the literature on endemic areas (2, 8, 10, 18, 25, 28, 30). Serum thyroxine-binding capacity and free serum thyroxine values were within the normal range. We were not able to detect abnormal iodinated serum compounds with the chromatographic methods employed.

If it is assumed that these subjects are in iodine equilibrium with their environment, the daily excretion of iodine will serve as an index of iodine ingestion. A close similarity exists between iodine content in water and food. Iodine content of water is extremely low throughout the area. Thus there is good reason to believe that there is dietary deficiency of iodine, particularly if we compare these figures with those reported in the literature from other iodine deficient areas (2, 10, 18, 25).

Extremely low serum cholesterol values and high serum globulin concentrations have no ready explanation, but are probably correlated with the characteristics of the diet: high-protein and low-fat content and vitamin deficiency, and perhaps genetic factors. Our studies suggest that this Mapuche tribe is probably a closed population with minimal gene inflow from outside the group and with a high degree of inbreeding.

SUMMARY

The Mapuche Indians living in an isolated reservation in the Chiquillihuin area of the Argentine Andes are a group of about 200 people who are occupied primarily in the breeding of goats. They inhabit 4,000 acres of arid land at a mean altitude of 1,500 meters at 39°30' south latitude. This area is isolated six months of the year and covered by heavy snowfalls in winter.

Seventy-five per cent of the population was examined, and 92.5 per cent of these had goiter. The incidence of goiter is equal among children and adults; nodular goiter predominates in females.

The mean diet of the adult population furnishes 1,800 calories per day, 22 per cent from protein sources, 18 per cent from fats, and the rest from carbohydrates. Serum gamma globulin values were elevated and the albumin-globulin ratio was 1.17. Serum cholesterol values were low and $PB^{127}I$ values were within the normal range but lower than controls.

PTU tasting was 97.3 per cent positive. Binding capacity for thyroxine (TBG and TBPA), as well as the serum free thyroxine were normal. No abnormal iodinated serum compounds were detected. Iodine content of water was extremely low throughout the area. The population contained 77 per cent with blood group 0, and the whole population was Rh positive. Genetic studies suggest that this Mapuche tribe is probably a closed population with a high degree of inbreeding.

From these data, the main causal factor of the endemia is a severe degree of iodine deficiency.

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CHAPTER 13

CHARACTERISTICS OF ENDEMIC GOITER IN A MAPUCHE INDIAN TRIBE IN CHIQUILLIHUIN, EL MALLEO, PROVINCE OF NEUQUEN, ARGENTINE REPUBLIC

II. IODINE KINETIC STUDIES

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In the endemic goiter area of Chuquillihuin, in the Andean province of Neuquen, Argentine Republic (5), 92.5 per cent of the Mapuche Indians are affected. Iodine deficiency appears to be the main causal factor. This Indian community is genetically isolated. The high incidence of goiter is equal among children and adults. The peculiar social and economic factors and the incidence of consanguinity prompted us to study iodine metabolism in the hope of finding additional information concerning this endemia. Results of the preliminary studies are presented here.

MATERIALS AND METHODS

Measurement of ^{131}I uptake by the thyroid were made in 72 members of the Chiquillihuin Tribe, between 14 to 62 years of age (Table 1). Thyroid uptakes were carried out at 1, 24, 48, and 72 hours. $PB^{131}I$ determinations were performed at 24 hours by methods described elsewhere (7, 8). Mean values and standard errors were obtained for every determination. A one-way analysis of variance was performed in each assay to test the significance of group differences, and paired group comparisons were done with Duncan's multiple range test to maintain the level of significance ($P < 0.05$) for the experiment (10, 23).

Iodine kinetic studies were performed in 18 subjects: three without goiter, six with diffuse goiter, and nine with nodular goiter. The methods

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for $PB^{127}I$ and urinary iodine have been described elsewhere (5). The methods of the genetic studies and the analysis of kinetic data were performed according to the mathematical approach of DeGroot (4). Blood and urine samples were collected until the 15th or 16th day. At the end of this period 30 I.U. of TSH was injected intramuscularly and samples of blood were taken 24 hours later to determine $PB^{131}I$ and $PB^{127}I$ (15). The symbols used to identify the different parameters were those utilized by DeGroot. We have added Q_G^4 to indicate the calculation of the thyroid exchangeable ^{127}I by the method of Nodine et al. (15). Similar kinetic studies were performed in five normal subjects of Buenos Aires.

RESULTS

Thyroid uptakes of ^{131}I and $PB^{131}I$ are summarized in Table 1. We have observed the following:

Table 1. ^{131}I thyroid uptake, Chiquillihuin Mapuche Indian Tribe, and $PB^{131}I$ (at 24 hours).

		Number of cases		^{131}I thyroid uptake (%) 1 hr.	24 hrs.	38 hrs.	72 hrs.	24 hrs. $PB^{131}I$ (%/l.pl.)
Without goiter		7	mean	18.4	65.4	65.6	65.3	0.11 (4 cases)
			S.E.	2.7	1.6	2.2	1.9	0.03
With goiter	Type 1	10	mean	20.1	61.4	61.9	62.0	0.10 (7 cases)
			S.E.	3.3	5.2	4.4	4.1	0.04
	Type 2	36	mean	22.0	65.9	66.2	65.7	0.29 (8 cases)
			S.E.	3.1	2.3	2.5	2.6	0.09
	Type 3	19	mean	17.1	59.1	60.1	59.3	0.72 (10 cases)
			S.E.	4.1	3.6	3.4	3.4	0.11
Total		72	mean	20.1	64.4	64.8	63.5	0.37

Goiters were classified according to WHO classification.

Thyroid uptakes were higher than those observed in Buenos Aires (the mean 24-hour uptake of 143 normal subjects of Buenos Aires was 45.7 per cent), and were much higher than those observed in Boston by DeGroot. The difference among the subjects of Chiquillihuin without goiter and those with different kinds of goiter was not significant.

$PB^{131}I$ values were within the normal range and were similar to normal values of our laboratory, in nongoitrous subjects and in those with goiter type 1, and the values increased in patients with goiter type 2 and 3. From the practical point of view all patients of the latter groups had nodular goiters.

Thyroidal clearances of ^{131}I (CG) in the 18 cases in whom the test was performed were high. The mean value, 102.7 ml per min (S.E.±14.1) was significantly different from that for the controls in Buenos Aires (35.2 ml per min) and from the values given by DeGroot. The mean value for plasma inorganic iodine fraction (PII) was 0.41 up per 1 (S.E.±0.06), whereas for our controls in Buenos Aires it was 1.68 µg per 1.

The mean Absolute Thyroid Uptake (AIU) of the Chiquillihuin Indians was 54.4 µg per day (S.E.±6.7) and was similar to the controls, who had a mean value 56 µg per day, and to the normal values presented by DeGroot of 52 µg per day. If in the calculation of AIU one uses Riggs's formula, the mean value obtained in the Chiquillihuin area was 52.8 µg per day, and in this case there was also no difference with our values in Buenos Aires or with those of DeGroot in Boston.

Of the 18 kinetic studies, only 12 could continue until the $PB^{131}I$ appeared to have reached a steady level. The other six were interrupted for various reasons.

The results of physical examination of these 12 subjects are summarized in Table 2. Only one normal subject without goiter remains in this group.

Table 2. Physical characteristics of subjects under kinetic studies.

Case	Age (years)	Sex	Wt. (kg)	Diagnosis and clinical data	Thyroid wt. (est. g)
1	65	F	59	Normal	20
2	35	F	65	Diffuse goiter Grade 1	50
3	53	M	67	Diffuse goiter Grade 2	90
4	53	M	61	Diffuse goiter Grade 2	100
5	37	M	75	Mn goiter Grade 2	100
6	19	F	47	Mn goiter Grade 2	90
7	41	F	77	Mn goiter Grade 3	150-200
8	38	F	48	Mn goiter Grade 3	200
9	52	F	72	Mn goiter Grade 3	180
10	64	M	72	Mn goiter Grade 3	250
11	50	F	78	Mn goiter Grade 3	200
12	45	F	55	Mn goiter Grade 3	150

The individual values and the mean value of the theoretical maximum thyroid uptake (U max) appear in Table 3, which also shows the iodide space (V_{Io}^2) in liters and in per cent of body weight, the thyroid ^{131}I clearance (CG) per min, the kidney ^{131}I clearance (CK) in ml per min, and the ratio of thyroid clearance to clearance by the thyroid and kidney combined. These results do not differ from the values found in the group of 18 patients referred to earlier

The $PB^{127}I$ urinary iodine, PII, $PB^{131}I$, and the thyroid ^{131}I uptake values are presented in Table 4. Serum and urinary specific activity and their

Table 3. Kinetic studies. Theoretical maximum thyroid uptake (U max); iodide space ($V^*_{I_O}$) in liters and in per cent of body weight; thyroid (CG) and kidney (CK) iodine clearance values.*

Case	U* max. Fr. dose	$V^*_{I_O}$	Iodide space[2] % body wt.	C G (ml/min)	C K (ml/min)	C G / CG+CK
1	0.86	19.5	33.0	151.0	23.1	0.87
2	0.85					
2	0.85	22.6	34.8	105.4	21.9	0.83
3	0.53	11.3	16.9	31.1	30.0	0.51
4	0.70	33.6	55.1	209.7	45.5	0.82
5	0.55	25.9	34.5	67.6	22.5	0.75
6	0.87	20.1	42.8	117.2	36.9	0.76
7	0.63	26.3	34.2	59.2	23.9	0.71
8	0.51	14.6	30.4	72.2	27.8	0.72
9	0.77	24.2	33.6	212.5	48.0	0.81
10	0.56	19.7	27.4	86.4	35.3	0.71
11	0.71	23.3	29.9	77.0	41.2	0.65
12	0.65	19.3	35.1	52.6	21.0	0.71
Mean	0.68	21.7	34.0	103.5	31.4	0.74
S.E.±	0.04	1.7	2.9	17.1	2.8	0.01

Table 4. Kinetic studies. $PB^{127}I$, plasma inorganic iodide fraction (PII), urinary iodine excretion, higher ^{131}I thyroid uptake at any time of the study, and $PB^{131}I$ 48-hour values.

Case	$PB^{127}I$ (μg/l)	PII (μg/l)	Urinary iodine (μg/d)	Higher ^{131}I thyr. upt. % dose	$PB^{131}I$ (48 hrs.) (% dose/l)
1	32	0.24	8	82	0.26
2	54	0.54	17	83	0.15
3	51	0.74	32	51	0.07
4	40	0.23	15	69	0.22
5	45	0.45	15	54	0.73
6	40	0.22	12	84	0.18
7	50	0.81	28	58	0.76
8	39	0.52	21	47	2.18
9	47	0.30	21	72	0.84
10	70	0.55	28	46	2.61
11	50	0.39	23	66	0.54
12	39	0.40	12	62	0.35
Mean	46.4	0.45	19	65	0.74
S.E.±	2.8	0.45	2.1	4.0	0.24

ratio appear in Table 5. $PB^{127}I$ and urine iodine values are in agreement with those found in the general population of Chiquillihuin in a previous study (5). $PB^{131}I$ values could be divided into two groups. The first, including cases 2, 3, 4, and 6, had values of $PB^{131}I$ at 48 hours which were in the normal range for our laboratory (0.02 - 0.22 per cent per liter), and 24-hour $PB^{131}I$ values under 0.20 per cent per liter. This probably indicates a slow turn-over of ^{131}I.

Table 5. Kinetic studies. Serum and urine specific activity.

Case	S/A serum	S/A urine	S/A $\frac{\text{urine}}{\text{serum}}$
1	0.043	0.040	0.94
2	0.009	0.015	1.66
3	0.005	0.005	1.00
4	0.016	0.010	0.63
5	0.026	0.033	1.27
6	0.016	0.018	1.05
7	0.026	0.023	0.89
8	0.036	0.033	0.93
9	0.029	0.025	0.86
10	0.047	0.039	0.83
11	0.018	0.033	1.83
12	0.015	0.033	2.20

Exchangeable thyroid ^{127}I was calculated by three methods, Q_G, Q_G^3, and Q_G^4, and three results were obtained in every case. Q_G^4, as was indicated by others (1, 4) showed the lowest values, and Q_G and Q_G^3 had a good correlation (Table 6). Cases 2, 3, 4, and 6 presented higher values for the thyroid exchangeable pool, corresponding to lower $PB^{131}I$ values. The other cases had lower pools in agreement with higher $PB^{131}I$ values. This indicated faster turnover. The total mean value of Q_G was 4,197 µg (S.E.$\pm$835). The four cases with slow turnover had a mean pool value of 7,383 µg, while the eight cases with fast turnover had 2,604 µg. These values in every case are lower than our findings in Buenos Aires (11,250 µg) and are lower than the values referred to by DeGroot (10,690 µg).

Iodide pool (Q_I) was reduced in all subjects in Chiquillihuin (Table 6). The extrathyroidal organic iodine pool (Q_B) was normal.

The results of the calculation of absolute thyroid ^{127}I uptake (AIU) and of the thyroid hormone secretion rate (H) are summarized in Table 7. The values obtained are in agreement with the data of others for endemic or non-endemic areas (1, 2, 4, 14). In these cases H appears to be higher than AIU. The ratio of observed urinary ^{131}I observed to predicted values had a mean of 2.1, and was similar to the findings in Buenos Aires (Table 7).

Table 6. Kinetic studies. Thyroid exchangeable ^{127}I and inorganic iodine pool.

Case	Exchangeable ^{127}I (μg)					Q_I (μg)
	Total	Q_B	Q_G	Q_G^3	Q_G^4	
1	2,135	264	1,871	1,920	1,240	4.7
2	10,190	490	9,700	8,530	7,820	12.2
3	10,200	478	9,722	9,790	5,810	8.4
4	5,715	342	5,373	4,130	2,370	7.7
5	3,000	473	2,527	1,840	1,650	11.7
6	5,000	263	4,737	4,850	3,230	4.4
7	2,780	540	2,240	1,980	1,405	21.3
8	2,230	262	1,968	1,170	2,910	7.6
9	3,030	474	2,556	2,437	1,297	7.3
10	1,800	705	1,095	700	760	10.8
11	4,545	545	4,000	3,445	3,870	9.1
12	4,875	300	4,575	3,770	2,220	5.6
MEAN	4,625	428	4,197	3,713	2,881	9.2
S.E.±	719	41	835	819	604	1.3

Table 7. Kinetic studies. Thyroid absolute uptake and thyroid hormone secretion values.

Case	Thyroid absolute ^{127}I uptake (AIU) (μg/d)			Thyroid hormone ^{127}I sec. (H) (μg/d)				Urinary ^{131}I Ratio $\frac{\text{observ.}}{\text{predict.}}$
	(1)	(2)	(3)	(2)	(3)	(4)	(6)	
1	49	82	53	136	79	62	70	2.1
2	96	172	82	352	181	108	113	3.6
3	36	49	33	244	109	97	102	2.3
4	49	107	70	93	32	57	79	1.0
5	18	97	45	75	45	103	130	1.1
6	80	66	38	305	107	99	104	4.2
7	48	134	69	117	67	116	143	1.3
8	22	35	55	51	40	67	76	1.5
9	70	91	92	96	105	125	149	2.6
10	41	63	68	79	53	90	128	0.9
11	56	76	43	166	146	97	110	2.7
12	22	41	30	142	74	65	83	2.5
MEAN	49	84	57	154	86	81	107	2.1
S.E.±	7	11	6	28	13	13	8	0.3

DISCUSSION AND CONCLUSIONS

Previous studies in the Chiquillihuin area indicated that the principal cause of the locally prevalent endemic goiter is probably iodine deficiency. The content of water and the low urinary iodine excretion are in accord with this conclusion. It was found in the present study that ^{131}I uptake by the thyroid is high, and similar to the findings of Stanbury et al. (21) in Mendoza Province not far from Neuquen, by us in Caacupé, Paraguay Republic (6), by Perinetti (17) in San Carlos de Bariloche, 300 kilometers from Chiquillihuin, by Oñativia (16) in Salta, and by Rozados et al. (20) in Misiones. The findings are similar to those of De Visscher et al (9) in Uele.

The PII values are consistent with iodine deficiency. High ^{131}I thyroidal clearance rates agree with the findings of Stanbury et al. in Mendoza. They reported that in Mendoza the mechanism for adaptation of the thyroid to iodine deficiency was mainly an increase of extraction of iodide from the serum. Our results are in agreement with this postulation.

Our values for PII are lower than those reported by Beckers et al. (1) from the area of Pedregoso (Chile), but are similar to values for nodular goiters of Pedregoso. The small number of cases, which included three of the six adults without goiter found in Chiquillihuin, did not permit a comparison of results in normal subjects and subjects with diffuse or nodular goiter.

The $PB^{131}I$ results showed two different patterns, as also reported by Ermans et al. (11) and by Weinstein et al. (23) in endemic areas. One pattern included 48-hour values in the normal range and reflected a slow iodine turnover; the other with high values indicated a faster iodine turnover. The existence of different types of turnover has been associated inversely to thyroid iodine content (3). Our results are in agreement with this finding.

The mean value of the iodine thyroid pool was lower than the mean value obtained in controls of Buenos Aires and the values observed by others (1, 4, 13, 18). These were slower and statistically significantly different in subjects with fast turnover and were higher in the four patients with slow turnover. Roche et al. (19) have postulated a faster iodine turnover in iodine-deficient goiter as a mechanism of adaptation, but results presented by Ermans et al. (11) from the Uele endemic area are not in accord with this point of view. Ermans observed that the mean iodine content of the glands in the Uele goiter area was in the range indicated by Berson and Yalow (2) for normal and hyperthyroid patients.

Our results indicate a lower content of iodine in glands of Mapuche Indians of the area of Chiquillihuin, even in those with a slow turnover (mean value for four cases 7,383 ug). Slower values were obtained in the assay of thyroid exchangeable iodine using Nodine's method (15). Following Beckers and others (1, 23) we utilize 30 IU of TSH for Nodine's calculation in order to mobilize all the intrathyroidal iodine that could be reached by TSH.

None of these methods estimate correctly the total thyroid iodine. Generally they underestimate the size of the iodine thyroidal pool (3, 12), especially the method of Nodine. This is apparent if one compares the results obtained with the chemical determination of iodine in thyroid tissue or by means of *in vivo* activation analysis (3, 12). The measurements do permit one to

compare thyroid function in different situations. From this point of view the patients of Mapuche have a diminished iodine pool, when compared with normal controls of Buenos Aires or the values indicated for an endemic area by Beckers et al. (1), Ermans et al. (12), and Weinstein et al. (23).

The mean value for the ratio between urine and serum specific activities at equilibrium was 1.17 (S.E.±0.14). This is higher than the mean value obtained in our controls of Buenos Aires (0.9) and higher than the values obtained by DeGroot. We found that the mean value for the ratio of urinary radioactivity: observed/predicted was 2.1 (S.E.±0.3). This is in the range found by DeGroot and in our normal controls.

The observation that the secretion rate from the thyroid is higher than absolute uptake, and that a ratio between urinary and serun specific activity and between observed and predicted urinary radioactivity are greater than unity, suggests a leakage of iodine, either as iodide or as an iodine compound rapidly degraded to iodide. This iodine spillage contributes to iodine deficiency and other factors in the pathogenesis of endemic goiter in this area.

SUMMARY

Iodine kinetic studies were performed in a tribe of Mapuche Indians in the region of Chiquillihuin, Province of Neuquen, Argentine Republic. This is an isolated community.

The high thyroid uptake (64.4 per cent at 24 hours), increased thyroid iodine plasma clearance, low iodine in the urine, low plasma inorganic iodide fraction, and negligible content of iodine in the water are indicative of iodine deficiency in this region.

Kinetic studies indicated a mean daily absolute iodine uptake which varied between 49 and 84 µg, depending on the method employed, and a mean thyroid hormone secretion between 81 and 154 µg I.

These data and the ratio between urine and serum specific activity and between observed and predicted urinary radioactivity indicate a leakage of iodine from the glands of these subjects. Iodide wastage and iodine deficiency appear to be the main pathogenic factors of the endemia.

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CHAPTER 14

ENDEMIC GOITER IN THE REPUBLIC OF PARAGUAY[1]

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Endemic goiter was known in Paraguay as early as 1820 (18). In 1870 Burton (4) reported that not a single family in Asunción could be considered free of goiter. At present the whole of Paraguay may be considered an endemic goiter area, with the mountainous areas having the highest morbidity.

The first reliable modern study of goiter in Paraguay was by Peña and Isasi Fleitas (14), in 1943-1946. This included hospital statistics and a population study of schoolchildren (ages 6 to 16). They found that 29.2 per cent of these children had goiter. In 1954 Isasi Fleitas (12) performed a second survey of 44,000 schoolage Paraguayan children and found percentages similar to those previously reported. In the region of the Cordillera, where the survey described here was conducted, he reported an incidence of 49.4 per cent. A nationwide campaign for eradication of goiter in Paraguay began in 1958. The method has been iodinization of table salt with potassium iodate at one part per ten thousand.

The present survey was carried out in the area of Caacupé, in the region of the Cordillera, 60 km from the capital city of Asunción. Caacupé is 600 meters above sea level and has a population of approximately 20,000. The main objectives were to verify the goitrogenic index in the area and to assess the results of salt iodization after six years. After six years of the anti-goitrogenic campaign, school surveys in rural areas show little variation, if any, from the original goitrogenic indices. There were remarkable increases in some mountainous areas, while in urban areas there was a substantial decrease.

METHODS

A total of 1,457 residents of Caacupé were examined. Most were natives of the region. This included 1,237 children, which comprised most of the schoolage population. The absence of goiter was classified as grade 0 and the goiter as grades I, II, or III, according to the WHO classification (9). Goiter was found in 63.7 per cent of the schoolage population. Most of these were grade I. This value can be compared to 49.4 per cent as reported by Isasi Fleitas in 1954 (12).

One hundred eight subjects were selected for additional studies. Included were 23 normal persons who would act as controls, 20 adults with grade I goiter, 28 with grade II, and 19 with grade III. Among the children there were 11 with grade I and seven with grade II. All were natives of Caacupé

1/ From the National Atomic Energy Commission. Buenos Aires, Argentina.

(Tables 1 and 2). Additional control subjects were selected in Asunción, where there has been a sharp decrease in the percentage of goiter. Food availability and cooking procedures are different in the city. These subjects included nine normal inhabitants and seven patients with grade I goiters. Two euthyroid cretins and three patients with Jod-Basedow were also observed but are not included in this study.

Table 1. Age and goiter incidence in Caacupé, Republic of Paraguay.

Age in years	Total number	Thyroid grade per cent		
		0	I	II
6	163	43	56	0.6
7	147	39	60	0.7
8	89	35	64	1.1
9	153	33	66	0.6
10	162	38	62	0.6
11	148	45	55	0
12	170	35	62	3
13	141	24	74	2.1
14	49	34	72	4
15	15	13	87	0
Totals	1,237	36.3	62.5	1.2

Table 2. Sex distribution of adult natives of Caacupé, correlated with goiter incidence.

Thyroid grade	Sex		
	Male	Female	Total
0	19	4	23
I	25	6	31
II	10	25	35
III	2	17	19
Totals	56	52	108

Tracer doses of ^{131}I between 50 and 100 microcuries were administered, except for children under 15 years of age who received 10 μc orally. Plasma radioiodine clearance, thyroid ^{131}I uptake at 2, 24, 48, 72, and 96 hours after administration of the tracer, $PB^{131}I$, butanol extractable fraction at 24 hours, conversion ratio, and effective thyroid $T_{1/2}$ for ^{131}I were performed in most of the patients (17, 19).

Total plasma iodine, protein-bound iodine, and urinary excretion of iodine were performed in all the adult subjects (6, 10). A thiocyanate test was

performed in seven patients by administration of 2 grams of the drug at 24 hours after the ingestion of the tracer. To 26 adults a dose of 200 microcuries of ^{131}I was administered in order to obtain plasma iodinated amino acid separation on paper (5), and column chromatography from blood samples extracted 24 hours after the tracer dose. Column chromatography of serum iodinated amino acids was performed in an anion exchange resin, Dowex 1-X8 (20-50 mesh), equilibrated with acetate buffer, and eluted with this same buffer in a pH gradient ending at glacial acetic acid (1, 2).

Averages and standard deviations for each determination in each (or all) groups have been calculated. A one-way analysis of variance was performed in each assay to test the significance of the group differences. Paired-group comparisons were done using the Duncan multiple range test to maintain the level of significance ($P < 0.05$) for the experiments (22).

RESULTS

A diet survey showed that the main component in Caacupé is manioc. This has a high content of thiocyanate which is mainly in the peel (25). For cooking, this peel is discarded and the rest is boiled for more than two hours. The broth is also discarded and never consumed. The small amount of thiocyanate left in the manioc after it is peeled is destroyed by this prolonged boiling. No other goitrogens were observed in the diet of the area. It was also found that in this region salt is added to the food after it is cooked and in any case is noniodized.

^{131}I uptake values appear in Table 3. It should be noted that some of the control adults and the adults with grade I goiter belong to a local religious seminary, whose economic level and alimentary habits are markedly different from those of the residents of the area and are quite similar to those of the inhabitants of Asunción. There is a statistically significant difference between groups 0 and I of adults as compared with groups 0 and I of children, the latter showing much higher uptakes than the former. The difference is less striking when group II adults are compared with group II children. Both adults and children of Caacupé showed a much stronger avidity for iodine than residents of Asunción of either group 0 or I, as can be seen in Table 4. As a whole the uptake values found in adults and children of Caacupé have little scatter. This was not the case with those of group III, where owing to the large size of the goiters, problems of geometry arose during the recordings.

$PB^{131}I$ values obtained from groups 0 and I of Caacupe are similar to those found in Asunción. Groups II and III had high values. This may possibly be attributed to the long duration of the disease, which together with the presence of nodules might produce significant changes in the intrathyroidal iodine compartments (Table 5).

High conversion ratios were found in every group. The 24-hour butanol extractable fractions were normal (Table 5).

The $PB^{127}I$ values in the natives of Caacupé were not different from those of 542 normal subjects from Buenos Aires (Table 5). Plasma iodine clearance showed statistically significantly ($P < 0.001$) high values in the rural population, especially in those of group II, when compared to controls, grade 0

Table 3. Thyroid ^{131}I uptakes for adults and children of Caacupé.

Thyroid grade	Number	^{131}I thyroid uptakes per cent			
		2 hr.	24 hr.	48 hr.	96 hr.
0	Adults 12	18±5	56±9	56±8	54±12
	Children 11	27±14	70±13	69±14	64±15
I	Adults 20	20±8	56±14	58±13	56±13
	Children 11	40±13	79±6	75±9	66±11
II	Adults 28	37±17	66±12	64±12	61±11
	Children 7	33±5	78±7	76±9	62±15
III	Adults 19	32±10	58±7	57±7	52±8

Table 4. ^{131}I thyroid uptake, $PB^{131}I$, and conversion ratio in 16 adult subjects of Asunción.

Thyroid grade	No. of cases	Thyroid uptake %				$PB^{131}I$ (24 hrs.) %/liter	Conversion ratio % in 24 hrs.
		2 hrs.	24 hrs.	48 hrs.	96 hrs.		
0	9	11±4	35±5	35±8	28±7	0.10±0.07	16±11
I	7	12±4	34±9	32±8	28±7	0.12±0.06	22±19

and I of Asunción. These plasma iodine clearances of grade II from Caacupé are not statistically significant when compared to their own rural grades 0 and I, although the figures obtained point to important differences (grade 0, 45 ml per min; grade I, 63 ml per min; grade II, 91 ml per min) (Table 5).

The intrathyroidal biological half-life is somewhat increased in rural grades 0 and I compared to euthyroid normal subjects, but the figures are without statistical significance. On the contrary, the half-life is diminished in grades II and III and is inversely related to the $PB^{131}I$, a metabolic pattern already described by one of us (Table 5).

In each one of the subjects tested the thiocyanate test was normal, as has already been reported for iodine deficiency goiter (23).

Paper chromatography of iodinated amino acids disclosed normal patterns with no qualitatively or quantitatively abnormal findings.

Table 5. Average values obtained in 57 adults of Caacupé.

Thyroid grade	No. of cases	PBI^{131} % dose/l	Conversion ratio 24 hrs., %	Butanol extractable fraction 24 hrs., %	Plasma clearance ml/min	Effective thyroid T1/2 in days	Plasma iodine micrograms/ 100 ml	PBI^{127} micrograms/ 100 ml	DUIO micrograms/ 100 ml
0	12	0.09	63	82	45	6.3	6.5	5.8	48
		±0.05	±22	± 7	±24	±1.9	±1.0	±0.8	±22
I	11	0.09	72	93	63	6.5	6.9	5.8	52
		±0.03	±23	±11	±35	±1.6	±0.7	±0.6	±16
II	20	0.29	74	81	91	4.5	5.4	4.7	24
		±0.21	±20	± 7	±69	±1.0	±1.0	±0.7	±10
III	14	0.36	81	88	52	4.9	5.9	5.4	21
		±0.17	±16	± 8	±19	±1.3	±1.6	±1.6	± 3

The average daily urinary iodine output values (DUIO) were comparable between grade 0 and grade I, and grade II and grade III of Caacupé (Table 5). The latter two showed a low DUIO, which is characteristic of endemic goiter areas. Groups 0 and I showed higher values. Upon closer inspection it was found that groups 0 and I were composed of two different populations, one comprising subjects from the local seminary and the other the rural population. In Table 6 the DUIO of these populations are compared. It can be seen that the low figures of the rural population are balanced by the higher DUIO of the seminary controls. Thus it is proved that grades 0 and I of the rural population are characteristic of endemic goiter areas.

Table 6. ^{131}I thyroid uptake, daily urinary iodine output, and daily thyroid hormone output.

Thyroid grade	No. of cases	Thyroid uptake in 24 hrs., %	DUIO micrograms/ 24 hrs.	Thyroid hormone secretion micrograms/24 hrs. (**)
Normal	7(**)	52	59	64
	5	61	24	38
I	7(**)	58	69	95
	4	55	37	45
II	20	67	25	51
III	14	54	21	25

** *Local seminary.*

The daily hormone output is also shown in Table 6, calculated using Riggs's formula (21). The daily hormone output is reduced as a whole, while there exists a clear-cut difference between the seminary and the rural populations. A tendency for an inverse relationship between the DUIO and the 48-hour thyroidal uptake was observed when individual cases were compared.

Iodine content of drinking water from the three principal sources of Caacupé (called A, B, and C) was determined by triplicate analysis. Triplicate analyses of samples of the city of Buenos Aires drinking water were performed at the same time (10). The results are shown in Table 7. The differences between the figures are evident and comparable to those in other endemic goiter areas.

Table 7. Iodine content of the drinking water of Caacupé and Buenos Aires.

Area	Source	Iodine content micrograms per liter
Caacupé:	A	0.5
	B	1.8
	C	1.5
Buenos Aires:	Four different sources	10.0
		9.0
		8.5
		11.5

DISCUSSION

Clinical studies have shown that the incidence of goiter in the childhood population of Caacupé is 63.7 per cent. This figure is comparable to that observed in Apóstoles, Argentina (20) and is higher than in La Paz, Bolivia (3). The high rate clearly demonstrates an endemic goiter area. The studies performed indicate a state of iodine deficiency in the region of the Cordillera, in the Republic of Paraguay. The high uptake curves and the increased plasma clearance of iodide are in favor of an increased thyroidal avidity for iodide, as can be seen in other endemic areas where the same etiopathology has been proposed. De Visscher's group (8, 11) observed in the Uele region low values of $PB^{127}I$ in subjects with endemic goiter. Our results show normal values of this parameter. The maintenance of normal levels in endemic goiter areas has already been mentioned for Europe by Terpstra (24) and for Argentina by Stanbury et al. (21) and Soto et al. (20). The decrease of the intrathyroidal biological ^{131}I half-life and the increased $PB^{131}I$ in grades II and III are indicative of an increased intrathyroidal turnover as a mechanism to compensate for the iodine deficiency. The chromatographic studies and measurements of the butanol-extractable fraction were normal, and did not suggest any abnormal intrathyroidal mechanism of hormonal biosynthesis or abnormal circulating thyroidal hormones. The results obtained with the thiocyanate test are in agreement.

Roche et al. (17) indicated a pathological response in the thiocyanate test in endemic goiter, especially when the drug was administered a few hours after the tracer dose. In our studies thiocyanate was given 24 hours after the tracer dose and the results agree with those of others.

The daily urinary excretion of iodine may be considered as an index of the iodine intake (13, 16, 21) in subjects in iodine equilibrium. The findings

in Caacupé were consistent with a deficiency in dietary iodine. Also, the low content of iodine in drinking water proves that the zone under study has an iodine deficiency which is the main cause of its goiter.

A tendency for an inverse relationship between the DUIO's and the 48-hour thyroidal uptakes was observed when the patients were compared individually. If it is assumed that these subjects were in iodine equilibrium it would seem possible to consider that the lower iodine intake was compensated by a higher thyroidal avidity. This is in agreement with the findings of Stanbury et al. (21). Lower ^{131}I uptakes and higher daily urinary excretion of iodine in the local seminary group, which enjoys a higher economic level and has better alimentary habits, while living in the same area as the rural population of Caacupé, proves the iodine deficiency hypothesis even further, although other etiopathological factors cannot be excluded, such as nutritional factors, poverty, etc. In point of fact, the local seminary group by itself demonstrated daily excretion rates for iodine which were in the border line for an endemic area (15), while the ^{131}I thyroidal uptake curves were higher than those found in normal areas.

SUMMARY

Rural and especially highland Paraguay is an endemic goiter zone. In the town of Caacupé the incidence of goiter in children reaches 63.7 per cent. Radioiodine uptake and other studies are in accord with iodine deficiency as the principal, if not the sole, causal factor in this endemia. Iodine content of drinking water in Caacupé is very low.

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SECTION IV

ENDEMIC GOITER IN BRAZIL

CHAPTER 15

ENDEMIC GOITER IN BRAZIL

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HISTORY

References to endemic goiter in Brazil go back to colonial times when the French scientist, Saint-Hillaire (1819), reported in his "Description of People and the Land of Brazil" that more than 80 per cent of the inhabitants of the State of Goiás presented an enlarged thyroid gland (13). In 1820 another traveler, Martius (17), noted the same in the State of Minas Gerais and observed that women were more affected than men. In the same year D'Orbigny gained the impression that four-fifths of all the men and women in a small valley in São Paulo had visible tumors of the neck (15).

Early in the present century Neiva and Pena (10) reported a high incidence of goiter in the upper São Francisco river. The number of person affected seemed to be lower as they traveled down the river toward the sea. They also mentioned a significant incidence of enlarged thyroids in areas where there was no Chagas' disease. This seems to be the first observation that there is no direct relationship between the two endemic disorders. Twenty years later Lobo-Leite (5) confirmed this impression, reporting negative complement fixation tests for Chagas' disease in most goitrous patients of Minas Gerais.

Iodine-want as an etiologic possibility for the Brazilian endemic was first seriously suggested by Viana (18) in 1938. He presented evidence for lack of environmental iodine (in food and water) in a number of towns in Minas Gerais State. In 1944 Lobo-Leite (6) was able to demonstrate the effectiveness of potassium iodine in preventing endemic goiter. He reported a decrease in the incidence of thyroid enlargement in schoolchildren from 44 per cent to 27 per cent after two years of continuous administration of the iodized salt. During the decade that followed many investigators reported the incidence of goiter among schoolchildren in São Paulo, Paraná, and Rio Grande do Sul (1, 3, 11, 14).

Official attention was first paid to the problem in 1953 when the Ministry of Health published a report on the incidence of endemic goiter in Brazil (16). It was only in 1956 that Pellon et al. (12) issued a complete study on the problem of endemic goiter. For this extraordinarily thorough study 1,129 towns were visited in 19 states and 886, 217 individuals were examined. More recently Aragão (2) established the geographic distribution of endemic goiter

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in Brazil, and Memoria (9) reported the incidence of non-tasters to phenylthiocarbamide (PTC) among normal and goitrous children in Minas Gerais State.

In 1964 Gandra (4) described his findings in 56,230 schoolchildren between the ages of 6 and 16 in 154 towns in São Paulo State. For this study radioiodine was used and the stable iodine concentrations in water and common salt were measured.

Lobo et al. (7, 8) in 1966 studied the relationship between endemic goiter and Chagas' disease and observed a number of abnormalities in thyroidal iodoproteins in endemic cretins.

Present Public Health Significance of Endemic Goiter

On the basis of the official report of Pellon et al. (12) it appears that nearly 11 million Brazilians are affected with endemic goiter. The survey revealed also three distinct areas of endemicity:

1) East meridional, south and west regions: high incidence.

2) North and northeast occidental regions: moderate endemicity

3) Northeast oriental and east setentrional: free of endemic goiter.

No correlation was found between the frequency of endemic goiter and altitude, but goitrous females outnumbered affected males by more than two to one. The differences between sexes was reduced with increasing degree of endemicity up to 40 per cent.

The present problem of endemic goiter in the various states of Brazil is presented in Table 1. Gandra (4) was unable to find a decrease in the incidence of goiter among schoolchildren in São Paulo State two years after the first survey. He concluded that the prophylactic program of endemic goiter must be revised and an increased iodine content of the diet must be put in practice by the public health system.

Table 1. Incidence of endemic goiter in various states of Brazil (12).

State	Incidence (per cent)
Mato Grosso	58.9
Minas Gerais	34.6
Goiás	34.0
São Paulo	30.0
Rio de Janeiro	28.8
Santa Catarina	28.4
Paraná	24.0
Espírito Santo	21.5
Rio Grande do Sul	19.5

Present Practice Regarding Prophylaxis

By a law of (14) August 1953, it became mandatory that iodine be added to salt in the proportion of 10 mg per kilogram of refined sodium chloride, either as sodium or potassium iodide. The law, however, reserved the use of iodized salt to Brazilian regions where endemic goiter among schoolchildren is more than 15 per cent for boys and 25 per cent for girls. This was critized, for it deprived many regions from the benefits of the law. The law made no reference to the use of iodate, which technically and economically has many advantages over iodide salts.

Gandra (4) first called the attention of public health officials to the lack of control of the concentration of iodine in common salt in São Paulo. Analyses were made of 869 samples of table salt (under different names) in 153 towns. The mean concentration of iodine was 4.57 ± 3.56 mg of iodine per kilogram of salt. In 57 samples more than 10 mg per kg were found, and in 163 the value of iodine was between 5 and 9.9 mg per kg. Of 16 brand names, more than 15 samples were collected at different places and towns. Only one brand name sample had a confidence interval that included 10 mg per kg. Accordingly, it was suggested that the current industrial mixture of iodine and sodium chloride was not homogeneous and that the entire system should be revised.

Nevertheless, it should be mentioned that the use of iodized salt in São Paulo since 1953 decreased the incidence of endemic goiter among schoolchildren from 27.08 per cent (4) to 19.29 per cent. This result is far from satisfactory. One of the reasons could be the heterogeneous concentration of iodine in common salt. If one assumes that the daily use of salt per person is 10 to 15 g (mean: 12.5 g) with a mean concentration of 4.5 mg of iodine per kg, then the population in São Paulo State receives 57.6 µg of iodine per day. Even when this figure is added to the iodine content of food and water it is still below the 300 µg per day recommended as an effective prophylaxis program. It may be hoped that these deficiencies will be rectified in the near future.

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CHAPTER 16

STUDIES ON THE CONCENTRATION OF PARTICULATE IODOPROTEIN, RNA, AND DNA IN NORMAL AND ENDEMIC GOITER GLANDS

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The thyroid gland is unique in that much of its protein-synthetic machinery is concerned with the formation of a large, complex iodinated glycoprotein, thyroglobulin. This protein is a required intermediate in the production of the chemically less complex hormonal iodine. It may comprise 70 or more per cent of the dry weight of the gland and serves as a reservoir for storage of thyroid hormones and their precursors in order that iodine may be conserved and buffered against changes in iodine supply from external sources. It is quite clear that when synthesis of thyroglobulin is blocked or modified owing to disorders of the thyroid gland, this buffering action may be seriously impaired.

Efforts of many investigators (13-15, 17, 19, 20, 21) have yielded an impressive amount of information on changes that occur in thyroid disease in the relative proportion of thyroglobulin and other iodinated proteins which are found somewhat variably in small amounts in normal thyroid tissue. One of these, an iodinated albumin-like protein may comprise as much as 1.3 per cent of the total iodine (19). Its sedimentation constant is approximately 4S. The fraction of the total iodoprotein which is 4S may be sharply increased in thyroid disease (17, 19, 21). The other is a dense particulate iodoprotein which is readly solubilized by trypsin and is present in large relative and absolute amounts in congenital goiter (14, 15), thyroid tumors (20), and adenomas (13, 14). The physiological role, if any, of this moiety of the thyroid iodoproteins is entirely unknown.

The present study is concerned with the occurrence, nature, and properties of the particulate iodoprotein found in enlarged thyroid glands from an endemic goiter region. Included are the results of DNA and RNA analyses on the same specimen and on normal glands. We have tried to establish a relationship between cellularity, protein synthesis or turnover, and the presence of particulate iodoprotein.

MATERIALS AND METHODS

Three normal glands were obtained from euthyroid patients at the time of neck surgery for parathyroid adenomas (patients S.N. and A.S.) and from the opposite lobe of a thyroid gland in which there was a solitary thyroid adenoma (patient A.C.). Microscopic examination disclosed a normal morphological picture.

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The five patients with endemic goiter (with one exception, S.G.) came from São Paulo State, Brazil, which is within the endemic goiter area delineated by the Brazilian Government. They had large hyperplastic glands that had been present for many years without significant changes in size or position. Only one of these patients had received some iodine drops as treatment (patient A. M.B.) but discontinued the medication more than one year before the present study.

Clinical and laboratory information on the endemic goiter patients appears in Table 1. These data included a high uptake of RAI, a negative TRC agglutination test, and a normal PBI. A pathological diagnosis of colloid goiter was rendered on four specimens; the fifth was an adenomatous goiter (patient S.G.). The total weight of the gland was estimated from the thyroid scintiscan.

Table 1. Clinical and laboratory data on goitrous patients.

Name	Age	Sex	PBI (µg/100ml)	RAI Uptake		Tanned red cell agglutination	Estimated weight of the gland (g*)	Histologic picture
				2 hrs.	24 hrs.			
M.R.	48	F	8.6	13.0%	36.0%	Neg.	147	Colloid goiter
A.M.B.	37	F	6.7	23.0%	56.0%	1:80	137	Colloid goiter
M.D.T.	39	F	7.8	34.5%	75.5%	1:40	96	Colloid goiter
S.G.	29	F	6.7	24.0%	68.0%	Neg.	72	Adenomatous colloid goiter
M.G.	48	F	6.2	19.0%	46.0%	Neg.	112	Colloid goiter

* *Based on thyroid scintiscan.*

The eight patients received a tracer dose of ^{125}I at 35 to 47 days before surgery and to two patients (M.R. and M.G.) another tracer dose of ^{131}I was given 24 hours before the operation. The glands were collected at the time of surgery in cracked ice, rinsed free of blood, and dissected to remove fibrous tissue from glandular tissue. In every case of endemic goiter two specimens were processed independently. The tissues were homogenized at 4°C in 0.26 m sucrose solution in an all-glass motor-driven homogenizer. The homogenates were made up to four times their original weight with 0.26M sucrose and an aliquot was separated for chemical determinations (^{127}I, protein, RNA, and DNA-phosphorus), and then centrifuged for 10 minutes at 700xg in a refrigerated centrifuge. The supernatant was removed, the sediment was resuspended, centrifugation was repeated, and the supernatants were pooled. The final sediment (nuclear fraction) was made to 50 per cent of the volume of the homogenate. The combined supernatant fractions were centrifuged at 105,000xg for 60 minutes at 0°C in the Spinco preparative ultracentrifuge and the fluid above the pellet was removed by aspiration to constitute the soluble protein fraction. The pellet was considered to be a mixture of mitochondria and microsomes and

separated for a mitochondrial fraction. Aliquots were taken for ^{127}I, ^{125}I, and protein determination as indicated.

Analytical Methods

Chromatography was done in Dowex resin columns, according to the Blanquet-Meyniel technique (3). Stable iodine determinations were made by the Benotti and Benotti (2) modification of the Zak method. Protein was determined by the method of Lowry et al. (10). Pancreatin and pronase hydrolysis of the thyroid proteins was performed with the sample brought to pH 8.4 in the presence of 10^{-3}M propylthiouracil and a few drops of toluene. The samples were stored at 37 C for 4 hours and then chromatographed. Separation of thyroid proteins was made on Sephadex G-200 column eluted with 0.15M NaCl according to Perelmutter et al. (16). Solubilization of particulate iodoprotein was attempted by a brief treatment with 0.4 per cent trypsin (15 min) at room temperature, followed by soybean trypsin inhibitor. The samples were centrifuged and the solubile fraction applied to Sephadex columns for gel filtration. RNA and DNA-phosphorus content of the thyroid tissue were determined following the method of Munro, as modified by Goldberg and his colleagues (7). These investigators demonstrated the unsuitability of colorimetric methods for nucleic acid estimation when applied to the human thyroid gland. When colloid storage is poor, as in thyrotoxicosis, interference with RNA and DNA estimations is less than that found in normal glands. It is likely that carbohydrates associated with thyroglobulin contribute to this interference. Therefore RNA was estimated by ultraviolet spectrophotometry and DNA content of the lipid extracted fraction was estimated by its content of phosphorus (7).

RESULTS

1. Relative Proportion of Labeled Iodine, Stable Iodine, and Protein in Goitrous and Normal Glands

In the goitrous glands close to one-fifth of the total labeled and stable iodine was present in the 700xg fraction. In the normal glands less than 10 per cent of the total iodine was present in this fraction. In most goitrous glands the proportion of stable iodine in the nuclear fraction was higher than the labeled iodine percentage. This was not the case for the normal tissue. The relative proportion of protein was also significantly higher in the goitrous tissue as compared with normal glands (Table 2).

2. Concentration of ^{127}I and Protein per Gram of Wet Weight in the Nuclear Fraction

In both goitrous and normal glands the absolute amount of iodine and protein was measured in the nuclear fraction and corrected to the total wet weight of each specimen. As can be seen in Table 3, the concentration of protein in the nuclear cut of the abnormal glands is considerably higher as compared to the same determination in the normal tissue (respectively 44.71 ± 9.55 and 14.72 ± 4.58). This was significant at a level of $p < 0.01$. The amount of stable iodine in the goitrous tissue, however, in the same fraction, was not significantly different as compared with the normal. Respectively 11.8 ± 6.53 mcg/g and 14.06 ± 3.48 were obtained for goitrous and normal glands. The ratio

Table 2. Relative proportion of labeled iodine, stable iodine, and protein in goitrous (patients 1-5) and normal glands (patients 6-8). Results in % of the total in the whole homogenate.

Patient	Labeled iodine (^{125}I)	Stable iodine (^{127}I)	Protein
1 - M.R.	17.1	22.80	25.0
2 - A.M.B.	14.3	9.26	33.3
3 - M.D.T.	12.4	17.47	29.9
4 - S.G.	26.2	28.72	27.6
5 - M.G.	12.5	18.40	18.6
Mean	16.53	19.33	26.8
S.D.	± 7.33	± 9.37	± 6.5
6 - S.N.	7.3	9.3	13.2
7 - A.S.	10.7	7.8	20.4
8 - A.C.	9.7	8.4	10.8
Mean	9.23	9.50	14.8
S.D.	± 1.83	± 1.2	± 4.7

Table 3. Concentration of protein and ^{127}I in the 700 x g sediment ("nuclear" fraction) in goitrous (patients 1-5) and normal glands (patients 6-8). Results in mg per g of wet weight.

Patient	Protein mg/g of wet weight	^{127}Iodine µg/g of wet weight	µg of ^{127}I/mg of protein
1 - M.R.	36.46	16.13	0.458
2 - A.M.B.	48.54	8.13	0.167
3 - M.D.T.	59.65	6.02	0.109
4 - S.G.	38.08	10.48	0.275
5 - M.G.	40.81	17.66	0.433
Mean	44.71	11.80	0.288
S.D.	± 9.55	± 6.53	±0.155
6 - S.N.	12.58	15.57	1.237
7 - A.S.	19.99	11.60	0.580
8 - A.C.	11.61	15.01	1.292
Mean	14.72	14.06	1.036
S.D.	± 4.58	± 3.48	±0.393
p	<0.001	>0.05	<0.01

of stable iodine per mg of protein is significantly lower in the abnormal tissue (0.288±0.155) as compared with the normal tissue (1.036±0.393).

When the same determinations were applied to the whole homogenate the results were quite similar. The goitrous glands had only 65.6±16.8 μg of ^{127}I per g of wet weight, whereas the normal tissue had 165.0±12.3 μg. The protein content of abnormal glands was significantly higher than in the normal tissue, and the ratio of stable iodine per mg of protein was significantly lower in the abnormal tissue as compared with the normal glands (Table 4).

Table 4. Concentration of ^{127}I and protein per gram of glandular tissue (wet weight) in goitrous (patients 1-5) and normal glands (patients 6-8).

Patient	μg of ^{127}I/g	μg of protein/g	μg ^{127}I/mg protein
1 - M.R.	73.4	145.8	0.503
2 - A.M.B.	87.8	145.8	0.602
3 - M.O.	34.5	199.5	0.172
4 - S.G.	36.5	138.0	0.261
5 - M.G.	96.0	219.4	0.437
Mean	65.6	169.7	0.395
S.D.	±16.8	± 37.1	±0.178
6 - S.N.	167.4	95.3	1.756
7 - A.S.	148.8	98.0	1.517
8 - A.C.	178.8	107.5	1.662
Mean	165.0	100.3	1.645
S.D.	± 12.3	± 6.41	±0.119
p	< 0.001	<0.05	<0.001

Thus the goitrous gland has three times less iodine in the gland per unit of weight, but in the nuclear cut the same amount of iodine as the normal tissue. The ratio of stable iodine to protein is quite low in both the nuclear fraction and in the whole homogenate of the goitrous glands.

3. Ratio of Labeled Iodine (^{125}I and ^{131}I) in Particulate and Soluble Proteins

In two patients (M.R. and M.G.) it was possible to measure the ratio of both ^{125}I and ^{131}I as related to protein. By the double-labeling technique we intended to measure the rate of turnover of labeled iodine in both particulate (700xg fraction) and soluble protein fractions. The ^{125}I was administered at least four weeks before surgery and ^{131}I was given 24 hours before the gland was removed. In both patients the particulate iodoprotein had a higher ratio four weeks after the tracer dose (^{125}I) than at 24 hours (^{131}I).

Particulate iodine was six to ten times higher in iodine after four weeks than at 24 hours after administration. This suggests a slow turnover of iodine in this insoluble iodoprotein (Table 5). For the soluble protein fraction the ratio at 24 hours was higher than at four weeks. This suggests a more active incorporation and release of iodine in the iodoproteins of the soluble fraction.

Table 5. Ratio of ^{125}I and ^{131}I to particulate (700 x g fraction) and soluble protein fraction (105,000 x g fraction). ^{131}I was administered 24 hours and ^{125}I four weeks before surgery. Results expressed as % of the administered dose per gram of protein.

Patient	Isotope	Particulate iodoprotein	Soluble iodoproteins
M.R.	131_I	0.056	1.096
	125_I	0.123	0.908
M.G.	131_I	0.048	0.985
	125_I	0.076	0.738

4. Concentration of Protein, RNA, and DNA-Phosphorus

There was an increased proportion of protein, RNA, and DNA-phosphorus per gram of wet weight of tissue in the five specimens of goitrous glands (Table 6). These values were compared with the same obtained for the normal glands and were found to be statistically different ($p < 0.05$). Thus the goitrous glands have more DNA-phosphorus and RNA per gram of tissue than the normal specimens. As an approximation to the content of the individual cell the use of DNA as a reference parameter is widely accepted. For this reason the other tissue constituents (protein and RNA) were expressed relative to the DNA content of the sample and the results given in Table 7.

Table 6. Concentration of protein, RNA, and DNA-phosphorus in goitrous and normal glands (number of specimens in parentheses). Results as mean ± SD in mg/g of wet weight. Statistical comparison by Student's t test.

	Normal glands (3)	p	Goitrous glands (5)
Protein	100.3 ± 6.41	<0.05	169.71 ± 37.11
RNA	0.917 ± 0.110	<0.05	1.988 ± 0.617
DNA-phosphorus	0.264 ± 0.126	<0.05	0.414 ± 0.128

The goitrous glands had a significant increase in RNA per mg of DNA-phosphorus but not in protein per mg of DNA, as compared with normal tissue. The increase in the RNA/DNA ratio is compatible with the increased protein synthesis or turnover occurring in the abnormal tissue.

Table 7. Concentration of protein and RNA in goitrous and normal glands in relation to the total concentration of DNA-phosphorus (number of specimens in parentheses). Statistical comparison by Student's t test).

	Normal glands (3)	p	Goitrous glands
Protein	440.64 ± 59.50	>0.05	587.94 ± 11.32
RNA	3.91 ± 1.33	<0.05	6.27 ± 1.95

Both the DNA-phosphorus content of the tissue and the RNA/DNA ratio were related to the absolute amount of particulate protein in each specimen of the goitrous glands (Table 8). Thus there seems to be no correlation between the amount of particulate protein and the abnormal proportion of RNA and DNA-phosphorus in these goitrous glands ($\alpha = 0.05$).

Table 8. Correlation between particulate protein, RNA/DNA ratio, and DNA-phosphorus in the goitrous glands.

Patient	Particulate protein mg/g of wet weight	DNA-phosphorus mg/g of wet weight	RNA/DNA ratio
M.R.	36.46	0.220	8.76
A.M.B.	48.54	0.439	7.36
M.D.T.	59.65	0.462	4.16
S.G.	38.08	0.566	4.45
M.G.	40.81	0.383	6.62
Correlation coefficient	-	r = 0.11*	r = 0.35*

** There is no significant coefficient of correlation between particulate protein versus DNA-phosphorus and RNA/DNA ratio. ($\alpha = 0.05$).*

5. Properties of the Solubilized Iodoprotein

The solubilized iodoprotein of the goitrous glands was applied to a column of Sephadex (G-200) gel and eluted in 3 ml fractions with 0.15M NaCl and collected by an automatic fraction collector. A peak was observed after 150 ml of effluent which was distinctly different from normal human thyroglobulin. This component had an ultracentrifugal sedimentation coefficient between 3.1 and 6.8S (18).

Both the particulate and the soluble proteins were hydrolyzed with pronase and submitted to column chromatography. The results appear in Figure 1. The particulate protein had a higher iodotyrosine/iodothyronine ratio as compared with soluble proteins and a significantly lower content of T_3/T_4. The solubilized iodoprotein did not react with goat anti-human thyroglobulin in Ouchterlony agar plates; both normal and trypsin-treated thyroglobulin exhibits

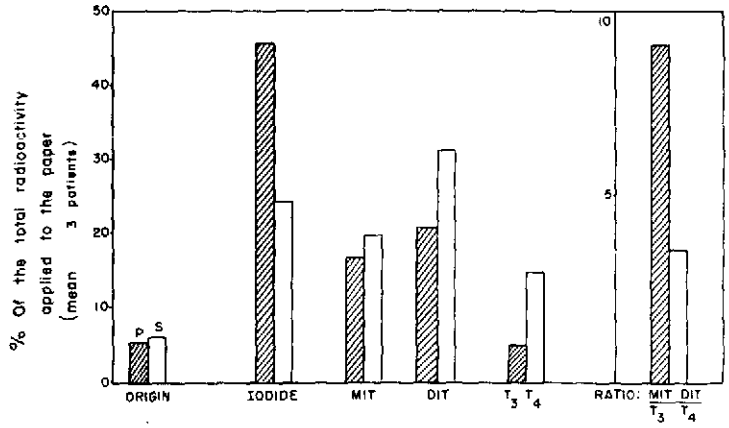

Figure 1. Column chromatography of hydrolyzed particulate (P) and soluble protein fraction (S). The results are expressed as per cent of the total radioactivity applied to the column and are the mean of three specimens. Note a lower relative proportion of T_3T_4 for the particulate protein and a higher iodotyrosine to iodothyronine ratio.

a definable line of precipitation in the same agar plate. Thus the solubilized iodoprotein behaved distinctly differently from normal human thyroglobulin in both chemical and immunological tests.

DISCUSSION

In the normal thyroid gland almost all the iodine is in soluble form as part of the thyroglobulin molecule. A small fraction sediments along with the cell nuclei and tissue debris at 700xg for 10 minutes. One of us (G.M.N.) has studied this component in five normal glands, and in these tissues less than 10 per cent was found in the nuclear cut (12).

In some abnormal tissue as much as 50 per cent of the total iodine may be found in this form (12). This component was first detected in a rat transplantable thyroid tumor by Robbins et al. (20) and later found in congenital goiter (13, 14, 20), thyroid adenomas (13, 14), and human thyroid tumor (13). In endemic goiter glands several abnormalities in protein biosynthesis have been reported. An appreciable increase of 4S protein was observed both in hyperplastic endemic goiters and in endemic cretinism. This soluble protein has electrophoretic mobility and immunological reactions similar to those observed with serum albumin (9). An increased MIT/DIT ratio was also observed (1). A similar finding was also observed in hyperplastic goiters produced in hamsters by iodine-deficient diets but not in colloid goiters (6). As judged by these studies chronic iodine-deficiency may induce an abnormal pattern in the distribution of iodine in the soluble proteins, characterized by an increase in an iodinated albumin-like protein.

Few studies have been devoted to insoluble proteins in these abnormal tissues. Beckers and De Visscher (1) reported their findings in four specimens of goitrous glands from the Uele region. These patients received labeled iodine three to seven days before surgery and, with one exception, iodine

prophylaxis for one year before the study was begun. In these glands 6.94 to 12.13 per cent of the total labeled iodine was particulate. Stable iodine concentrations in the particulate fractions were not reported. When these results are compared to those reported here it should be recalled that the time of labelling is important when particulate labeled iodoprotein is analized. We labeled the gland at least four weeks before surgery and this may be an explanation for somewhat different proportions of particulate protein in the tissue.

Westra et al. (22) observed in rats that the particulate labeled iodine rose sharply with time and that the percentage of thyroidal insoluble iodoprotein, which is approximately 10 in rats on an iodine-deficient diet, was lowered to 2.6 in the iodine-rich glands. These studies have also shown that in rats there is a fraction of particulate labeled iodoprotein which is relatively slowly labeled with the isotope and which turns over slowly. It appears that the percentage of thyroidal stable iodine in particulate form must be influenced by factors related to the cellularity and iodine content of the gland. In our patients there was a low iodine content of the thyroid tissue, both in goitrous and in normal glands. In nine normal individuals who underwent neck surgery Ermans et al. (5) reported a mean of 620 ± 66 µg of ^{127}I per gram of fresh thyroid tissue. This is almost four times higher as compared with the value we have obtained in our normal specimens (165.0 ± 12.3) and almost ten times higher than the value found in goitrous glands. It should be stressed that our patients were on a chronic iodine-deficient diet and this could be the main reason for the low iodine content in the gland. If the conclusions of Westra et al. (22) are valid for the human thyroid gland, then the higher absolute and relative proportion of particulate protein found in our goitrous glands is related to chronic iodide deficiency. It is also possible that in these hyperplastic thyroids the percentage of particulate (i.e., intracellular) iodine is actually elevated because of the preponderance of cells.

The data obtained from analysis of the goitrous glands for RNA and DNA-phosphorus have shown that there is actually an increase in both parameters in the abnormal tissue as compared with the normal specimens. It has previously been shown in animal experiments that an increase in RNA content, but not in DNA content, follows thyrotropin injection, whereas the former falls after hypophysectomy, without change in the latter (8, 11). Thus the data relative to DNA are in accord with an increased cellularity in the hyperplastic glands, but no correlation was found between the increased DNA content and the absolute or relative proportion of particulate iodoprotein in the goitrous glands. Two reservations must be made regarding these findings. First no account has been taken of polyploidy, which almost certainly occurs in these abnormal glands. The second is the problem of lymphocytes and plasma cells that may be found infiltrating the tissue. Goldberg et al. (7) demonstrated a higher proportion of RNA and DNA-phosphorus in chronic thyroiditis and attributed this to the large proportion of lymphocytes infiltrating the thyroid tissue. Our data on normal glands fall within the range of the normal group of Goldberg et al. (7). Their specimens were removed within a short time of death from patients without thyroid disease. We have also compared our data on goitrous glands to those published by Goldberg and his colleagues for thyrotoxic glands. They are quite similar except for an increase in protein content in the latter as compared with the former. This suggests that the endemic goiter glands are also highly stimulated to increased cellularity and protein synthesis similarly to thyrotoxic glands. Increased protein synthesis

or turnover in the goitrous glands is suggested by the higher RNA/DNA ratio as compared with normal thyroid tissue.

As judged by our data and from previously reported findings (1, 9), the increased protein synthesis in the endemic goiter glands seems to be related to the production of abnormal iodoproteins. Furthermore, double labeling of the gland demonstrated that the ratio of labeled iodine to particulate protein is higher at four weeks than at 24 hours of a tracer dose. This suggests that this abnormal component has a slow turnover and that it is gradually labeled to a considerably higher content of iodine because of a slower loss of label, as compared with the soluble proteins. Moreover, the chromatographic distribution of labeled amino acids seems to be quite different as compared with soluble proteins.

The physiological significance of particulate thyroidal iodoprotein with sluggish turnover rate is unknown. They may constitute "an adventitious concomitant of thyroid hormone production" (4). The particulate iodoprotein apparently is not metabolically available and thus a considerable amount of iodine is sequestered into this abnormal component. If one considers that these glands have a very low content of iodine available for normal hormone synthesis, the production of this iodoprotein constitutes such a drain on the iodine economy of the gland that growth of the gland is required in order to provide sufficient normal hormone for daily needs. It is tempting to relate the presence of large amounts of particulate iodoprotein in endemic goiter to a basic pathologic process that aggravates chronic iodine deficiency.

SUMMARY

Thyroid glands were obtained from three normal subjects at the time of parathyroid surgery and from five patients with endemic goiter. These specimens were examined for distribution of iodine and of labeled iodine among the various particulate and soluble fractions and for iodinated protein content.

Approximately one-fifth of the total labeled and stable iodine was easily sedimentable in the goitrous glands, whereas only a small fraction was sedimentable in the homogenates of the normal thyroids.

The iodine concentration was smaller in the goitrous gland. Turnover of iodine was slow in the insoluble and easily sedimentable iodoprotein. There was more DNA-phosphorus and RNA per gram in the goitrous glands than in the normal specimens.

The easily sedimentable particulate iodoprotein had a higher ratio of iodotyrosines to iodothyronines than did the soluble iodoproteins. When solubilized it did not react with antihuman thyroglobulin.

The physiological significance of the easily sedimentable particulate thyroidal iodoprotein with sluggish turnover is unknown. Evidently it is not metabolically available and represents a considerable sequestration of iodine in the thyroid in endemic goiter.

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CHAPTER 17

STUDIES ON ENDEMIC GOITER AND CRETINISM IN BRAZIL[1]

I. EPIDEMIOLOGICAL SURVEY IN MATO GROSSO

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Endemic goiter and cretinism are common in the middle-west region of Brazil. This area corresponds to a large plateau about 800 meters above sea level (range 500 to 1,100 meters) which separates the two largest hydrographic basins of South America: those of the Amazon and La Plata. Scarcely inhabited (less than 0.5 inhabitants per square kilometer), the Mato Grosso plateau, where this epidemiological study was done, is limited by the jungle to the north and by a large plain, which is flooded by the Paraguay River during at least five months of the year, to the south (Figure 1). The climate is warm, with a distinct rainy season (2 to 3 meters of water per year). The soil is of sedimentary origin. This area is about 2,000 kilometers from the Atlantic Ocean and is linked to the coastal and more developed region of Brazil by airlines or by unpaved roads that reach Brasilia or São Paulo. Nevertheless, the poor economic conditions of its inhabitants (annual per capita income of US$98.00) makes its population a fixed and stable group.

A survey was made in 11 towns of the Mato Grosso State. Full family data were collected. All families were interviewed by at least one member of a team. The information obtained in 30 per cent of the families was checked by a different interviewer. These data were analyzed by two members of the genetics research team before coding and computer processing.

Goiter was determined by palpation and inspection. The classification of the World Health Organization (WHO) was adopted (16). In this classification goiter 1 is palpable and readily visible with the head thrown back and the neck fully extended; goiter 2 is easily visible with the head in normal position, and goiter 3 is one visible at a considerable distance.

RESULTS

The analysis of the data obtained in 1,525 families (9,590 individuals) showed the following:

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Sex ratio: Male/female = 1.06

Fertility: 5.92 pregnancies per couple. This figure did not change when one or both parents had goiter.

Prenatal mortality: (Frequency of abortion and stillbirths per pregnancy: 7.13 per cent. The presence of goiter in one or both parents did not change this number significantly ($X^2 = 6.70$; $P > 0.05$).

Prevalence of cretinism and deafmutism per live-born sibs: 0.5 per cent of cretinism and 0.33 per cent of deafmutism.

Prevalence of congenital malformations per live-born sibs: 1.87 when the inbreeding coefficient F was 0 and 5.15 when F was greater than 0; no significant differences were obtained when one or both parents had goiter. ($X^2 = 5.19$; $P > 0.10$).

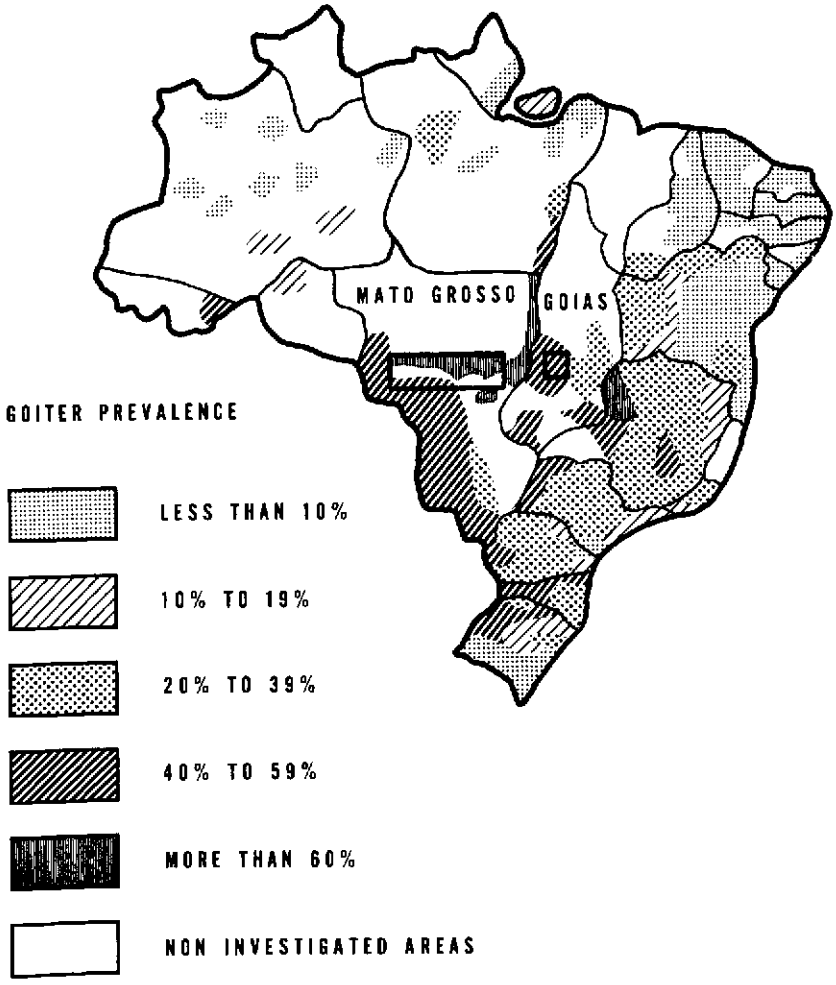

Figure 1. Endemic goiter in Brazil. The rectangle shows areas where endemic goiter and cretinism were studied in Mato Grosso and Goiás States.

Table 1 presents the prevalence of goiter for age and sex. Girls had a higher frequency of goiter earlier than the boys and although in the males there was a decrease in goiter frequency after 14 years, this was not found in women, in whom goiter prevalence remained almost the same. There was no statistical difference between goiter prevalence after age 9 in the female ($X^2 = 5.02$; $P > 0.50$); this difference was, however, highly significant in males ($X^2 = 57.63$; $P < 0.01$).

The distribution of goiter 1, 2, and 3 according to the age and sex of patients is shown in Figure 2. There was a decrease in the frequency of goiter 1 after 20 years and an increase in the relative frequency of goiter 2. Goiter 3 was not found before 20 years in females and before 40 years in males.

The prevalence of goiter according to ethnic group and sex is shown in Table 2. A significantly higher incidence of goiter was found in the mulatto or Negro group, as compared to the white group.

The statistical analysis of the prevalence of goiter in the three groups was highly significant in males ($X^2 = 21.49$; $P < 0.01$) and females (X^2 30.52; $P < 0.01$). However, there was no statistically significant difference between Negroes and mulattoes ($X^2 = 1.79$; $P > 0.10$ for males and $X^2 = 0.1$; $P > 0.70$ for females).

Table 3 shows the prevalence of goiter according to place of marriage and place of birth. A higher prevalence of goiter is found when the patients were born or when they married in rural areas as compared to the frequency observed when they were born or married in an urban area or outside the endemic goiter region. The differences are statistically significant, respectively in regard to place of marriage and place of birth ($X^2 = 5.48$; $P < 0.02$ and $X^2 = 20.52$; $P < 0.01$, respectively).

The prevalence of goiter was determined in patients living in different socioeconomic situations. This was assessed by the housing condition, the density of individuals per room of the house, profession of the husband, and education of the spouses (Table 4). A significantly higher frequency of goiter is found when the housing condition is poorest ($X^2 = 34.61$; $P < 0.01$), when the husband is an unskilled worker (X^2 40.98; $P < 0.01$), when the couple is illiterate ($X^2 = 26.82$; $P < 0.01$), and when there are more than two individuals living in the same room of the same house ($X^2 = 44.07$; $P < 0.01$).

The distribution of white, Negro, and mulatto in relation to the place of birth and place of marriage is shown in Table 5.

A higher frequency of Negroes is found in the worst socioeconomic conditions, as presented in Table 6 ($X^2 = 71.41$; $P < 0.01$ for profession of the husband; $X^2 = 87.26$; $P < 0.01$ for education of the couple, and $X^2 = 46.33$; $P < 0.01$ for density of individuals per room of the house).

Table 7 shows the distribution of white and Negro in relationship to housing condition, density of individuals per room of the house, and profession of the husband. A statistically significant higher frequency of Negroes is found in the poorest situation (as presented in Table 6). Nevertheless,

Table 1. Prevalence of goiter by age and sex.

Age group	Female					Male				
	T	N	%	X^2	P	T	N	%	X^2	P
0 - 5	527	53	10.1			534	58	10.9		
5 - 9	488	217	44.5			470	222	47.2		
9 -12	222	131	59.0			210	113	53.8		
12 -14	126	75	59.5			85	54	63.5		
14 -17	155	95	61.3			97	51	52.6		
				5.02	>0.05				57,63	<0.01
17 -20	70	44	62.9			42	22	52.4		
20 -30	457	274	60.0			140	51	36.4		
30 -40	334	219	65.6			170	64	36.6		
> 40	525	310	59.1			466	150	32.2		
Total	2,904	1,418	48.8			2,214	785	35.5		

T = Number of patients examined.
N = Number of patients with goiter 1+2+3 (WHO classification).

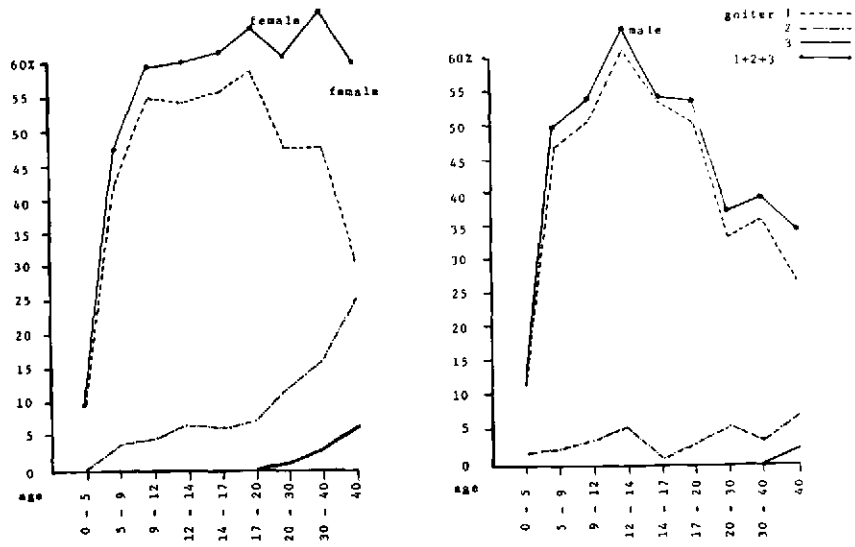

Figure 2. The distribution of goiter 1, 2, and 3 (WHO classification) according to age and sex in Mato Grosso State.

Table 2. Prevalence of goiter by ethnic group and sex in the parents.

Ethnic group	Female					Male				
	T	N	%	X^2	P	T	N	%	X^2	P
White	348	172	49.4			251	61	24.3		
Mulatto	700	458	65.4			349	133	38.1		
				30.52	<0.01				21.49	<0.01
Negro	364	240	65.9			219	96	43.8		
Total	1,412	870	61.6			819	290	35.4		

T = Number of patients examined.
N = Number of patients with goiter 1+2+3 (WHO classification).

Table 3. Prevalence of goiter by place of marriage and of birth.

Region	Place of marriage					Place of birth				
	T	N	%	X	P	T	N	%	X	P
Endemic region:										
Urban area	1,448	741	51.2			899	445	49.5		
Rural area	179	108	60.3			472	289	61.2		
				5.50	>0.05				20.53	<0.01
Outside the endemic region	603	311	51.6			828	411	49.6		
Total	2,230	1,160	52.0			2,199	1,145	52.1		

T= Number of patients examined.
N = Number of patients with goiter 1+2+3 (WHO classification).

Other comparisons:

Place of marriage: Rural x urban: $X^2 = 5.44$, $P < 0.02$; rural x outside the region: $X^2 = 4.23$, $P < 0.05$; urban x outside the region: $X^2 = 0.12$, $P > 0.70$.

Place of birth: Rural x urban: $X^2 = 17.63$, $P < 0.01$: rural x outside the region: $X^2 = 16.77$, $P < 0.01$; urban x outside the region: $X^2 = 0.004$; $P > 0.95$.

Table 4. Prevalence of goiter by housing conditions, density of individuals per room of the house, profession of the husband, and education of the spouses.

Code	Housing conditions					Density per room					Profession					Education				
	T	N	%	X^2	P	T	N	%	X^2	P	T	N	%	X^2	P	T	N	%	X^2	P
1	1,122	653	58.2			1,748	973	55.7			863	523	60.6			1,084	624	57.6		
				34.61	<0.01				44.07	<0.01				40.98	<0.01				26.82	<0.01
2	1,114	510	45.8			473	182	38.5			1,370	639	46.6			1,134	528	46.6		
Tot.	2,236	1,163	52.0			2,221	1,155	52.0			2,233	1,162	52.0			2,218	1,152	51.9		

T = Number of patients examined.
N = Number of patients with goiter 1+2+3 (WHO classification).

Code:	1	2
Housing conditions	Very poor	Reasonable or good
Density of individuals in the house	> 2 per room	0-2 per room
Profession of the husband	Unskilled worker	Trained worker
Education of the spouses	Illiterate	Literate

Table 5. Prevalence of goiter by distribution of white, mulatto, and Negro groups in relation to the place of marriage and of birth of the parents.

Region	Place of marriage									Place of birth								
	T	White		Mulatto		Negro		X^2	P	T	White		Mulatto		Negro		X^2	P
		N	%	N	%	N	%				N	%	N	%	N	%		
Endemic region:																		
Urban area	1,930	494	26	884	46	552	29			1,149	324	28	538	47	287	25		
Rural area	255	52	20	117	46	86	34			581	118	20	278	48	185	32		
								19.88	<0.01								19.79	<0.01
Outside the endemic region	854	271	32	372	44	211	25			1,188	339	29	522	44	327	28		
Total	3,039	817	27	1,373	45	849	28			2,918	781	27	1,338	46	799	27		

T = Number of patients examined.
N = Number of patients with goiter 1+2+3 (WHO classification).

Table 6. Prevalence of goiter by socioeconomic conditions in the Negro group.

Code	Profession of the husband					Education of the couple					Density of individuals per room of the house				
	T	Negro				T	Negro				T	Negro			
		N	%	X^2	P		N	%	X^2	P		N	%	X^2	P
1	1,317	742	56.3			608	439	72.2			791	473	59.8		
				71.41	<0.01				87.26	<0.01				46.33	<0.01
2	337	103	30.6			852	406	47.7			859	370	43.1		
Total	1,654	845	51.1			1,460	845	57.9			1,650	843	51.1		

T = Number of patients examined.
N = Number of patients with goiter 1+2+3 (WHO classification).

Code:	1	2
Profession of the husband	Unskilled worker	Trained worker
Education of the couple	Illiterate	Literate
Density of individuals in the house	> 2 per room	0-2 per room

Table 7. Prevalence of goiter by ethnic group and socio-sanitary conditions.

Ethnic group	Housing conditions						Density of individuals per room of the house						Profession of the husband					
	Very poor (1)			Reasonable or good (2)			>2 (1)			0-2 (2)			Unskilled worker (1)			Trained worker (2)		
	T	N	%	T	N	%	T	N	%	T	N	%	T	N	%	T	N	%
White (W)	371	173	46.6	226	60	26.6	336	152	45.2	262	81	30.9	209	92	44.0	389	141	36.2
Negro (N)	381	236	61.9	202	100	49.5	464	281	60.6	119	55	46.2	333	205	61.6	250	131	52.4
Total	752	409	54.4	428	160	37.4	800	433	54.1	381	136	35.7	542	297	54.8	639	272	42.6

T = Number of patients examined.
N = Number of patients with goiter 1+ 2+3 (WHO classification).

Statistical analysis

		W x N
Housing conditions	(1)	$X^2_2 = 18.02$ $p < 0.01$
	(2)	$X^2 = 23.04$ $p < 0.01$
Density of individuals in the house	(1)	$X^2_2 = 18.58$ $p < 0.01$
	(2)	$X^2 = 8.10$ $p < 0.01$
Profession of the husband	(1)	$X^2_2 = 16.09$ $p < 0.01$
	(2)	$X^2 = 16.52$ $p < 0.01$

	Housing conditions (1x2)	Density of individuals (1x2)	Profession of the husband (1x2)
Negro	$X^2 = 6.74$, $p < 0.01$	$X^2 = 13.25$, $p < 0.01$	$X^2 = 4.83$, $p < 0.05$
White	$X^2 = 22.24$, $p < 0.01$	$X^2 = 12.60$, $p < 0.01$	$X^2 = 3.44$, $p < 0.10$

Table 8. Prevalence of goiter by water supply.

Water supply	T	N	%	X^2	P
Spring	69	19	27.5		
Well	222	78	35.1		
				7.66	>0.05
River	142	53	37.3		
Public system	283	101	35.7		
Total	716	251	35.0		

T = Number of patients examined.
N = Number of patients with goiter 1+2+3 (WHO classification).

comparing separately the goiter frequency in white and Negro, one can demonstrate that the socioeconomic situation influences in a significant way the goiter prevalence since both groups (white and Negro) show a higher frequency in the worst economic and living conditions. The only exception relates to whites, which do not show significant difference of the prevalence of goiter in relationship to the profession of the husband ($X^2 = 3.44$; $P > 0.05$).

The frequency of goiter studied in a group of 716 individuals in respect to the water supply shows (Table 8) that there is no statistically significant difference when the water was supplied by spring, well, river, or public system ($X^2 = 7.66$; $P > 0.05$).

DISCUSSION

Endemic Cretinism

When endemic goiter is severe, endemic cretinism is generally found. Eugster (7) reported that as high as 3.5 per cent of all children in a Swiss village were cretins (cretin rate of his survey ranged from 0.6 to 3.5 per cent).

The studies published by the Sardinian Cretinism Commission (17) reported 7,083 cretins in a population of 2,651,000 (the cretin rate varied from 0.01 to 2.79 per cent).

Three types of cretins were described by the Sardinian Commission:

1) Those with only vegetative faculties.

2) Those with vegetative and reproductive faculties.

3) Those endowed with vegetative and reproductive faculties and capable of some communication through imperfect language carried on by words and gesture.

DeQuervain and Wegelin (6) reported also that in an endemic goiter region there are different types of cretinism: "le crétinisme endémique est loin de présenter toujours le même tableau. Il y a d'abord des differences de degré." Nevertheless there is a remarkable similarity (Figure 3) in cretins studied in different areas of the world (1, 6, 15).

A number of authors (3, 8) do not accept the relationship between iodine deficiency and cretinism. They present the findings of generally normal ^{131}I thyroid uptake and $PB^{127}I$ as evidence against this relationship. However, one of the strongest arguments in favor of a deficiency of iodine as the causative factor in endemic cretinism is the remarkable lowering of the incidence of the disease by the establishment of iodine prophylaxis.

In our survey 0.5 per cent of the population studied in Mato Grosso presented cretinism and/or deafmutism: 0.17 per cent cretinism and 0.33 per cent deafmutism. In Goiás, where endemic goiter and cretinism are also severe, the prevalence of deafmutism is 0.8 per cent (12).

Several authors believe that even minor degrees of hypothyroidism, which may be practically symptomless, may seriously affect the reproductive process (4, 9), either by decreasing the fertility of affected women or by the production of abortion. Kemp (11) investigated the cause and incidence of unexplained stillbirths among the female white population of Vancouver and showed that there was a marked decrease in occurrence of stillbirths in women treated with iodine during the last three months of pregnancy. Hoet (10) treated a group of patients with a history of pregnancy wastage (congenital malformations, miscarriages, and stillbirths) with thyroid extracts during subsequent pregnancies and showed improvement. More recently Naumoff and Shook (15) studied a group of patients who had at least one early spontaneous abortion and showed in 48.4 per cent of the cases a response to administration of thyroid stimulating hormone characteristic of low thyroid reserve.

In our results the occurrence of goiter in one or both parents did not change the fertility rate, the prenatal mortality, or the prevalence of congenital malformations. Thus we may assume that during pregnancy there is not even a mild degree of hypothyroidism in our cases.

Stanbury (18) believes that cretinism is the result of a functional thyroid deficiency occurring during embryogenesis or early postnatal life. The normal thyroid function observed later in these patients would be the result of a greater supply of iodine. The difference between the neurological picture observed in these patients and in athyreotic hypothyroid cases can be explained by assuming that in thyroid agenesis the mother is normal and there

Defective female child studied by Choufoer, Van Rhijn, and Querido (1) in New Guinea, 1965.

Endemic cretin studied by Carlos Chagas in an endemic goiter region in Brazil (Minas Gerais), 1909.

Myxedematous cretin studied by De Quervain and Wegelin (6) in Switzerland.

Endemic cretin studied by Labo et al. in Mato Grosso, Brazil.

Typical endemic cretin as observed by Stanbury et al. (14) in Tocachi, Ecuador.

Endemic cretin studied by Labo et al. (12) in Chapada, Mato Grosso, Brazil.

Figure 3. Comparison of cretinism in Brazil with the disease as seen in New Guinea, Switzerland, and Ecuador.

is enough placental transfer of sufficient maternal thyroid hormone. In endemic regions, however, the lack of iodine would make the hormonal level of the mother low, and thus it would not be possible to have adequate transfer of thyroxine to the fetus. The reduced iodine supply to the fetus would produce then a transitory phase of hypothyroidism. Our results, however, do not seem to show the occurrence of a transient maternal hypothyroidism during pregnancy. The relationship of endemic cretinism and embryonal development is not clear and merits further research.

Endemic Goiter

Endemic goiter (1 + 2 + 3, WHO classification) was found in about 49 per cent of the female and 35 per cent of the male population of Mato Grosso (Table 1). The prevalence of goiter decreases after 14 years in the males (a more marked fall is observed after 20 years); this is not found in the female.

Clements (2) reported also that there is a rise in the rate of thyroid enlargement up to 12 years of age in both sexes, and this continues in the girls into early adult life; in boys the rate commences to fall after 14 years.

A higher prevalence of goiter was found in our series when the social status of the family was lower (assessed by several indices, i.e., housing conditions, density of individuals per room in the house, education and profession of the spouses).

Although a higher proportion of Negroes lived in worse social and sanitary conditions, analyzing the goiter prevalence in Negroes or whites living in different social conditions permitted us to show that both factors really interfere with goiter prevalence.

A higher prevalence of goiter was found when patients were born and when they were married in the rural areas in the endemic zone, as compared to patients coming from villages or from outside the region. Although there are more Negroes living in rural areas, socio-sanitary conditions are worse there and both factors are probably interfering with goiter prevalence.

McCarrison noted (13) that the incidence of goiter in the villages along the rivers of the Himalayas increased progressively downstream. The water was used for drinking, as well as for bathing and sewage disposal. He suggested that gastrointestinal organisms which polluted the water were responsible for the goitrogenesis. McCarrison fed the residue of a filtration of feces to native subjects and induced goiter; no goiter appeared when the fecal residue was boiled before feeding, or when individuals were treated with intestinal antiseptics.

Recently Vought et al. (19) reported a higher prevalence of goiter in Negroes of all ages and in both sexes as compared to the white population of northern Virginia. They proposed the hypothesis that unprotected water supplies may exert a goitrogenic effect. The higher prevalence of goiter in the Negro group was explained by the fact that fewer Negro families than whites had access to protected water supplies. No difference was found in the iodine intake of white and Negro populations. These authors proposed that the goitrogenic factor may be of a microbial nature since they found that 70 to 90 per cent of private springs and dug wells yielded *E. coli* on analysis by standard methods.

Our results (Table 8) did not show significant differences in the goiter prevalence in relationship to the water supply. However, a bacteriological study of the water collected in different towns of Mato Grosso was not done.

The analysis of our data shows that there is a definitely higher incidence of goiter in Negroes, as compared to whites living in the same region, and a higher prevalence of goiter in worse socioeconomic and sanitary conditions.

The relationship between sanitary conditions and goiter is not clear and needs further investigation.

SUMMARY

Endemic goiter and cretinism are common in the midwestern region of Brazil. Data were obtained from 1,525 families and analyzed for factors related to the prevalence of these diseases.

Cretinism was found in 0.17 per cent and deafmutism in 0.33 per cent of the population.

Goiter (1+2+3, WHO classification) was found in 61 per cent of the female and 35 per cent of the male population. The prevalence of goiter decreased after age 14 in the males; this was not found in the females in which there was a decrease in the relative frequency of goiter 1 after 20 years and an increase in the frequency of goiter 2. Goiter 3 was not found before age 20 in females and before age 40 in males.

The frequency of goiter was higher in the mulatto and Negro groups than in the white group, and goiter was more frequent in rural than in urban areas. Goiter was significantly correlated with socioeconomic status. There was no relationship between goiter prevalence and water supply.

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CHAPTER 18

STUDIES ON ENDEMIC GOITER AND CRETINISM IN BRAZIL

2. GENETIC STUDIES[1]

Luiz Carlos Galvão Lobo,[2] A. Quelce-Salgado,[3] and A. Freire-Maia[4]

The use of seaweed as an efficient remedy for goiter was attributed to the Chinese. A relationship between iodine and goiter was suspected soon after Courtois (10) isolated this element in 1811 from ashes of seaweed. Coindet (8), Boussingault (2), and Chatin (4) were among the first to stress the use of iodine in the treatment of goitrous patients.

In 1915 Marine (18) stated: "The primary effect on the thyroid of iodine want is the production of hyperplasia; the supplying of adequate amounts of iodine will cause involution of almost any hyperplastic thyroid."

In general, analyses of drinking water or of 24-hour collections of urine give low values in endemic goiter areas. This is not always true. Reports have shown high frequency of goiter in areas where iodine deficiency is not marked (9, 12, 27), or a low incidence of goiter in regions where iodine deficiency is severe (5, 23). Goiter is generally observed in mountainous areas distant from the sea. Nevertheless, there are several studies showing the occurrence of endemic goiter in coastal regions (3, 9, 17).

Although the relationship between thyroid hyperplasia and iodine deficiency is well established, several authors stress the fact that in an endemic goiter region there are remarkable differences in both goiter occurrence and goiter size in individuals living in the area. No difference in clinical history has been found in these individuals, except for a higher percentage of goitrous relatives in the goitrous subjects (24, 25).

Several studies have stressed the importance of hereditary factors in goiter prevalence. One of the earliest was that of Riebold (22) who, stressing the higher prevalence of goiter in women, concluded that there is probably a sex limited-gene producing the disease. Siemens (26) pointed out that although endemic goiter is the result of various conditions, such as infections and iodine lack, sporadic goiter is an inherited disease. The differences in the frequency of goiter according to sex were explained as merely reflecting

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the more important role played by the thyroid in the female. von Pfaundler (28) in 1924 showed that goiter in Bavaria tended to appear in sibs with unexpected frequency. According to him the sensitiveness of the thyroid to external conditions may be passed from generation to generation. Bauer (1) in 1926 concluded that goiter was produced by an autosomal dominant sex-limited gene.

In 1932 Davenport (11) studied ten families starting with nongoitrous propositi and 97 families with goitrous propositi. The study involved a total of 2,268 males and 2,462 females. No difference in goiter prevalence was found when well water or city water was used by the patients. The prevalence of goiter was 4.63 per cent in males and 24.78 per cent in females. Family analysis allowed him to conclude that goiter can be produced under suitable environmental conditions, whenever the individual possesses a dominant sex-linked gene G' combined with a dominant autosomal gene G".

ENDEMIC CRETINISM

The term cretinism has been widely used all over the world and has been applied to describe either endemic or sporadic patients. The lack of a precise definition of cretinism comes from our ignorance regarding the etiopathogenesis of this disease. On the basis of the recommendations of the Pan American Health Organization Scientific Group on Research in Endemic Goiter (21), a cretin may be described as an individual with irreversible changes in mental development, born in an endemic goiter area, and presenting a combination of some of the following syndromes not explained by other causes: neuromuscular disorders; abnormalities in hearing and speech, leading in some cases to deafmutism; impairment of somatic development; and hypothyroidism. Although the relationship between endemic goiter and cretinism has been always emphasized, some (7, 16) have stressed the fact that endemic cretinism does not occur in all localities of an endemic goiter region. Some areas have a long history of endemic goiter without cretinism (the Jura region, for example).

The reports of McCarrison and of the Sardinian Cretinism Commission stressed that the prevalence of cretinism is higher in areas where consanguineous marriages occur more frequently (19, 20). The prevalence of cretinism decreases when one of the spouses comes from outside the endemic goiter region. These observations tended to validate the hypothesis that cretinism is produced by genetic factors (6). On the other hand, Eugster (14) refuted this possibility by showing the discordance among monozygotic twins in respect to cretinism. The report of a mating of two cretins resulting in a normal child is also an argument against the possibility of recessive inheritance.

Frazer's studies (15) in Yugoslavia do not seem to support the possibility of a simple genetic hypothesis in the genesis of cretinism. Furthermore these studies stress the fact that genetic determinations in goiter intervene only in a secondary manner to affect susceptibility to the primary environmental cause.

MATERIALS AND METHODS

Our study is based on the results of a goiter and cretinism survey made in the state of Mato Grosso (15). A total of 1,525 families and 6,683 sibs

were studied. Genetic data were obtained by a group of eight locally trained interviewers, under the supervision of two members of the genetic group. All questionnaires were revised and 30 per cent of them checked by a different interviewer. The collected data were codified by the genetic team and processed in a computer.

RESULTS

Endemic Goiter

The prevalence of goiter by inbreeding coefficient F and by sex appears in Table 1. The differences in the frequencies in both groups were not statistically significant ($X^2 = 0.293$, $p > 0.50$ for the females, and $X^2 = 0.133$, $p > 0.70$ for the males).

Table 1. Prevalence of goiter by inbreeding coefficient.

	Females					Males				
	T	N	%	X^2	P	T	N	%	X^2	P
F = 0	1,279	785	61.4			729	253	34.7		
F > 0	96	57	59.4	0.293	>0.50	40	15	37.5	0.133	>0.10
Total	1,375	842	61.2			769	268	34.8		

T = Number of patients examined.
N = Number of patients with goiter 1+ 2+ 3 (WHO classification).

A significantly higher prevalence of goiter was found in Negroes and mulattoes as compared to the whites in different socioeconomic conditions (Table 2).

The study of mortality in the sibs in relationship to goiter in the parents is shown in Table 3. There is no statistically significant relationship between frequency of abortion and occurrence of goiter in the parents. However, the differences in the frequency of stillbirths in relationship to goiter in the parents is statistically significant.

A significant difference was also found in relation to the number of sibs that died after birth, but in this case the occurrence of goiter in both parents decreased the mortality in the sibs.

There was no statistically significant difference in the total mortality of the sibs when the parents had no goiter or when both parents had goiter ($X^2 = 0.025$, $p > 0.80$).

Table 4 shows the prevalence of anomalies in the sibs in relationship to goiter in the parents; no statistically significant differences were found ($X^2 = 5.21$, $p > 0.10$).

Table 2. Prevalence of goiter by ethnic group and socio-sanitary conditions.

Ethnic group	Housing conditions						Density of individuals per room of the house						Profession of the husband					
	Very poor (1)			Reasonable or good (2)			>2 (1)			0-2 (2)			Unskilled worker			Trained worker		
	T	N	%	T	N	%	T	N	%	T	N	%	T	N	%	T	N	%
White (W)	371	173	46.6	226	60	26.6	336	152	45.2	262	81	30.9	209	92	44.0	389	141	36.2
Mulatto (M)	679	409	60.2	363	179	49.3	690	415	60.1	358	176	49.2	421	265	62.9	626	325	51.9
Negro (N)	381	236	61.9	202	100	49.5	464	281	60.6	119	55	46.2	333	205	61.6	250	131	52.4
Total	1,431	818	57.2	791	339	42.9	1,490	848	56.9	739	312	42.2	963	562	58.4	1,265	597	47.2

T = Number of patients examined.
N = Number of patients with goiter 1+ 2+3 (WHO classification).

Statistical analysis

Housing conditions		W x M x N
	(1)	$X^2_2 = 22.64$, $P < 0.01$
	(2)	$X^2 = 35.72$, $P < 0.01$
Density of individuals in the house	(1)	$X^2_2 = 23.83$ $P < 0.01$
	(2)	$X^2 = 21.79$ $P < 0.01$
Profession of the husband	(1)	$X^2_2 = 22.59$ $P < 0.01$
	(2)	$X^2 = 27.28$ $P < 0.01$

Table 3. Mortality in the sibship (abortions, stillbirths, and deaths after birth) in relationship to goiter in the parents.

Parents	Pregnancies	Abortions	%	Stillbirths and live-born sibs	Stillbirths	%	Live-born sibs	Death sibs after birth	%	Pregnancies	Total mortality	%
A	3,060	267	8.7	2,543	96	3.8	2,436	311	12.8	3,060	674	22.0
B	494	54	10.9	441	22	5.0	419	60	14.3	494	136	27.5
C	3,918	356	9.1	3,297	156	4.7	3,138	306	9.7	3,918	818	20.9
D	1,009	106	10.5	826	46	5.6	780	68	8.7	1,009	220	21.8
X^2		5.49			9.13			21.73			11.48	
P		> 0.05			< 0.05			< 0.01			< 0.01	

Parents	Male		Female
A=	non-goiter	x	non-goiter
B=	goiter	x	non-goiter
C=	non-goiter	x	goiter
D=	goiter	x	goiter

Table 4. Prevalence of anomalies in the sibship in relationship to goiter in the parents.

Parents	N	T	%	X^2	P
A	51	2,002	2.5		
B	8	349	2.3	5.21	0.10
C	78	2,660	2.9		
D	9	664	1.3		
Total	146	5,675	2.6		

N = frequency of sibs with congenital abnormalities.
T = total number of sibs.

Parents	female x	male
A	non-goiter	non-goiter
B	non-goiter	goiter 1+2+3
C	goiter 1+2+3	non-goiter
D	goiter 1+2+3	goiter 1+2+3

The frequency of goiter in the sibs in relationship to goiter in the parents and profession of the father is shown in Table 5.

A significantly higher prevalence of goiter in the sibship was observed when one or both parents had goiter as compared to the prevalence observed when no parent had goiter. These differences are statistically significant in both socioeconomic groups (the father is an unskilled or is a trained worker). Nevertheless, the frequencies observed when the father is an unskilled worker are statistically higher than those obtained when the father is a trained worker.

Endemic Cretinism

The prevalence of endemic cretinism in the population was 0.17 per cent and the prevalence of deafmutism 0.33 per cent. The number of patients examined was not sufficient to permit a genetic analysis. However, we studied the sibs of six endemic cretins. All were clinically normal; radioisotope studies of two sibs showed normal thyroid function, and radiological examinations made immediately after birth in one case and at four months in another showed normal bone age. Since the father was unknown in all cases, no family data could be obtained.

DISCUSSION AND CONCLUSIONS

There is no effect of inbreeding on goiter prevalence in the population studied (Table 1). This indicates that endemic goiter is not produced by a

Table 5. Prevalence of goiter in the sibs in relationship to goiter in the parents and to the profession of the father.

Goiter parents	Unskilled worker(1)					Trained worker(2)					Profession (1+2)				
	T	N	%	X^2	P	T	N	%	X^2	P	T	N	%	X^2	P
A	1,564	163	10.42			610	56	9.18			2,174	219	10.07		
B + C	1,311	292	22.27	115.82	<0.001	337	45	13.35	22.27	<0.01	1,648	337	20.45	141.35	<0.001
D	819	215	26.25			124	29	23.15			943	244	25.87		
Total	3,694	670	18.14			1,071	130	12.14			4,765	800	16.79		

T = Number of patients examined.
N = Number of patients with goiter 1+2+3 (WHO classification).

A *Absence of goiter in both parents.*
B+C *Goiter in the father or the mother.*
D *Goiter in both parents.*

rare autosomal recessive gene. This hypothesis could be excluded also by the high prevalence of goiter in the area.

Both ethnic factors and socioeconomic conditions influence the prevalence of goiter. Mulattoes and Negroes have a higher prevalence of this disease than white persons living in the same region. People living in worse socioeconomic conditions also have a higher frequency of goiter, as compared to those living under better situations. These facts point to an interaction between genetic and environmental influences in goitrogeneses. This hypothesis is confirmed also by analysis of family data (Table 5).

Analysis of mortality frequencies in sibs (Tables 3 and 4) shows that there is a higher increase in prevalence of stillbirths only when one or both parents have goiter. This can mean the occurrence of natural selection at this level.

Analysis of family data indicates that the prevalence of goiter in sibs is in direct relationship to the occurrence of goiter in the parents. We can observe in Table 5 that when one or both parents have goiter the prevalence of this disease in the sibs is higher than the prevalence obtained when there is no goiter in the parents ($X^2 = 141.35$, $p < 0.001$). This observation points out the familial influence, probably genetic, in the production of endemic goiter.

In order to study the influence of socioeconomic factors on this observation, the population was split in two: (1) those living in poor socioeconomic situations, as assessed by the profession of the husband; and (2) those living in better socioeconomic conditions. In both groups there is a higher prevalence of goiter in sibs when one or both parents have goiter, as compared to the frequency observed when there is no goiter in the parents ($X^2 = 115.8$, $p < 0.001$ for socioeconomic condition 1, and $X^2 = 22.27$, $p < 0.01$ for socioeconomic condition 2). Nevertheless, the prevalence of goiter was still higher when the family lived in a worse socioeconomic situation ($X^2 = 19.84$, $p < 0.01$). These observations indicate the interference of both genetic and environmental factors in the genesis of endemic goiter.

The small number of cretins studied, the lack of information about the parents in several cases, the unknown fatherhood of a few cretin sibs evaluated by us, and the fact that very often these patients are encountered in institutions where there is generally no information about families made impossible a genetic study on the cretins examined in Mato Grosso.

SUMMARY

A total of 1,525 families and 6,683 sibs were studied in the State of Mato Grosso, Brazil. There appeared to be no relationship between goiter and the inbreeding coefficient.

Stillbirths among goitrous mothers had a higher incidence than in non-goitrous mothers, but there was no difference in postnatal mortality. There was a significantly higher prevalence of goiter in sibships when one or both parents had goiter, irrespective of socioeconomic class.

The sibs of six endemic cretins found in this population were all clinically normal.

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CHAPTER 19

STUDIES ON ENDEMIC GOITER AND CRETINISM IN BRAZIL

3. THYROID FUNCTION STUDIES[1]

Doris Rosenthal, Luiz Carlos Galvão Lobo,[2] Moacyr A. Rebello, and Jacques Fridman

Studies of thyroid function were performed in 397 inhabitants of an endemic goiter areas. Forty of these were cretins. They lived in the middle-west region of Brazil in six different townships (Table 1). This region is known to be an area of endemic goiter and cretinism (12); it is a plateau approximately 800 meters above sea level (see Chapter 17, Figure 1).

METHODS

All patients were examined by at least one, and usually by two members of the research team. No patient presenting symptoms of hyperthyroidism was found.

Radioiodine thyroid uptake was measured 24 hours after the administration of a 20-50 μc dose of ^{131}I. Blood samples were obtained 24 and 48 hours after radioisotope administration for the determination of $PB^{131}I$ conversion ratio, triiodothyronine resin uptake (9), $PB^{127}I$, $BE^{127}I$, $BI^{127}I$, $T^{127}I$, and antithyroglobulin autoantibodies (4).

In those subjects on whom iodine kinetic studies were performed a 100 μc dose of radioiodine was administered intravenously. Thyroid uptake was measured and blood samples taken at different times up to 72 hours. Radioiodine determinations were performed locally; the other tests were done in Rio de Janeiro.* In some instances radioiodine uptake was repeated after the administration of 100 μg of 3,5,3'-l-triiodothyronine for a 10-day period (suppression test), and after thyroid stimulation by 30U exogenous TSH** (stimulation test).

Serum and urine (24-hour collections checked for completeness by creatinine determination) were kept frozen until analysis. The thyroid glands of seven endemic cretins (Goiás) and 21 goitrous patients from Brasilia and

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* ^{127}I determinations were done at the Boston Medical Laboratory.

** Ambinon, Organon Laboratories.

Table 1. Patients studied in endemic goiter areas.

Towns	Non-cretins	Cretins	Total
Mato Grosso (MT)			
Agua Fria	8	-	8
Diamantino	54	8	62
Chapada	80	-	80
Livramento	90	8	98
Federal District (DF)			
Brasilia	7	-	7
Goiás (GO)			
Goiânia	118	24	142
Totals	357	40	397

Rio de Janeiro were cooled immediately after surgery and the iodoproteins (labeled by prior administration of a 1 mc dose of ^{131}I) were studied by several techniques (7, 11).

RESULTS

Thyroid Function Tests

(1) Non-cretins: Thyroid function tests on patients with endemic goiter and in controls living in a nonendemic goiter area (Rio de Janeiro) appear in Table 2 (9). There are marked differences among the results obtained in the townships of the endemic area. In the State of Mato Grosso a higher ^{131}I mean uptake value was found in Agua Fria and Diamantino, small and isolated villages (there is no statistically significant difference between the results obtained in these two towns), than in Chapada and Livramento, villages situated nearer the state capital, to which they are connected by better roads. Urinary ^{127}I varied in accordance, showing lower values in Diamantino. $PB^{131}I$, conversion ratio, $PB^{127}I$, $BE^{127}I$, $BI^{127}I$, and $T^{127}I$ values were not statistically different in the villages of the area.

A small number of patients were observed in Brasilia as part of a general iodine kinetic study. The uptake values were higher than those obtained by Ulyssea (13) in normal individuals living in Brasilia, but were comparable to the values found in goitrous patients. Studies were also done in the Goiás State (6) in the city of Goiânia.

Table 2. Thyroid function tests in endemic goiter patients.

Locality	N	I^{131} uptake(%)	PBI^{131}(%/l)	RC(%)	PBI^{127}(ug%)	Urinary I^{127} (ug/24h)	BEI^{127}(ug%)	BII^{127}(ug%)	TI(ug%)
MT									
1 Agua Fría	8	72.0± 5.4	0.24±0.24	63.5±21.9	-	-	-	-	-
1 Diamantino	54	68.1±16.4	0.10±0.06	50.5±25.8	3.82±0.88	47.5±10.3	2.87±0.56	0.65±0.13	4.75±1.22
"t"		ns	ns	ns					
2 Chapada	80	57.7± 7.3	0.21±0.07	49.9±26.6	5.26±1.55	47.8±37.8	4.36±1.45	0.76±0.20	5.58±1.45
2 Livramento	90	49.4±13.5	0.18±0.21	52.7±24.9	4.24±1.37	77.0±11.0	3.44±1.70	0.72±0.11	4.07±0.54
"t"		ns	ns	ns	ns	p < 0.01	ns	ns	ns
"t" 1 x 2		p < 0.05	ns	ns	ns		ns	ns	ns
GO									
Goiânia	118	49.00±19.00	0.26±0.18	51.5±25.0	5.79±1.90	82.0±44.0	-	-	-
"t" x MT 1		p < 0.01	ns	ns	p < 0.01	p < 0.01			
x MT 2		ns	ns	ns	ns	p < 0.01			
DF									
Brasilia	7	63.16±10.12	-	-	4.82±1.47	100.4±14.6			
"t" x MT 1		ns	-	-	ns	p < 0.01			
x MT 2		p < 0.01	-	-	ns	p < 0.01			
x GO		p < 0.05			ns	p < 0.02			
References									
Brasilia (13) E	417	48.6±14.0	-	-					
DG	41	58.1±18.0	-	-					
NG	96	52.4±19.0	-	-					
Rio (10) E	858	38.2±13.7	0.10±0.10	30.5±23.2	5.50±1.00				
DG	429	39.8±15.4	0.12±0.12	31.8±23.1					
NG	639	38.4±16.6	0.11±0.08	35.7±24.8					

N = Number of patients studied.
E = Euthyroid.
DG = Diffuse goiter.
NG = Nodular goiter.
ns = Non-significant.

Statistical analysis showed that differences between the mean results of thyroid uptake in Chapada, Livramento, and Goiânia were not significant, whereas the differences between the values found in these towns and those obtained in Agua Fria, Diamantino, and Brasilia were significant.

The mean result of the $PB^{127}I$ determinations in Mato Grosso (all towns) is statistically different from the mean value from Goiás. Both areas are statistically different from Rio de Janeiro in relation to radioiodine thyroid uptake, but there are no differences among the mean values of $PB^{127}I$ in Goiânia, Brasilia, and Rio de Janeiro.

Although none of the patients showed clinical signs of hyperthyroidism, in 13 cases of nodular goiter there was no decrease in the thyroid uptake after triidothyronine suppression tests. Presumably these patients had autonomous thyroid nodules.

Triidothyronine resin uptake showed normal mean values in Chapada, Diamantino, and Livramento. A statistical analysis of all results showed that there was a direct and significant correlation betwen these values and those of the $PB^{127}I$ ($P < 0.01$), as might be expected.

(2) Cretins: Forty endemic cretins were studied; 16 were in Mato Grosso (Diamantino and Livramento) and 24 in Goiás (Table 3). All showed oligophrenia, short stature, and neuromuscular disorders, as described previously (8, 10). The age range was between 18 and 65 years; there were nine men in the total group. Deafmutism was present in 19 patients. Nodular goiter was found in 26, diffuse goiter in six, and in eight others the thyroid was palpable.

Cytogenetic studies in eight cretins showed in all but one, reported elsewhere (5), a normal karyotype. Spasticity was the typical neurological finding in these cretins. Pneumoencephalograms done on nine revealed no noteworthy abnormalities; electroencephalograms showed diffuse anomalies with slow waves.

The results of thyroid function tests were compared with those found in non-cretin inhabitants of the same area. In Goiás, where the goiter endemicity is not as severe as in Mato Grosso and the iodine intake is higher, no significant differences were obtained between the values from cretins and controls. On the other hand, the thyroid ^{131}I uptake was significantly lower in the cretins of Mato Grosso, as compared to controls living in the area, as well as to cretin and non-cretinous patients living in Goiás.

An analysis of Table 3 shows that although the mean values for thyroid radioiodine uptake of non-cretins varied in accordance with iodine intake, as judged by the urinary ^{127}I excretion, this was not true for the cretinous subjects.

There was no hypothyroidism in these patients. They respond well to TSH stimulation, as can be seen in Table 4. In a few instances in which $PB^{127}I$ was determined an increase over the basal level after TSH stimulation was found. This indicated the existence of a functional reserve. Triiodothyronine suppression tests in six subjects showed a normal response.

Table 3. Thyroid function tests in cretins and non-cretins.

Locality	N	I^{131} uptake (%) $\bar{X} \pm \delta$	PBI^{127} (ug%) $\bar{X} \pm \delta$	Urinary I^{127} (ug%) $\bar{X} \pm \delta$
MT				
1) Diamantino				
Non-cretins	54	68.1 ± 16.4	3.82 ± 0.88	47.5 ± 10.3
Cretins	8	24.7 ± 13.2	3.11 ± 1.63	-
"t"		$p < 0.01$	ns	
2) Livramento				
Non-cretins	90	49.4 ± 13.5	4.24 ± 1.37	77.0 ± 11.0
Cretins	8	20.9 ± 7.3	3.04 ± 1.25	-
"t"		$p < 0.01$	$p < 0.01$	
"t" Non-cretins		$p < 0.01$	$p < 0.05$	$p < 0.01$
"t" Cretins		ns	ns	-
TOTAL MT				
Non-cretins		-	4.39 ± 1.48	-
Cretins		22.82 ± 10.7	3.07 ± 1.38	
"t" Cretins x non-cretins		-	$p < 0.01$	
GO				
Non-cretins	118	49.00 ± 19.0	5.79 ± 1.90	82.0 ± 44.0
Cretins	24	32.62 ± 4.7	5.50 ± 0.84	-
"t"		ns	ns	-
"t" MTxGO				
Non-cretins		-		-
MT(1)xGO		$p < 0.01$	$p < 0.01$	$p < 0.01$
MT(2)xGO		ns	$p < 0.05$	ns
Cretins		$p < 0.01$	$p < 0.01$	-

N = Number of patients studied.

$\bar{X}$ = Mean.

δ = Standard deviation.

ns = Non-significant.

Table 4. Stimulation test.

	N	Initial uptake (%) $\bar{X} \pm \delta$	Uptake after 30 U -TSH* $\bar{X} \pm \delta$
MT. Non-cretins with low uptake	4	12.8 ± 4.8	83.4 ± 15.3
MT. Cretins	6	23.8 ± 5.5	70.6 ± 8.4
Cretins x non-cretins		$p < 0.02$	ns
GO. Cretins	9	41.1 ± 14.9	63.1 ± 2.8
"t" Cretins MT x GO		$p < 0.01$	ns

N = Number of patients studied.

$\bar{X}$ = Mean.

δ = Standard deviation.

ns = Non-significant.

* = Ambinon, Organon.

Plasma iodoamino acids were analyzed by chromatography in five cases (7, 10). In only one case iodotyrosines were found in the plasma in addition to thyroxine. No plasma iodoproteins were found.

STUDIES ON IODINE KINETICS

Iodine kinetics were studied in three goitrous non-cretins and eight cretins living in Diamantino (Mato Grosso) and in nine goitrous non-cretins living in the Brasilia region. The results obtained were compared with those from three subjects with autonomous thyroid nodules studied in Brasilia, and from one hypophysectomized and three goitrous patients evaluated in Rio de Janeiro (Table 5).

The results obtained in goitrous non-cretins examined in Brasilia and Rio were comparable with those found by Beckers (1) in patients with sporadic goiter in Belgium. There was, however, a low value for the half-time of fall in the plasma iodide in the cretins and non-cretins examined in Mato Grosso. The iodide space was smaller in these patients, and since the rate constant of thyroidal accumulation of iodide was not higher in the cretins, an increase of the rate constant of the renal disposal of iodide was postulated. A higher rate constant of renal disposal of iodide was also found in the hypophysectomized patient studied in Rio, but in this case there was a lower value of the rate constant of thyroidal accumulation of iodide. Cassano et al. (2, 3) have also reported a higher renal clearance of iodide in goitrous patients.

Table 5. Kinetic studies of iodine metabolism (mean values).

Locality	$K_1 + K_5$	K_1	K_5	V_1	t 1/2 T^*_4	K_3	I^{131} Uptake	PBI^{127}	I^{127} Urine
DIAMANTINO									
Cretins (C)	59.3%/h	15.3%/h	44.0%/h	12.1L	5.76 d	12.1%d	24.7%	3.11µg%	
Non-cretins (G)	42.3%/h	26.6%/h	15.8%/h		-	-	68.1%	3.82µg%	47.5µg/24h
"t" test	ns	$p < 0.05$					$p < 0.01$	ns	
BRASILIA									
Goitrous (G)	25.7%/h	16.8%/h	11.5%/h	28.71	6.80 d	10.7%d	63.2%	4.82µg%	100.4µg/24h
Aut. nodules	47.1%/h	34.3%/h	12.7%/h	17.11	-	-	63.7%	5.20µg%	98.2µg/24h
"t" test	$p < 0.05$	$p < 0.05$	ns	$p < 0.05$			ns	ns	ns
RIO DE JANEIRO									
Goitrous	27.4%/h	18.2%/h	9.2%/h	15.41	-	-	65.3%	-	-
Hypophysect	30.6%/h	6.4%/h	24.1%/h	-	-	-	19.0%	-	-
"t" test	ns	$p < 0.05$	$p < 0.05$				$p < 0.05$		
"t" TEST									
G,Bras x G.Dia.	$p < 0.01$	$p < 0.01$	ns				ns	ns	$p < 0.01$
G,Bras x C.Dia.	$p < 0.01$	ns	$p < 0.01$	$p < 0.01$	ns	ns	$p < 0.01$	ns	
G,Bras x G.Rio	ns	ns		$p < 0.05$	ns		ns		

K_1 = Constant rate of thyroidal iodide accumulation.

K_5 = Constant rate of renal disposal of iodide.

K_3 = Constant rate of hormonal iodide.

V_1 = Iodide space.

T_4* = Tagged thyroxine.

ns = Non significant.

Thyroid hormone catabolism was normal in all cases, as shown by the determination of the biological half-life of injected labeled thyroxine in cretin and non-cretin goitrous patients treated with carbimazole.

THYROIDAL IODOPROTEINS

Studies in nine endemic cretins and in 12 goitrous patients showed a definite increase in the relative proportion of an iodinated protein with physical-chemical and immunological characteristics of human serum albumin. In each case a marked hyperplasia of the thyroid was found, as well as a higher concentration of 3-monoiodotyrosine as compared to 3,5-diiodotyrosine. A detailed description of these finding has been published elsewhere (8, 11).

ANTITHYROIDAL AUTO-ANTIBODIES

Circulating antithyroidal auto-antibodies were determined using the technique of hemagglutination of tanned and formolized red cells sensitized to thyroglobulin. The sera of 404 goitrous patients were examined; positive results were obtained in 52. Only five had a titer of 1/2,500 and two of 1/25,000. No cretin showed a titer higher than 1/25 (Table 6).

The results observed in goitrous patients of Mato Grosso and Goiás were compared with those found in euthyroid subjects and in patients with diffuse and nodular goiter studied in a nonendemic goiter area (Rio de Janeiro). Statistical analysis showed that the frequency observed in the endemic goiter region was significantly lower than that observed in the control region ($X^2 = 25.61$, $P < 0.01$).

SUMMARY

No evidence of significant thyroid dysfunction has been found in a group of endemic cretins from central Brazil. These subjects were clinically euthyroid, and their neuromuscular disorders were of a different type from those found in congenital hypothyroidism. The $PB^{127}I$ levels in Mato Grosso and Goiás were not significantly different as compared to normal subjects living in the same area.

Nevertheless there are significant differences between the two groups in some aspects of the iodide cycle, and these differences are greatest in the townships where iodine deficiency is most marked. Thyroid uptake values of non-cretins were inversely proportional to iodine intake, but this was not found to be true in cretins; these patients maintained remarkably similar levels of thyroid uptake in areas of dissimilar iodine supply.

No significant differences were found in thyroid hormone catabolism between goitrous cretins and non-cretins living in the same or different areas. A significant difference in the rate of renal iodide disposal was found between the goitrous cretins and non-cretins of Diamantino. This may be explained by assuming that the thyroid is working at maximal capacity in the cretins. This explanation seems doubtful, since a normal response was obtained after thyroid stimulation by exogenous TSH. Since chromatographic studies of plasma from the cretins failed to show abnormal iodinated proteins, we can assume that the PBI increase observed after TSH stimulation was due to the thyroid hormones.

Table 6. Antithyroglobulin auto-antibodies (TRC).

Positive (titer)

Region	Negative		5		25		250		2500		25000		250000		Total positive		Total subjects
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	
Mato Grosso +	168	92.8	8	4.8	3.	1.8	1	0.6	1.	0.6	-	-	0	0.0	13	7.2	181
Goiás +	184	82.5	15	6.7	12	5.4	6	2.7	4.	1.8	2	0.9	0	0.0	39	17.5	223
Rio ++																	
Euthyroid	209	82.3	9	3.5	19	7.5	9	3.5	5.	2.0	3.	1.2	0	0.0	45	17.7	254
Diffuse goiter	175	74.8	24	10.3	12	5.1	14	6.0	7.	3.0	2.	0.8	0	0.0	59	25.2	234
Nodular goiter	308	77.4	32	8.4	30	7.6	14	3.5	1.	2.5	3.	0.7	1	0.2	90	22.6	398

N= Number of patients studied.
\+ Endemic goiter area.
++ Nonendemic goiter area.

The positive response to TSH found in our group of endemic cretins as well as the increased rate of renal iodide disposal found in the hypophysectomized patient studied in Rio de Janeiro indicates a possible pituitary involvement in endemic cretinism which may merit further investigation. Although the neurological state of the cretins studied has been defined, the relationship of these findings to embryonal development as governed by the availability of thyroid hormone is not clear and also needs further research.

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SECTION V

ENDEMIC GOITER IN CHILE

CHAPTER 20

ENDEMIC GOITER IN CHILE

José Barzelatto, M.D.[1]

The first known historical facts concerning endemic goiter in Chile are dated at the beginning of the 19th century, when some reports stated that it was uncommon and recent (16, 22), while others indicated that it was very frequent (15, 27). The popular term for goiter in Chile is "coto," a word of Quechua origin which was present in the language of the Indians prior to the Spanish Conquest. There has been a prolonged controversy as to whether endemic goiter was present in pre-Columbian times (16, 17, 23, 29).

It is dangerous to draw conclusions regarding goiter from non-medical documents. This is especially true in Chile, which was consecutively the frontier of the Inca and the Spanish Empires in their wars to dominate the Araucanians. Organized medicine began in Chile in 1833. In that year the Chilean government created the Medical School of the University of Chile and sent a group of young people to study medicine in Europe. The Chilean Medical Society was founded in 1869 and three years later began to issue its journal, the Revista Médica de Chile. In its pages, in 1884, one finds the first Chilean medical publication on endemic goiter; it remarked on the high prevalence of the disease in a rural area near Santiago, attributed the cause to iodine deficiency, and made a plea for national legislation for the addition of potassium iodide to salt (18). It is unfortunate that this sound advice still has not become a reality. During the first half of the present century a few scattered papers called attention to endemic goiter as a problem circumscribed to some isolated and small communities (2, 16, 22, 27).

Radioiodine was introduced in Chile for the study of thyroid disease in 1954. A high proportion of euthyroid patients from all over the country, with or without goiter, were found to have high thyroid ^{131}I uptakes. This suggested widespread iodine deficiency (3). Since then a series of systematic surveys have demonstrated the existence of endemic goiter in many provinces in central and southern Chile: Coquimbo (12), Santiago (11), Linares (13), Ñuble (13), Malleco and Cautín (24), and Aisén (1).

The results of all epidemiological surveys for goiter in the country are summarized in Table 1. The data indicate the prevalence as well as size and characteristics of the samples. The summary does not show the great variations found from one location to another within the same area. In general, it can be said that prevalence is low along the coast, and increases eastward toward the Andes. Urban populations are usually not affected. A good example is the

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Table 1. Endemic goiter surveys in Chile.

Province	Examined No.	Goitrous %	Date of survey	Sample examined
Santiago*	39,433	11.0	1954-1955	28% primary schoolchildren (11)
Coquimbo*	8,232	19.0	1958	Primary schoolchildren (12)
Linares*	7,746	33.5	1958	Primary schoolchildren (13)
Ñuble*	7,127	24.6	1958	Primary schoolchildren (13)
Aisén*	123	69.1	1959	53% primary schoolchildren Alto Palena (1)
All*	5,351	1.3	1960	0.07 of total population of Chile (19)
Santiago*	400	2.7	1961	94% total population of Caleu Valley (8)
Cautín and Malleco*	683	25.1	1963	0.3% total Indian population (24)
Easter Island*	183	3.8	1963	17% total population (14)
Concepcion**	22,273	3.2	1964	5% total population over 6 years (20)
Ñuble**	21,845	9.4	1967	17% total population over 6 years (21)

* *Only visible goiters included, following World Health Organization recommendations.*
** *Palpable goiters included, following Pérez et al. (26).*

Province of Santiago (11), where prevalence by counties ranges from 0 to 37 per cent. Some schools near the coast have 0 per cent, in contrast to many of over 50 per cent among those located in the Andes. In the greater Santiago area the prevalence is 5 per cent, while the total is 11 per cent for the whole province. The same is true for the Province of Coquimbo (12), where the prevalence of goiter in the seashore cities of La Serena and Coquimbo is 4 and 2 per cent, respectively, while counties located far from the sea like Salamanca, Lomo Alto, and Punitaquihad 38, 34, and 30 per cent, respectively. Such a distribution is also seen in the rest of the Latin American countries (22).

A special comment concerns the Province of Ñuble, where a prevalence of visible goiter of 24.6 per cent was reported among schoolchildren in 1958 (13), while a second survey in 1967 recorded an incidence of 9.4 per cent of all goiters, both visible and also palpable (21). Furthermore, if only the 15,616 children from six to 15 years of age were considered, practically all of whom attended the same school surveyed in 1958, the incidence was 8.1 per cent, and if goiters that were not visible were excluded so as to make things more comparable, the figure went down to 4.1 per cent. Although the studies were made by different observers, it is reasonable to conclude that the incidence of goiter has spontaneously diminished in Ñuble during the last ten years.

From all the surveys listed in Table 1, the ICNND Report (19) seems to be in frank disagreement with the rest. Such a situation is not suprising since the samples, although taken from all over the country, include only urban

populations. A similar study made in Uruguay by the ICNND concluded that a severe goiter endemic existed in this country, which is well known to be one of the least affected in America (6).

As to the etiology of endemic goiter in Chile, iodine deficiency seems to be the main factor; the participation of natural goitrogens and of genetic factors is discussed in other papers presented in this volume. In 1935 soil and water samples taken in a goitrous district of O'Higgins Province, near Santiago, showed a lower content than samples taken in the capital, considered not to be a goitrous region. Mean values were 115 and 554 microgams per kilogram of soil and 0.64 and 2.4 micrograms per liter of water, respectively. Simultaneous measurements of iron content showed double concentration in the goiter area when compared with Santiago, while there was no difference in the amount of copper (5). In 1956 samples of soil and drinking water were taken in 20 different places in Santiago Province. There was an inverse relationship between iodine content of the samples and the incidence of goiter (7). A similar study, limited to samples of drinking water, corroborated these findings a few years later, and demonstrated no correlation between calcium content and goiter prevalence (25). Iodine deficiency has also been demonstrated in the goiter endemic of the Pedregoso Reservation in the Province of Cautín, as judged by low concentration in drinking water and an average daily urinary excretion of 33 micrograms (4).

In spite of all these finding iodine prophylaxis programs are still not effective in Chile. In 1959 the Ministry of Public Health and Social Security authorized the sale of iodized salt (9), but this has been completely ineffective in gaining any significant distribution of iodine. New decrees have made the measure compulsory, starting 1 July 1968 (10). It is interesting to consider that 85 per cent of the salt consumed in Chile is the product of natural mines located near Iquique, one of the two northern ports through which Chile exports nitrate and iodine to the rest of the world.

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CHAPTER 21

STUDY OF ENDEMIC GOITER IN THE AMERICAN INDIAN

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Endemic goiter in the Americas can be traced back to pre-Columbian times (5, 14, 15, 43). During recent decades the disease has been demonstrated in practically every country in the Americas. From the review published by Kelly and Snedden (21) in 1960, it can be estimated that at least 20 per cent of the population is affected by goiter. All races are affected, but unfortunately there has been little precision about racial distribution in most surveys. Most American Indians are mixed in different degrees and have been included in variable proportions. Those living in primitive conditions, who are presumably less mixed, have been surveyed for goiter in only a few instances (35, 46).

Iodine deficiency, as judged by urinary excretion, has been found in practically every instance of endemic goiter where it has been measured (2, 17, 18, 19, 24, 26, 36, 41, 44). This is not surprising since with the exception of the nitrate deposits in the north of Chile (20) iodine is not abundant on the surface of this continent. Whenever the diet has been supplemented with iodine, the incidence of endemic goiter has been greatly reduced. Well-documented examples are those of the U.S.A. (22), Mexico (40), Guatemala (39), Colombia (6), and Argentina (33).

The concept that endemic goiter is caused solely by iodine deficiency is probably no longer tenable. The complexity of the etiology of the disease is illustrated by the finding of groups of Indians without goiter who are iodine deficient (37), and the persistence of endemic goiter in children born in areas where iodine prophylaxis was underway (13). Natural dietary goitrogens may play a role in the pathogenesis of some endemias (11, 23, 32, 34). A possible example is the "piñón," the nut of the Araucaria araucana, eaten by the Pewenche Indians in south central Chile (42).

Genetic mechanisms have not been documented in the pathogenesis of endemic goiter. Enzymatic defects have not been demonstrated in its etiology. Concentration of subjects with endemic goiter within families has been reported in America (16, 25) as elsewhere (7, 27). A positive association of nodular goiter with the distribution of non-tasters for phenylthiocarbamide (PTC) has been reported in a mixed population from Brazil (28). Search for this

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association among American indians is difficult because of the small proportion of non-tasters usually found among them, as was the case in a study of the Pewenche (10).

The impact of endemic goiter on public health is unclear. Severe goiter endemias are associated with endemic cretinism, deafmutism, and neurological defects. There are no adequate studies of comparable populations with and without endemic goiter in respect to mortality and fertility differentials. The same is true for the individuals within an endemic goiter area.

Comparative studies of endemic goiter among different groups of American Indians could provide an excellent opportunity to study the pathogenesis of endemic goiter. They presumably have very different genetic endowment and they are under the influence of different environments. Since the interplay of all these factors in the genesis of endemic goiter is probably unique in each population, studies in depth in certain communities may be rewarding, apart from providing the basis for rational programs of prophylaxis.

ENDEMIC GOITER IN PEDREGOSO, CHILE

Pedregoso, is a geographically isolated valley located at an altitude of 1,310 meters in the Chilean Andes. The climate is cold. According to a census made in 1965, 592 Pewenche Indians live there by relatively primitive agriculture and some cattle raising. A permanent component of the diet is the nut of the Araucaria araucana, a pine tree which is abundant in the surrounding forests. The available genetic data show that these Indians are less mixed than other groups of the Araucanian complex to which they belong (8, 10, 29, 30, 38) and constitute a highly inbred population in which one-fourth of the marriages are consanguine (10).

The prevalence of endemic goiter in Pedregoso is 66.8 per cent in a sample comprising 81 per cent of the total population (Table 1 and 2). The remaining 19 per cent, mainly young people, were not examined in our house-to-house survey because they were involved in diverse activities in nearby places. The distribution according to sex and age is shown in Table 1. The frequencies of clinically nodular and large goiters are also recorded in this table. In this last group we have included goiters estimated to weigh 60 grams or more and all those with retrosternal extensions. As in most other studies women at all ages are more goitrous than men. The fact that the frequency does not diminish after puberty and the finding of nodular goiters in the first decades of life are indications of the severity of the endemia. On the other hand huge goiters were exceptional and only one cretin and no deafmutes were found.

It was of interest to inquire whether the increase in size and in nodularity seen in relation to age are independent effects. Nodularity increases with age in both sexes, appearing earlier among women (Table 2). Large goiters are more frequently nodular than small ones with advancing age, but some individuals have small nodular goiters in the first decades. It is obvious that in order to study the influence of other etiological factors upon endemic goiter variability, sex, age, size, and nodularity should be considered separately. A number of facts reinforce this conclusion: (1) Goiter is more severe among women, as judged in this endemia by greater frequency, larger

Table 1. Goiter in Pedregoso.

Age (years)	Men					Women				
	Examined		Goitrous			Examined		Goitrous		
		Goitrous		Nodular	Large		Goitrous		Nodular	Large
	No.	%	No.	%	%	No.	%	No.	%	%
0- 9	63	25.4	16	0.0	6.2	71	39.4	28	3.6	0.0
10-19	55	72.7	40	2.5	2.5	55	72.7	40	15.0	15.0
20-29	37	62.2	23	26.1	21.7	44	95.5	42	23.8	45.2
30-39	28	67.9	19	47.4	42.1	33	100.0	33	54.6	30.3
40-49	16	75.0	12	66.7	50.0	17	94.1	16	81.2	68.8
50+	29	76.9	22	68.2	63.6	28	96.4	27	77.8	81.5
Total	228	57.9	132	29.5	26.5	248	75.0	186	37.1	36.6

Table 2. Nodularity and goiter size in Pedregoso.

Age (years)	Men				Women			
	Small goiters		Large goiters		Small goiters		Large goiters	
		Nodularity		Nodularity		Nodularity		Nodularity
	No.	%	No.	%	No.	%	No.	%
0- 9	15	0.0	1	0.0	28	3.6	0	0.0
10-19	39	2.6	1	0.0	34	14.7	6	16.7
20-29	18	22.2	5	40.0	23	13.0	19	36.8
30-39	11	36.4	8	62.5	23	39.1	10	90.0
40-49	6	50.0	6	83.3	5	80.0	11	81.8
50+	8	25.0	14	92.8	5	40.0	22	81.8
Total	97	14.4	35	71.4	118	21.2	68	64.7

size, and more nodularity, as well as by the more precocious appearance of these characteristics. (2) Men frequently stay out of Pedregoso for long periods, working in nearby towns or farms, while women and children tend to stay on in the Reservation. (3) Since most of the goiters become nodular with age, nodularity among younger individuals may have different implication than in older subjects. (4) Because of retrosternal extensions, estimation of goiter size among older individuals has greater error--this may have contributed to error when assessing nodular goiters.

One aspect of goiter variability in Pedregoso is pregnancy. In each decade women with either large of nodular goiters have had a larger than average number of pregnancies (Table 3). Rather than an effect of gestation, this may be only an age-dependent process, since at comparable ages men also have an increase in large and nodular goiters. Men and women of less than 30 years of age are not included in Table 3, although they showed these same tendencies. The differences are only significant for men with large, versus those with small goiters, in the older group ($t_{(31)} = 2.337$; $p < 0.05$). The average number of descendents of men without goiter is slightly larger than of goitrous men at these two age groups.

Inbreeding also affects goiter in Pedregoso (Table 4). The prevalence of goiter among children under ten years of age whose parents are consanguine is significantly smaller than in the two other classes studied: for children whose parents were born in Pedregoso but consanguinity was not detected, $X^2_{1\ (Yates)} = 4.275$ ($p < 0,05$), and for children whose parents were born in different reservations, $X^2_{1\ (Yates)} = 4.275$ ($p < 0.05$). The opposite trend seen among persons over 20 years of age is not significant. Inbred adults show a consistent and significant tendency to have less nodular goiters ($X^2_{1\ (Yates)} = 6.264$; $p < 0.025$) when compared with those whose parents were born in different reservations. Other comparisons show no significant differences. Thus it may be said that inbreeding in Pedregoso favors goiter developing at a later age and goiters which are somewhat less prone to nodules.

Phenylthiocarbamide tasting ability was determined by the technique of Harris and Kalmus in 255 persons in Pedregoso. Since only 4.3 per cent were non-tasters, this characteristic could not be properly related to goiter. Nevertheless, a comparison made among tasters showed that both men and women with nodular goiters, in the third and fourth decades of life, had significantly lower mean threshold values when compared with those with diffuse goiters (9).

Height was correlated with goiter (Table 5). Among goitrous persons larger goiters were found among taller subjects in both sexes in the third and fourth decades. An analysis of variance combining all these groups showed that this relation is significant at the 5 per cent level ($f_{(1,106)} = 4.04$). On the other hand, nongoitrous men of the same age are, in general, taller than goitrous ones, but this difference is not significant even when the comparison is made only with males with small goiters ($t_{(50)} = 1.857$)

Surveys in Pedregoso revealed two relevant nutritional facts concerning goiter. Not only is there iodine deficiency as judged by an average daily urinary excretion of 33.2 micograms (2), but also the people consume all year round "piñón" (1), which is goitrogenic in rats (42). Subjects with large or nodular goiters have lower mean urinary iodide excretion (Table 6) than those

Table 3. Fertility and goiter in Pedregoso.
(Total offspring, abortions excluded)

Goiter	Women					
	30-39 years			40 years or more		
	No.	$\bar{x}$	s_e	No.	$\bar{x}$	s_e
Diffuse	11	4.73	0.406	7	7.43	0.922
Nodular	15	5.73	0.547	31	8.29	0.605
Small	18	4.89	0.427	9	7.33	1.414
Large	8	6.25	0.620	29	8.38	0.531
	Men					
Diffuse	9	4.88	0.389	11	7.55	0.790
Nodular	6	6.00	0.775	22	9.59	0.973
Small	9	5.00	0.408	13	7.00	1.104
Large	6	5.83	0.792	20	10.20	0.841

Table 4. Inbreeding and goiter in Pedregoso.
(Men and women)

Age (years)	Inbreeding*	Examined		Goitrous		
		No.	Goitrous %	No.	Nodular %	Large %
0- 9	+	35	14.3	5	0.0	0
	?	72	38.9	28	3.6	3.6
	-	27	40.8	11	0.0	0
10-19	+	18	61.1	11	18.2	9.1
	?	56	80.4	45	4.4	11.1
	-	35	65.7	23	13.0	4.3
20-29	+	8	87.5	7	0.0	14.3
	?	53	84.9	45	24.4	40.0
	-	19	63.2	12	33.3	33.3
30-39	+	5	100.0	5	40.0	20.0
	?	42	88.1	37	54.0	35.1
	-	14	71.4	10	50.0	40.0
40+	+	4	100.0	4	50.0	50.0
	?	43	86.0	37	70.3	73.0
	-	33	87.9	29	82.8	65.5

* + = Parents born in Pedregoso, consanguinity detected.
? = Parents born in Pedregoso, consanguinity not detected.
- = Parents born in different reservations.

Table 5. Height and endemic goiter in Pedregoso (cm).

	Age (years)	Nongoitrous			Goitrous										
					Diffuse			Nodular			Small			Large	
		No.	$\bar{x}$	s_e	No.	$\bar{x}$	s_e	No.	$\bar{x}$	s_e	No.	$\bar{x}$	s_e	No.	$\bar{x}$	s_e
MEN:	20-29	14	162.1	1.85	17	159.8	1.22	6	160.0	2.81	18	159.4	1.41	5	161.5	1.00
	30-39	9	164.2	2.41	9	160.8	1.18	8	163.1	1.47	11	160.7	1.02	6	164.2	1.67
	40 +	10	159.5	1.10	11	159.8	1.37	20	159.0	1.48	13	159.0	1.04	18	159.4	1.15
WOMEN:	20-29	2	150.0	2.50	32	150.2	0.90	10	150.5	1.86	23	148.4	1.07	19	152.5	1.01
	30-39		-----		15	150.8	1.54	17	149.0	1.12	22	149.3	1.02	10	151.0	1.07
	40 +	2	150.0	2.50	9	151.9	1.76	30	149.8	0.86	9	150.3	1.88	30	150.3	0.85

Table 6. Urinary iodide and goiter in Pedregoso.
(Micrograms per day)

	No.	$\bar{x}$	s^2	50 µg or more (%)
Nongoitrous	12	36.6	388.99	25.0
Goitrous:				
Diffuse	30	38.1	545.13	26.7
Nodular	37	31.2	324.20	13.5
Small	32	38.5	560.13	31.2
Large	35	30.4	288.24	8.6

with small or diffuse goiters, respectively. These differences are better demonstrated by the percentage of subjects in each group excreting 50 micrograms or more. Using this system, statistical significance at the 5 per cent level is reached when comparing large against small goiters ($X^2_{I\ (Yates)} = 4.162$); nongoitrous subjects do not differ significantly from those with diffuse and small goiters. On the other hand, there is a direct relation between mean ingestion of "piñón" and thyroid size (Table 7); nongoitrous subjects showed the lowest consumption. Since there is a wide dispersion of the values observed, it is preferable to consider the proportion of subjects eating larger amounts, say 100 or more grams daily. The same trend is seen, the difference being significant when comparing subjects with large thyroids against the rest ($X^2_{I\ (Yates)} = 4.061$; $p < 0.025$). Diffuse and nodular goiters do not differ with this approach.

Kinetic studies of iodine metabolism were performed in Pedregoso (3). The most striking findings were: (1) slightly high values for net iodine intake by the thyroid and peripheral consumption of thyroid hormones; (2) a considerable "iodine leak" since the total output of iodine by the thyroid is five to ten times the amount incorporated into thyroid hormones; (3) increased amounts of endogenously labeled triiodothyronine (T_3), iodinated polypeptides (NBEI) and iodotyrosines in the blood. These facts, plus increased radioiodine uptake and $PB^{125}I$ levels, indicate an accelerated turnover of radioiodine by the gland and considerable inefficiency in handling iodine in terms of the percentage that is used for the synthesis of thyroid hormones. Some of these observations have also been made in other goiter endemias in America (31, 41) and elsewhere (12).

Metabolism of radioiodine is correlated with variation in goiter size and nodularity. The $PB^{125}I$ reflects the rate at which the thyroid incorporates radioiodine into circulating iodinated compounds. It is shown in Table 8 that nodular goiters have significantly higher values for this measurement, and the same is true for $NBE^{125}I$. Earlier we have shown that nodular goiters have the widest range of values for the thyroidal iodine pool, including both the smallest and the largest figures observed (4). This may explain why $PB^{125}I$ values have the greatest variance among nodular goiter, since small pools may determine the high values observed. In support of this view, the thyroidal iodine pool among 21 subjects in Pedregoso, calculated by the isotopic equilibrium method of Riggs (4) shows a significant inverse relationship with the

Table 7. "Piñón" ingestion and goiter in Pedregoso. (Grams per day)

	No.	$\bar{x}$	s^2	100 g or more (%)
Nongoitrous	22	46.0	1604.67	4.5
Goitrous:				
Diffuse	43	61.6	3315.72	18.6
Nodular	50	75.7	13665.77	16.0
Small	41	57.5	2921.71	9.8
Large	52	78.4	13462.44	23.1

Table 8. $PB^{125}I$ and $NBE^{125}I$ in Pedregoso. (% dose/Lt serum)

	$PB^{125}I$			$NBE^{125}I$		
	No.	$\bar{x}$	s^2	No.	$\bar{x}$	s^2
Nongoitrous	10	0.17	0.0135	7	0.012	0.0^4176
Goitrous:						
Diffuse	30	0.18	0.0204	19	0.012	0.0^4823
Nodular	34	0.50	0.1874	29	0.028	0.0^38736
Small	30	0.31	0.1147	23	0.022	0.0^37575
Large	34	0.39	0.1529	25	0.022	0.0^35058
F for nodularity		13.709^{ooo}			8.005^{oo}	
F for size		2.323			0.097	

^{oo} $p<0.010$.

^{ooo} $p<0.001$.

Table 9. Endogenously labelled triiodothyronine and iodotyrosines in Pedregoso.

	T_3		Iodotyrosines	
	No.	%	No.	%
Non goitrous	8	0.0	14	21.4
Goitrous:				
Diffuse	11	36.4	28	10.7
Nodular	25	36.0	34	41.2
Small	14	35.7	26	19.2
Large	22	36.4	36	33.3

$PB^{125}I$ values observed in these subjects ($\gamma = 0.472$; < 0.025). A similar correlation has been observed in euthyroid patients in Scotland (45). Furthermore, small iodine pools could also explain high $NBE^{125}I$ values since this parameter shows a significant positive correlation with $PB^{125}I$ ($\gamma = 0.50$; $p < 0.001$).

Endogenously labeled triiodothyronine was present in the same proportion among all goiters and absent in nongoitrous subjects (Table 9), although such difference was not significant ($X^2_{1\ (Yates)} = 2.549$). On the other hand endogenously labelled iodotyrosines were present in a significantly higher percentage of the Indians with nodular goiters when compared with those with diffuse goiters ($X^2_{1\ (Yates)} = 5.711$; $p < 0.01$). There was no significant difference between subjects with small and large goiters. Labelled iodotyrosines were found in a smaller proportion of nongoitrous persons than goitrous ones, particularly when compared to nodular goiters. Subjects with labelled iodotyrosines had significantly higher $PB^{125}I$ values ($t_{(46)} = 2.31$; $p < 0.05$); this was not true for those with labelled triiodothyronine. The demonstration of iodotyrosines can be directly ascribed to small thyroid iodine pools, as suggested by the fact that six subjects in whom they were present had an average pool of 1.97 mg$\pm$0.327, while the other five in whom they were not demonstrated, had an average value of 6.22 mg$\pm$1.757 ($t_{(4)} = 2.37$; $p < 0.05$).

DISCUSSION

Multiple causes probably participate in the etiology and pathogenesis of endemic goiter. Iodine deficiency is the predominant etiological factor in most endemias, but other environmental influences may enhance the effects of iodine deficiency, and in some instances, possibly cause endemic goiter in the presence of an adequate amount of iodine. While genetic factors have not been shown to influence the prevalence of endemic goiter, a role cannot yet be excluded.

In Pedregoso we have examined three aspects of the variability of goiter: presence, size, and nodularity. These interact in some measure, but each is characterized by a particular constellation of statistically significant factors. Nodularity is related to the two clearly defined genetic aspects studied so far, inbreeding and PTC tasting ability, and to some radioiodine parameters. Inbred people develop less nodular goiters; Indians with nodular goiters have lower thresholds for tasting PTC; and radioiodine studies show high $PB^{125}I$ and $NBE^{125}I$ values and a greater proportion of endogenously labelled iodotyrosines in subjects with nodular goiters. All these radioiodine findings can be linked to the predominance of small thyroidal iodine pools existing among nodular goiters. On the other hand, large goiters are associated with the two well-defined environmental factors: consumption of less iodine and more "piñón." Size is also positively associated with two complex factors, height and fertility. It is noteworthy that precocious appearance of goiter is negatively influenced by inbreeding, just as with nodularity.

In view of the complexity and number of interacting factors, it would seem necessary to apply to data of these kinds a multivariate analysis by means

of which proper values could be attached to main factors, as well as to the many kinds of interactions present. Two principal facts limit this possibility for our data, namely, the small size of our sample, especially since certain measurements have been recorded in subsamples which do not always comprise the same individuals, and since the categories for goiter contain different types of error in a nonrandom way and have different meanings. For example, the relations with age are difficult to study since small diffuse goiters at an early age may develop into large or nodular goiters.

The Pedregoso data suggest a need to extend these studies to other similar Pewenche communities, but even then comparisons with entirely different situations using similar types of analysis would be necessary in order to determine the bearing of such findings on the general problem of endemic goiter. Is endemic goiter the adaptation of man to iodine deficiency? Our data lead us to suggest that subjects with certain types of goiter are better adapted than others. The fact that Indians with large goiters are taller and have more children could be in line with such an interpretation, even if we are unable to interpret the mechanisms involved.

SUMMARY

Iodine deficiency exists in practically all of the endemic goiter areas studied in the Americas and constitutes its main cause. Since endemic goiter does not always accompany iodine deficiency, and may exist when iodine intake is seemingly normal, other etiological factors may play a role.

The American Indian population provides an excellent opportunity to study the variability of endemic goiter. Studies in the relatively isolated Pewenche reservation of Pedregoso in Chile have disclosed that sex and age influence the distribution and characteristics of goiter. Iodine deficiency is present and the diet includes a nut that is goitrogenic for rats. Both these factors are related to goiter size. Height and fertility also correlate positively with goiter size in both sexes. Inbreeding and PTC tasting status are related to the presence of nodules. These nodules condition the existence of small functional thyroid iodine pools, and this in turn is reflected in different radioiodine parameters.

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CHAPTER 22

ENDEMIC GOITER IN PEDREGOSO (CHILE)

EXPERIMENTAL GOITROGENIC ACTIVITY OF "PIÑON"

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Edmundo Covarrubias, M.D.,[3] and José Barzelatto, M.D.[2]

During a study of goiter among the Pewenche Indians of the Pedregoso Reservation (2, 3, 5) a dietary survey of 113 subjects, representing approximately one half of the adult population, showed that all except one ate daily during the whole year around, the nut of the Araucaria araucana ("piñón"). Fifteen per cent of them ate more than 100 g per day, and among these subjects 5.9 per cent had no goiter, and 64.7 per cent a goiter estimated as weighing over 50 grams. These figures contrasted with 21.9 per cent and 40.6 per cent, respectively, among the Indians eating less than 100 g daily (1).

These facts lead us to investigate the possibility of a goitrogenic action of "piñón" that could be a coadjuvant factor in the pathogenesis of endemic goiter in Pedregoso, where we had already shown iodine deficiency to be the main cause (2). We report here the results of feeding rats with the nut of the Araucaria araucana, incorporated into a nutritionally adequate diet. Preliminary experiments with rats fed exclusively on "piñón" showed that the diet did not interfere with the increased uptake due to iodine deficiency and suggested an independent goitrogenic activity of "piñón", but malnutrition interfered with the interpretation (14).

MATERIALS AND METHODS

Sixty young female rats, with an average initial body weight of 143 g and a standard deviation of 12.65, were fed a Remington diet (13) modified by adding yeast at a 10 per cent concentration and, for 30 of these animals, modified also by substituting cornmeal for cooked "piñón" flour. Table 1 shows the composition of these two diets, including the iodine content; protein values were calculated from nitrogen determinations multiplied by a factor of 5.7.

All animals were grey rats belonging to the AxC strain obtained originally from the Alton Oschner Foundation and kept by inbreeding. Animals were maintained in groups of four or five per cage and exsanguinated under ether anesthesia by aspirating from the abdominal aorta with a heparinized syringe.

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Table 1. Diet composition.

Diet	Proteins (% of dry weight)	Lipids (% of dry weight)	Ash (% of dry weight)	Water (%)	Iodine (μg/g)
Remington	23.1	2.1	3.0	10.3	0.06
Rem-"Piñón"	20.1	2.5	3.1	9.4	0.09

Experiment I

Twelve rats were fed "piñón" incorporated into the Remington diet, while another 12 were fed the Remington diet without "piñón." All these animals were fed ad libitum and had free access to distilled drinking water, but for half of each group, KI was added to a concentration of 0.65 mg per liter. From the average water consumed it was estimated that the amount of iodine added daily to the diet was approximately 5 micrograms per animal. The diet contributed a daily intake of iodine which was less than 1 microgram in all four groups of animals.

After seven weeks the rats were weighed, sacrificed, and their thyroids dissected and weighed in a torsion balance. These animals were previously injected intraperitoneally with 1 ml of 1 mg per cent propylthiouracil, followed 30 minutes later by 10 microcuries of carrier free ^{131}I injected by the same route. Animals were sacrificed one hour after the radioiodine injection.

The radioactivity present in each gland was measured in a scintillation well counter and referred to a standard of the administered dose. In the same way concentration of radioiodine in 1 ml of plasma of each animal was determined. Precipitation of these plasmas as well as precipitation of the homogenate of one lobe of the thyroid with 5 per cent trichloroacetic acid showed no detectable ^{131}I in the precipitate. Thyroid uptake was expressed as per cent of dose per ml. T/S ratios and thyroidal iodine spaces were calculated following Ramalingaswami et al. (12).

Experiment II

A total of 36 rats were divided into four equal groups and submitted to the same experimental dietary conditions as in experiment I. After eight weeks the rats were sacrificed and weighed and their thyroids dissected. After weighing, one lobe was homogenized and pools of two made for ^{127}I content by a modification of Barker's method (4). Five of these pools had to be discarded because of accidental gross contamination with iodine.

For statistical purposes all data were submitted to two-way analysis of variance, using a logarithmic transformation when analyzing radioiodine uptake, T/S ratio, and thyroidal iodine space. In addition, some paired comparisons were made by the use of "t" test.

RESULTS

Tables 2 and 3 show the changes in body and thyroid weight in animals eating "piñón" at two levels of iodine intake for periods of seven and eight weeks. It can be seen that both "piñón" and iodine intake, as separate factors, did not change significantly the increase in body weight of the animals, although the animals fed "piñón" tended to have smaller increases.

Table 2. Changes in body and thyroid weight after seven weeks of eating "piñón" at two levels of iodine intake (Experiment I).

Diet	Estimated ^{127}I intake (µg/day)	Average increase in body weight %			Thyroid weight (mg)		Thyroid weight (mg/100 g)	
		N	mean	s.e.	mean	s.e.	mean	s.e.
Remington	< 1	6	13.0	3.583	34.3	1.02	19.8	0.77
Rem-"Piñón"	< 1	6	18.3	3.583	29.2	0.75	18.6	0.58
Remington	5 to 6	6	21.1	1.45	25.4	1.01	15.8	0.51
Rem-"Piñón"	5 to 6	6	10.5	1.55	30.8	0.97	19.8	0.79

N = No. of animals.

F of diet	0.497	0.892	3.575
F of iodine	0.169	0.759	0.024
F of interaction	9.770^{oo}	11.843^{oo}	7.607^{o}

^{o} $P < 0.05$.
^{oo} $P < 0.01$.

Table 3. Changes in body and thyroid weight after eight weeks of eating "piñón" at two levels of iodine intake (Experiment II).

Diet	Estimated ^{127}I intake (µg/day)	Average increase in body weight %			Thyroid weight (mg)		Thyroid weight (mg/100 g)	
		N	mean	s.e.	mean	s.e.	mean	s.e.
Remington	< 1	9	18.6	1.578	27.8	1.01	16.6	0.62
Rem-"Piñón"	< 1	9	12.7	1.555	30.1	1.19	18.7	0.91
Remington	5 to 6	9	14.8	5.237	24.7	0.89	14.4	0.53
Rem-"Piñón"	5 to 6	9	9.5	2.037	28.9	0.85	18.2	0.62

N = No. of animals.

F of diet	3.546	3.796	18.244^{ooo}
F of iodine	1.275	1.693	3.912
F of interaction	0.011	0.299	1.494

^{ooo} $P < 0.001$.

The rats eating "piñón" had in general larger thyroids either if weight is considered as an absolute value or relative to body weight. This influence is significant after eight weeks ($P < 0.001$).

In both experiments when iodine was added to the diet a decrease in absolute and relative thyroid weight was observed in the animals eating the Remington diet, but not when "piñón" was added. Such a difference is reflected in the interactions of the analysis of variance, but was significant only in Experiment I.

Radioiodine studies performed at seven weeks are reported in Table 4. One animal was discarded because of grossly deviating results. Radioiodine uptake is significantly diminished by "piñón" at a low level of iodine intake. As expected all uptakes were very low when an excess of iodine was added to the diets. On the contrary, iodine intake did not influence plasma level, while "piñón" increased this value significantly. When relating these two parameters as a T/S ratio, there is an overwhelming effect of iodine intake, but there is also a clear tendency of "piñón" to diminish this ratio at a low level of iodine intake. A "t" test ($t = 7.650$; d.f. = 9) showed a highly significant value ($P < 0.001$ for this last difference, even though the analysis of variance did not show a significant effect of "piñón" intake upon this parameter. When the T/S ratio is related to thyroid weight, as iodide space, both the level of iodine intake and the ingestion of "piñón" show a significant effect by analysis of variance. It is of interest that the effect of "piñón" is compatible with the tendency to lower values for T/S ratio and thyroidal iodide space even at a high level of iodine intake, even though the "t" test was significant only for the T/S ratio at this level of iodine intake ($t = 3.239$; d.f. = 9; $P < 0.01$).

The ^{127}I content in the thyroids of these animals appears in Table 5. The effect of the ingestion of "piñón" in diminishing the iodine content of the glands is evident at both levels of iodine intake. The significant interaction observed by the analysis of variance reflects the fact that thyroid iodine content remains relatively constant at both levels of iodine ingestion when the animals are fed "piñón", while ^{127}I content increases by a factor of two when iodine intake is increased in the control animals.

DISCUSSION

The data which are reported here indicate that there is a relatively weak goitrogenic action on the rat thyroid when "piñón" is incorporated into the diet. This increase in thyroid weight is clearly significant after eight weeks of feeding under the experimental conditions employed. Furthermore this effect is not overcome by an iodide supplement, as was the case for the control rats fed a Remington diet.

These studies do not explain the mechanisms through which the "piñón" exerts its goitrogenic action. A double effect might be assumed, since the iodide pump is somewhat depressed while the supplement of iodide does not prevent the goitrogenic action. Also, the iodine content of the thyroid remains low even when iodine is added to the Remington "piñón" diet (Table 4). A double action has been previously suggested to explain the goitrogenic activity of other edible nuts (9, 10). It would not be surprising that there are two or more goitrogenic substances in the "piñón", since a combination of small amounts of different substances seems to explain better the goitrogenic action of many foods (6). Furthermore, goitrogens may act directly upon the thyroid at one or more steps of hormone synthesis and also can interfere with the peripheral utilization of thyroid hormone (7).

Table 4. Radioiodine studies after one hour in animals fed "piñón" for seven weeks (Experiment I).

Diet	Estimated ^{127}I intake (µg/day)	Thyroid uptake (% dose) N	mean	s.e.	Plasma level (% dose/ml) mean	s.e.	Thyroid/plasma ratio mean	s.e.	Thyroidal iodide space mean	s.e.
Remington	< 1	6	2.47	0.150	0.75	0.122	97.9	7.73	3333.2	193.15
Rem-"Piñón"	< 1	6	0.80	0.084	0.90	0.038	31.2	4.26	900.9	114.04
Remington	5 to 6	5	0.03	0.003	0.68	0.073	1.8	0.07	45.6	7.72
Rem-"Piñón"	5 to 6	6	0.03	0.006	0.89	0.057	1.1	0.25	37.7	6.99
F of diet			42.461 ooo		11.753 oo		1.36		55.16 ooo	
F of iodine			1122.741 ooo		0.264		154.85 ooo		821.51 ooo	
F of interaction			8.616 oo		0.414		19.92 ooo		0.62	

N = Number of patients.

oo $P < 0.01$
ooo $P < 0.001$

Table 5. Total iodine content of pools of two lobes of different animals, in Experiment II.

Diet	Estimated ^{127}I intake (μg/day)	Total iodine content (μg) N	mean	s.e.
Remington	< 1	3	1.04	0.084
Rem-"Piñón"	< 1	3	0.59	0.180
Remington	5 to 6	2	2.02	0.272
Rem-"Piñón"	5 to 6	3	0.48	o.125

N = No. of subjects.

F of diet	33.58^{ooo}
F of iodine	2.88
F of interaction	14.38^{oo}

oo P < 0.01
ooo P < 0.001

Demonstration of goitrogenic action of "piñón" does not prove a significant role in the pathogenesis of endemic goiter in Pedregoso. Nevertheless, this result justifies an effort to isolate the goitrogenic substances contained in the "piñón" in order to evaluate their importance in this endemia. It has been demonstrated experimentally that goitrogens can potentiate the effect of iodine deficiency upon the thyroid (7), and there is present evidence which strongly suggests that goitrogens play a coadjuvant etiological role in some human goiter endemias (8, 11).

SUMMARY

The nut of the Araucaria araucana (piñón) has been suspected of playing a goitrogenic role in the pathogenesis of endemic goiter in an Indian Reservation. Rats were fed "piñón" incorporated as flour into a Remington-type diet to avoid malnutrition. "Piñón" diet showed goitrogenic activity that was not due to iodine deficiency. On a low iodine intake, ingestion of "piñón" had some inhibitory effect upon the iodide pump as reflected by diminished T/S ratio and thyroidal iodide space. Thyroid ^{127}I content was lower among animals being fed "piñón", and this value did not change on a high iodine intake. Possible mechanisms of action are discussed.

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CHAPTER 23

GENETIC QUESTIONS RELATED TO THE GOITER ENDEMIA OF PEDREGOSO (CHILE)

Edmundo Covarrubias,[1] José Barzelatto,[2] and Ruth Guiloff[1]

The physiological adaptation of the thyroid gland to an imbalance between thyroid hormone production and body requirements often results in the appearance of goiter. Thyroid hormone metabolism is regulated by a complex of enzymes, and this implies the participation of multiple structural and regulatory genes. Different types of enzymatic defects corresponding to some of these many steps, have been described in sporadic goiter (26).

Environmental modifications may also lead to the development of goiter. Certain compounds, some of them contained in food, do so by interfering with specific enzymatic steps and thus create true phenocopies. Insufficient supply of iodine is an outstanding cause of goiter in many populations. There are situations where an increased hormone production is required, such as exposure to cold and pregnancy. These may potentiate the effects of lack of iodine or the presence of natural goitrogenic substances in the diet or both. Thus one may suspect that natural selection was intense for men due to thyroidal dysfunction occurring in the harsh conditions of life prevailing in the Pleistocene. The consequence of this can be considered in terms of old genetic systems of adaptation, and these may still be active.

Genetic factors in endemic goiter have been investigated for many years, especially in the Alpine endemia. As reviewed by Davenport (10) in 1932, most efforts have been directed toward demonstrating simple mendelian mechanism by means of extensive pedigree studies. Dominant genes, either autosomal or sex-linked, as well as recessive genes, have been postulated. Davenport himself suggested the interaction of a dominant sex-linked gene and a dominant autosomal one. Nevertheless, none of these simple approaches adequately explains the distribution of endemic goiter in families. An extreme and opposite view has been taken by Eugster (11), who denies any participation of genetic factors in endemic goiter. His data reveal the importance of environmental causes. Most genetic studies have only considered the presence or absence of goiter and not other aspects of goiter variability such as size and nodularity as we have done in Pedregoso (5). Present knowledge of thyroid physiology and of the bearing of different environmental causes in the etiology of endemic goiter serve to suggest a polygenic basis influencing endemic goiter variability.

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In this particularly complicated situation, population analysis techniques may provide more relevant information. While family concentration of goitrous subjects has been demonstrated in different endemias (7, 20, 22) the analysis of demographic patterns related to goiter has not usually been performed, in spite of the value of these kinds of data for defining the role of natural selection (21).

The search for associations between gene markers and endemic goiter has proved promising in the case of the ability to taste phenylthiocarbamide (PTC). Non-tasters have a higher frequency of nodular goiter (2, 6), a finding interpreted as an increased susceptibility for development of nodules (23).

Our previous studies of the goiter endemia in Pedregoso (4) have allowed us to establish criteria for defining goiter variability in this community (5), which have been applied to analysis of family and fertility data, as well as the association with PTC tasting ability. The first results of this analysis are reported here.

ANALYSIS OF SIBSHIPS

In Pedregoso a total of 129 sibships including 454 subjects have been examined by one of us (J.B.). Size of the thyroid was estimated and presence of palpable nodules recorded. The first question concerned the possibility of a trend for goiter size to concentrate within sibships. As reported previously (4, 5), sex and age influence goiter variability in Pedregoso in such a way that a correction is necessary in order to compare adequately the different subjects. The distribution of estimated size by sex and age (Figure 1) reveals a tendency for values to increase and disperse with age. Therefore we decided to transform the estimated weight to "z" values, taking into account the mean and standard deviation per decade for each sex (Table 1). Goiters estimated to weigh over 100 grams and those with retrosternal extension were given an arbitrary value of 110 grams. Sibships were separated into decades according to the oldest sib examined, in order to have even more comparable groups and a better correction for age. In this manner we intended to fix the time effect of environmental causes on goiter development. A non-parametric analysis of variance was performed on the sibships grouped as recorded in Table 2, in order to search for a comparison between intra- and intersibships variance. A highly significant result is observed for the total 82 sibships of three or more members. The same analysis performed per decade shows also highly significant results when the oldest sib examined belonged to either the second or the third decade of life. This analysis was restricted for statistical convenience to sibships of minimal size of four.

A graphical expression of size distribution in families is recorded in Figure 2 for the 82 sibships. For this purpose the "z" values have been coded in a scale of 25 units. It is seen that most sibs in the majority of sibships tend to form clusters and that only a few individuals in some sibships have greatly different thyroid sizes. In the older groups a tendency for a greater dispersion of values is also observed.

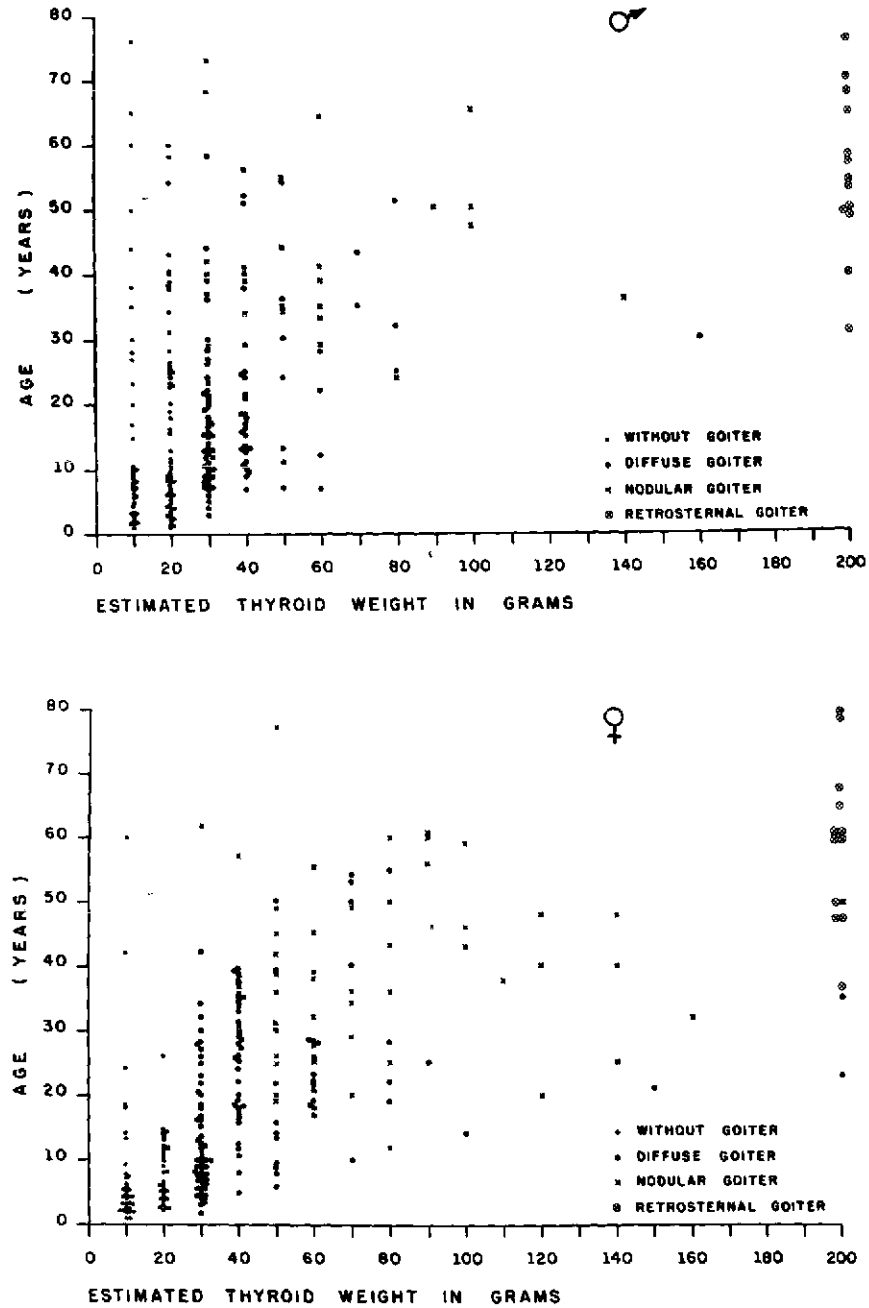

Figure 1. Age-specific distribution of estimated thyroid weights in Pedregoso. Goiters estimated to weigh over 200 grams and those with retrosternal extension are graphed at the 200 gram level. Nonpalpable thyroids were given an arbitrary value of 10 grams and the normal palpable glands one of 20 grams (top, males; bottom, females).

Table 1. Estimated thyroid weight in Pedregoso.
(grams)

Age (years)	Women N	$\bar{x}$	s	Men N	$\bar{x}$	s
0- 9	71	21	10.3	63	20	10.3
10-19	56	35	18.3	54	30	17.4
20-29	45	54	24.8	38	32	17.4
30-39	33	54	24.1	27	46	29.8
40-49	16	71	32.0	16	53	35.9
50+	27	83	29.5	29	63	43.6

N = Number of subjects.

Table 2. Non-parametric analysis of variance of thyroid size in sibships in Pedregoso.

Sibships: Age older sib examined (years)	Smaller size included	Sibships: No.	Subjects included: No.	H	P
0- 9	3	11	38	12.275	0.250 - 0.500
10-19	4	13	66	35.063	<0.001
20-29	4	10	55	27.033	0.001 - 0.005
30-39	4	15	88	22.697	0.050 - 0.100
40+	4	13	71	9.354	0.500 - 0.750
All	3	82	378	155.999	<0.001

NODULARITY IN FAMILIES

In order to analyze the distribution of nodularity in families we have selected 52 pedigrees, where both parents had been examined and whose age was over 30 years (Table 3). When both parents had nodular goiters their children had a prevalence approximately three times the value observed when none of the parents had nodules. An intermediate figure characterized offspring who had only one parent with nodular goiter. This heterogeneity is significant for the total of the observations, whether nongoitrous and diffuse goitrous children are pooled ($X^2_{(2)} = 7.359$: $p < 0.05$) or not ($X^2_{(4)} = 13.423$; $p < 0.01$). When these pedigrees are separated into two groups according to the age of the younger parent, the same tendencies are observed, although they are not statistically significant.

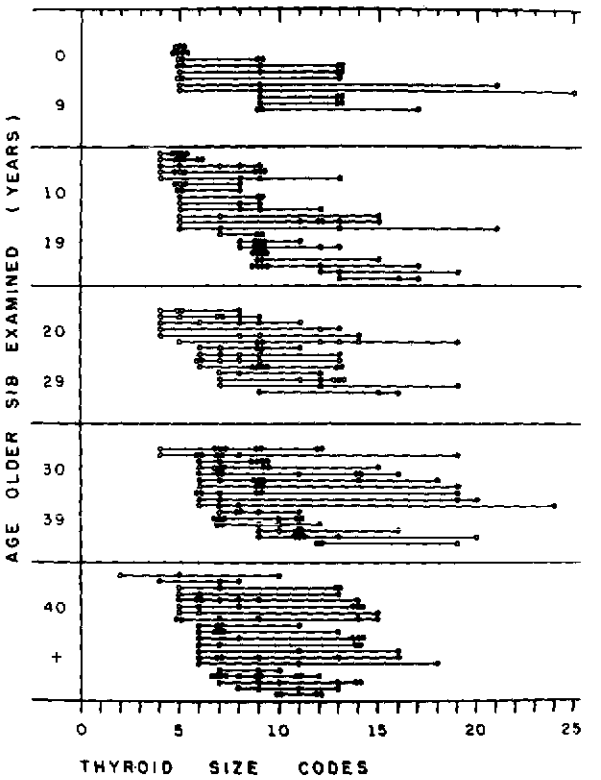

Figure 2. Thyroid size distribution in families for 82 sibships in Pedregoso. Each horizontal line represents a sibship; the beads are its members. See text for explanation of thyroid size coding.

Table 3. Family distribution of nodularity in Pedregoso.

PARENTS		SIBSHIPS	OFFSPRING			
Age of younger	Nodularity		Total	Non-goitrous	Diffuse goiter	Nodular goiter
		No.	No.	%	%	%
30-39	Both	5	18	67.7	27.8	5.5
	One	15	57	73.7	22.8	3.5
	None	5	19	68.4	31.6	0.0
40 +	Both	12	56	16.1	58.9	25.0
	One	9	51	29.4	54.9	15.7
	None	6	32	21.9	68.7	9.4
Total	Both	17	74	28.4	51.4	20.3
	One	24	108	52.8	38.0	9.3
	None	11	51	39.2	54.9	5.9

SOME DEMOGRAPHIC TRENDS

The available data on the reproductive history of women and men over 30 years in Pedregoso is summarized in Tables 4 and 5. Abortions were not included because the information obatined was obviously incomplete. Stillbirth figures seemed reliable and are considered. Women and men are studied separately because of the high number of polygynous marriages and because in many families only one parent was examined. In each sex and in the two age groups, higher fertility rates were observed among subjects with nodular or large goiters when compared to those with diffuse or small goiters respectively. Men without goiter had rates similar to those of subjects with large or nodular goiters. It is of interest that men and women of the older group with nodular goiters have strikingly larger variances than the rest. The rates of total offspring mortality and of death before one year among subjects 40 years of age or older show parallel tendencies to their fertility rates. Among subjects of 30 to 39 years of age no clear differences were observed among the different groups, except for women with nodular goiter. They show trends which are consistent with those observed among older individuals with nodular goiter when compared with those with diffuse goiters.

The sex ratio is lower when the fathers have nodular or large goiters than when they have diffuse or small goiters. Nongoitrous fathers have children with an intermediate figure for the sex ratio. The opposite trend is observed among children of goitrous mothers, except for size in the older group.

When a "t" test is applied to each of the differences already described, the only one that proves to be significant is the one between the fertility rates of the older men without large and small goiters ($t_{(31)} = 2.337$; $p < 0.02$).

PHENYLTHIOCARBAMIDE (PTC) TASTING ABILITY

The threshold for PTC tasting were determined among 253 subjects (3) using the sorting test described by Harris and Kalmus (16). The frequency distribution according to age, sex, and goiter is presented in Table 6. In common with observations on other American Indians, only a few non-tasters were found in Pedregoso (4.3 per cent), and hence it is not possible to look for meaningful relations with thyroid characteristics (9).

Nodularity has been related to the non-tasting status (2, 6, 17, 19, 23). We have extended the search for such a relation to the taster group, looking for differences in mean threshold values among subjects with nodular goiters and the other two groups. For this purpose we have chosen only subjects of 20 to 39 years of age, since nodular goiter is rare in younger individuals and too frequent among older ones. In Table 7 it is seen that persons with nodular goiters have the lowest mean values for each sex. An analysis of variance (Table 8) showed that both nodularity and sex affect significantly the PTC thresholds. The nongoitrous men do not differ significantly from the other two groups of men. Another argument reinforcing the difference between subjects with nodular and diffuse goiters is the fact that the entire distribution of thresholds among all tasters with nodular goiters is shifted to lower values (Figure 3).

Table 4. Fertility and offspring mortality related to goiter among women in Pedregoso.

MOTHER			OFFSPRING						
Goiter	Age (years)	N*	Total ever born			Total dead		Dead 1 year	
			$\bar{x}$	s^2	Sex ratio	$\bar{x}$	s^2	$\bar{x}$	s^2
Diffuse	30-39	11(10)	4.73	1.818	85.7	1.09	0.891	0.40	0.489
Nodular	"	15(15)	5.73	4.495	95.5	1.47	2.695	0.94	1.495
Small	"	18(17)	4.89	3.281	87.2	1.39	2.604	0.88	1.485
Large	"	8 (8)	6.25	3.071	100.0	1.12	0.411	0.62	0.554
Diffuse	40 +	7 (6)	7.43	5.952	85.7	1.29	0.905	0.33	0.267
Nodular	"	31(26)	8.29	11.346	100.8	1.77	2.214	0.73	1.485
Small	"	9 (8)	7.33	18.000	127.6	1.67	1.750	0.38	0.554
Large	"	29(24)	8.38	8.172	91.3	1.76	1.940	0.75	1.500

* *Figures in parentheses indicate mothers included as reliable for data on death under one year.*

Table 5. Fertility and offspring mortality related to goiter among men in Pedregoso.

FATHER			OFFSPRING						
Goiter	Age (years)	N*	Total ever born			Total dead		Dead 1 year	
			$\bar{x}$	s^2	Sex ratio	$\bar{x}$	s^2	$\bar{x}$	s^2
None	30-39	6 (6)	5.83	10.967	59.1	1.00	2.400	0.67	1.467
Diffuse	"	9 (8)	4.88	1.361	91.0	1.33	3.500	0.75	1.929
Nodular	"	6 (6)	6.00	3.600	56.5	1.00	1.600	0.50	0.700
Small	"	9 (9)	5.00	1.500	87.5	1.22	3.847	0.67	1.750
Large	"	6 (5)	5.83	3.767	59.0	1.17	1.367	0.60	0.800
None	40 +	10(10)	9.40	6.711	100.0	1.80	2.178	1.10	1.433
Diffuse	"	11(10)	7.55	6.870	130.6	1.27	0.818	0.30	0.456
Nodular	"	22(19)	9.59	20.820	81.9	1.95	2.998	0.84	1.585
Small	"	13(12)	7.00	15.830	122.0	1.31	1.564	0.33	0.606
Large	"	20(17)	10.20	14.130	109.3	2.00	2.737	0.88	1.610

* *Figures in parentheses indicate fathers included as reliable for data on death under one year.*

Table 6. Taste sensitivity to PTC and goiter in Pedregoso.

Age (years)	Thyroid	N	Thresholds (%)														
			<1	1	2	3	4	5	6	7	8	9	10	11	12	13	14
0-19	Non goitrous	♂16	6.2							12.5	18.7	18.7	25.0	18.7			
	Goitrous	♂27								7.4		48.1	33.3	3.7	3.7		3.7
	Non goitrous	♀12										58.3	25.0	8.3	8.3		
	Goitrous	♀22		4.5						4.5	13.6	18.2	31.8	18.2	4.5	4.5	
20-39	Non goitrous	♂15	13.3							13.3		26.6	46.7				
	Diffuse	♂17					5.9				23.5	23.5	41.2		5.9		
	Nodular	♂ 9								11.1	22.2	66.7					
	Diffuse	♀40				2.5				2.5	12.5	22.5	30.0	20.0	5.0	5.0	
	Nodular	♀22								9.1	9.1	31.8	36.4	13.6			
40 +	Non goitrous	♂10		10.0						10.0	20.0	10.0	30.0	20.0			
	Diffuse	♂10									20.0	30.0	50.0				
	Nodular	♂19	10.5						10.5		5.3	15.8	36.8	21.1			
	Diffuse	♀ 9									22.2	33.3	33.3			11.1	
	Nodular	♀25	4.0	4.0							12.0	28.0	36.0	16.0			

N = Number of subjects.

Table 7. Taste sensitivity to PTC and goiter in Pedregoso.

Age (years)	Thyroid	Sex	Non-tasters		Tasters		
			N	%	N	$\bar{x}$	s.e.
20-39	Nongoitrous	M	15	13.3	13	9.23	0.303
	Diffuse	M	17	5.9	16	9.38	0.272
	Nodular	M	9	0.0	9	8.56	0.438
	Diffuse	F	40	2.5	39	9.90	0.220
	Nodular	F	22	0.0	22	9.36	0.242

Table 8. Effect of nodularity and sex on PTC thresholds in Pedregoso. (Tasters only, 20 to 39 years)

Source of variations	S.S.	D.F.	M.S.	F	P
Nodularity	7.53	1	7.53	5.121	<0.05
Sex	6.92	1	6.92	4.708	<0.05
Interaction	0.35	1	0.35	0.240	
Subtotal	14.80	3	4.93		
Within	120.65	82	1.47		
Total	135.45	85			

Finally, no correlation was observed among PTC tasting threshold of tasters and thyroid size coded as previously explained.

DISCUSSION

Two significant tendencies of thyroid variability have been demonstrated in families of Pedregoso: the intersibship variation of size is greater than the intrasibship one, and there is a direct relation between the frequency of children with nodular goiter and the presence of this characteristic among the parents. Findings such as these do not prove by themselves the participation of genes, since environmental causes such as infection, dietary deficiency, or toxic agents can also have a family distribution, and all of these have been implicated in the etiology of goiter.

Variation of goiter within sibships must be due to individual differences in the interplay of genetic and environmental causes. Subjects with aberrant thyroid size (Figure 2) may be mainly the consequence of genic segregation in either a simple or a polygenic system, or the result of a peculiar convergence of environmental goitrogenic circumstances, or, more, probably, the product of the interaction of both systems. Presumably the influence of environmental causes accumulates with time and hence intrasibship variation may increase when the members grow older. The fact that our

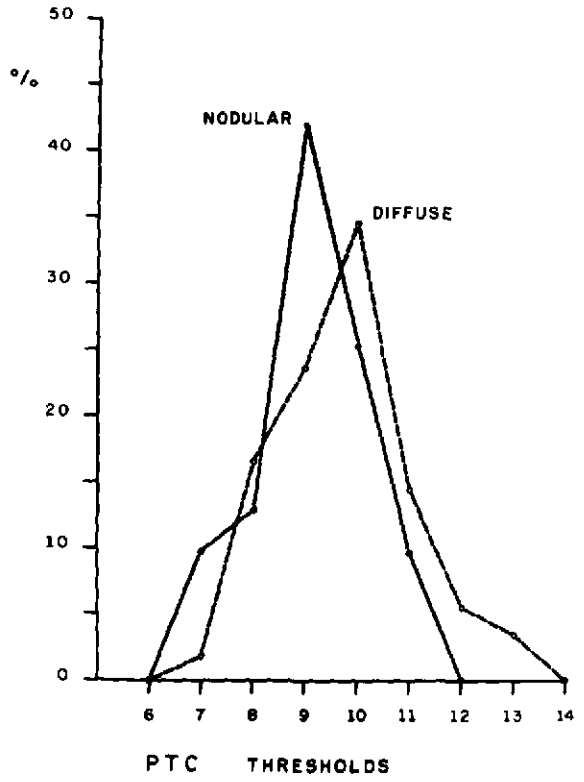

Figure 3. Frequency distribution of PTC thresholds among tasters of 20 to 39 years of age, according to type of goiter.

statistical analysis for family concentration of thyroid size shows highly significant results only in younger groups of sibs suggests the importance of genetic factors at this age. The lack of significant results observed when the age of the older sib examined was only 9 years can be explained in view of the small size of these sibships and the low prevalence of goiter in this period of life.

The family tendency to nodularity may be interpreted as due to differences in the age of appearance of the nodular goiters. Nodularity seems to be the end-result of the natural history of practically every goiter, but the precocity of its appearance could have a different meaning, possibly genetic. In this respect it is interesting to note the opinion of a number of investigators, mentioned by Costa (8), that "iodine prophylaxis is more effective in attenuating and retarding the appearance of endemic goiter than in combating its occurrence," and especially the existence of nodularity.

The genetic argument for nodularity is reinforced in Pedregoso by its significant relation to the thresholds of PTC among tasters in the 20 to 39 year age group, the age when most of the precocious nodular goiters appear. This new relation fits with the idea that the T gene for PTC tasting ability is partially dominant over its allele, as is suggested by the finding of Kalmus

of a higher threshold value for the taster homozygotes (TT) when compared with the heterozygote (Tt) (18). Thus, not only are the non-tasters (tt) positively associated with nodular goiter, either sporadic (17, 19) or endemic (2, 6, 23), but possibly also the heterozygous tasters. Such a hypothesis would require further studies in families. Another argument in its favor is our observation of a lower prevalence of nodular goiter among subjects born to consanguine marriages (5), which presumably include a higher frequency of homozygotes for the PTC genes.

The biochemical mechanism involved in the relationship of nodularity and the PTC gene is still a matter of conjecture. Differences between tasters and non-tasters in the peripheral metabolism of goitrogenic substances have failed to show clear results (25). On the other hand, a difference in the activity of a saliva peroxidase has been demonstrated between them (13) and the suggestion has been made that a similar circumstance could operate in the thyroid (25). Furthermore we have shown in Pedregoso striking differences in iodine kinetic studies among nodular goiters (5) and it would be worthwhile to attempt to correlate these with PTC studies.

Since the PTC phenotypes have been found among anthropoid apes, R.A. Fischer (12) has postulated that it constitutes "a stably balanced and enduring dimorphism" where the heterozygote should have a selective advantage over the homozygote. The natural selection mechanism involved could be somehow related to goiter (1). It is of interest in this respect that the study of reproductive histories in Pedregoso raises the possibility of fertility positively associated with either nodularity, goiter size, or both, independently of sex. This trend is accompanied by a less clear one for higher offspring mortality. Such evidence constitutes only indirect support to the thesis that PTC heterozygotes have some selective advantage. A direct study of the reproductive performance in relation to PTC thresholds would be the correct approach to solve this question.

The participation of PTC genes is probably only one of many genetic mechanisms influencing goiter variability. Since it is clear from epidemiological data that an important part of goiter variation is due to sex, many authors (10) have postulated either a gene localized in the sex chromosomes or simple limitation of the process by sex.

In past (11) and recent (14) publications skeptical attitudes concerning genetic participation in endemic goiter can be found. These are probably the consequence of the complexity of population findings together with problems of definitions, methodology, and statistical analysis. In order to arrive at our results we have made an effort to establish definitions appropriate to the Pedregoso endemia (5). Yet we are aware of the limitations of our analysis, which is based on statistical models whose applicability to the complex population structure of Pedregoso is debatable. In this respect the main problem is sampling of a small population where most of the subjects are genetically related in some way. These analytical difficulties have been recently commented upon by Neel and Salzano (24) and Gershowitz et al. (15) in their studies among the Xavante Indians.

The genetic mechanisms involved in endemic goiter in Pedregoso can be visualized as being the result of a polygenic system including at least one pair of genes (PTC genes) that creates a polymorphism which is probably

balanced. Thoday (28) has expressed optimism on the possibility of analyzing polygenic systems in man by the use of special population models. He has been able to individualize the genes of a polygenic system in Drosophila, showing that they are not as numerous as they were previously supposed to be. Such an approach seems promising in future research on endemic goiter. In principle we are of the opinion that observed variability in any population represents the outcome of a unique combination of environmental and genetic causes (27). Among them we can distinguish some that are widespread, such as lack of iodine and the PTC genes, and some that are peculiar to a certain endemia, such as natural goitrogens and the details of the genetic structure of the population.

SUMMARY

Variability of endemic goiter has been correlated with genetic data among the Pewenche Indians of Pedregoso, Chile. An analysis of thyroid characteristics in families reveals that sibships differ significantly in thyroidal size variation and that there is a positive correlation between nodularity of parents and offspring. Adults with nodular goiters, when compared with those with diffuse goiter, are characterized by: significantly lower threshold values for tasting phenylthiocarbamide among tasters; a lower amount of inbreeding; and higher fertility rates.

It is suggested that genetic factors in endemic goiter have a polygenic basis, one of whose components, the PTC genes, constitute a balanced polymorphism.

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SECTION VI

ENDEMIC GOITER IN COLOMBIA

CHAPTER 24

STUDIES ON THE PATHOGENESIS OF ENDEMIC GOITER IN THE CAUCA VALLEY, COLOMBIA, SOUTH AMERICA

Eduardo Gaitán[1] and Heinz W. Wahner[2]

HISTORY OF GOITER IN COLOMBIA

Accounts of the history of endemic goiter in Colombia have been published in the recent past by Kelly and Snedden (21), Rueda Williamson and Pardo Téllez (36), and Callejas-Arboleda et al. (4). Endemic goiter was first mentioned in Colombia as early as 1568. It was in 1831 that the French scientist Boussingault (3), as a result of observations on goiter and iodine content of salt samples from certain localities of Colombia, pioneered the idea that salt should be iodized in order to prevent goiter. The Department of Nutrition of the Inter-American Cooperative Public Health Service (Servicio Cooperativo Interamericano de Salud Pública) began in 1946 a four-year survey throughout the entire country to determine the geographic distribution and frequency of goiter among 180,000 schoolchildren. An overall prevalence of 53 per cent was found. The goiter endemia was most severe throughout almost the whole length of the valleys of the Magdelena and Cauca Rivers. The magnitude of the problem prompted official action, and in 1950 the "Concesión Salinas" of the Republican Bank in collaboration with the National Institute of Nutrition initiated the iodization of the natural salt deposits at Zipaquirá, at a level of 1 part of iodine per 25,000 parts of salt (40 ppm). Most of the production was at first distributed as a pilot study among five towns in the Department of Caldas, where the goiter prevalence was 81 per cent among schoolchildren during the 1946-1950 survey. Two years later the frequency had fallen to 40 per cent (17), and in 1965 it was claimed that less than 1 per cent of the school population was affected by goiter (Rueda Williamson and Pardo Téllez (36). Surveys were also made in 1959 and 1965 in the town of Mariquita (Department of Tolima) by a different group of investigators (Callejos Arboleda et al. (4). While prevalence rates of 51 per cent in the school population of this town had been reported in 1950 (17), 27 per cent of individuals 1 to 18 years of age had goiter in 1959, and 25 per cent in 1964. Salt samples contained 40 to 60 ppm of iodine on both occasions. Similar results have been obtained in the Cauca Valley by Gaitán et al. (14), and they will be discussed later in the text.

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Iodinization of the salt supply of the entire country according to the recommendations of the WHO Study Group on Endemic Goiter (51) was given official sanction in 1955 (Decree No. 0591 of 10 March), and since then its use has been compulsory throughout the national territory (10, 36). Forty-eight per cent of the consumable salt supply was iodized in 1959, 76 per cent in 1964, and 85 per cent in 1966. The National Institute of Nutrition is at present officially controlling the salt iodization program. The actual content of iodine is 50 to 75 ppm.

Endemic goiter in the Cauca Valley has been investigated in some depth during the past ten years. Histopathological characteristics were described by Correa and Castro (7), and comparison of goiter pathology from the Cauca Valley with that observed in a nonendemic area was made by Welsh and Correa (50). Studies of thyroid pathophysiology (12, 13, 48) and intrathyroidal iodine metabolism (13, 49) in endemic goiter and on its relation to carcinomas of the thyroid gland (47) have also been reported.

This paper will deal with studies on the pathogenesis of endemic goiter in the Cauca Valley and particularly in the town of Candelaria in the Department of Valle del Cauca. This area of 20,430 square kilometers is located in southwest Colombia, at altitudes ranging from sea level to 4,000 meters. The Valley of the Cauca River, an agricultural and cattle-raising area with an altitude of 1,000 meters, extends from 400 to 600 miles north of the equator and lies 70 miles inland parallel to the Pacific coast, between two mountain chains of the Andes system. Candelaria is a small town located in this valley. It has a mestizo population of about 25,000 inhabitants; 4,500 live in the urban area.

RESULTS AND LIMITATIONS OF TWELVE YEARS OF IODINE PROPHYLAXIS

A high incidence of endemic goiter was reported in the Cauca Valley in 1915 (8). Prevalence rates of 80 per cent or more were found among schoolchildren in certain localities of the Department of Valle del Cauca in 1948 to 1950 (17). Goiter was observed predominantly in the agricultural and cattle area along the Cauca River. Less goiter was found in the coffee, mining, and forest areas.

The prevalence* rates of goiter ranged between 16 and 98 per cent in the school population of five towns surveyed in the Cauca Valley in 1948 to 1950 (Figure 1). These figures were found to be markedly reduced in three of the towns when a similar examination was conducted in 1960. The earlier percentage frequency of goiter in Puerto Tejada (98 per cent), Guacari (95 per cent), and Candelaria (85 per cent) had decreased respectively to 32, 30, and 23 per cent. However Buenaventura, with 21 per cent and Zarzal with 16 per cent of goiter in 1948 to 1950, had not experienced any significant change by 1960. Prevalence rates of goiter in the general population of the same towns in 1959-1960 corresponded approximately to those found in the school population. Fifteen urine samples, taken randomly during the surveys in Zarzal and Guacari in 1959 and 1960, showed iodine concentrations of 68 to 228 µg per gm

* Prevalence of goiter is the relative frequency of goiter at a certain time.

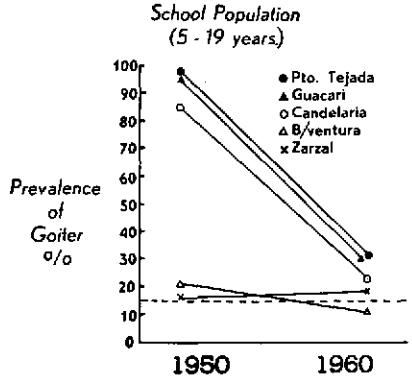

Figure 1. Prevalence rates of goiter in the school population of five towns in the Department of Valle del Cauca, in 1950 and 1960. Introduction of iodized salt in 1955.

creatinine. Salt samples obtained from local stores in Candelaria and Guacari contained respectively 23 and 32 μg of iodine per gm dry weight.

Since the iodization program had been started in 1955, it was not clear in 1960 whether the goiter endemia was continuously declining or whether the decline had reached a stable level. To answer this question a longitudinal epidemiologic study in the general and school population of Candelaria was carried out between November 1959 and November 1967.

The results obtained in the school population appear in Figure 2. During this period of eight years the prevalence rates, as determined at frequent intervals by cross-sectional surveys, had remained constant and around 30 per cent with a range of 20 to 50 per cent. For correlation with prevalence figures, urinary iodine excretions were measured at frequent intervals in representative samples of the school population examined during these surveys. Mean values were between 195 and 328 μg per day. This amount of iodine is considered more than adequate.

Various parameters of iodine metabolism in goitrous and nongoitrous children from Candelaria on six different occasions are compared in Table 1. In no instance was there a statistically significant difference in urinary iodine excretion between goitrous and nongoitrous children. Thyroidal ^{131}I uptakes and PBI concentrations were also always similar in both groups. The mean and range values of thyroidal uptakes were within low normal limits and in accordance with the high urinary iodine excretions. PBI concentrations were always within the normal range.

The "attack rate"* of goiter was determined for 242 schoolchildren from April to December 1965. A rate of 12.8 per cent was encountered for this eight-month period. Mean urinary iodine excretions were respectively 316 (81 to 646) μg per day and 302 (96 to 745) μg per day, and prevalence rates of

* "Incidence" or "attack rate" of goiter is the relative frequency of occurrence of goiter during a certain period.

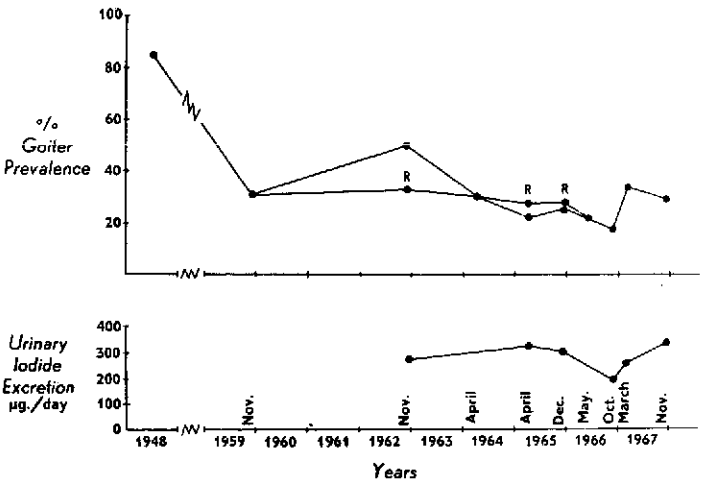

Figure 2. Longitudinal epidemiologic study in the school population of the town of Candelaria. Introduction of iodized salt in 1955. Prevalence rates of goiter are correlated with urinary iodine excretions. R: Children from rural areas.

goiter 20.8 per cent and 28.9 per cent in April and December of that year. During this period 16.1 per cent of schoolchildren had remained with their goiters.

Twenty-three salt samples randomly taken from the houses of children with and without goiter in the survey conducted in Candelaria in November 1962 showed a mean iodine concentration of 33.3 (range 11.4 to 51.5) µg per gm dry weight. This concentration corresponds to one part of iodine in 30,000 (20,000 to 90,000) parts of salt, which is more iodine than recommended by the WHO Study Group on Endemic Goiter (51).

The percentage frequency of goiter in another school population of similar age distribution and socioeconomic conditions to those of Candelaria but living 20 miles away was determined in May 1966. While at that time in Candelaria 22 per cent of goiter was found among 100 schoolchildren, only 4.7 per cent was observed among 202 schoolchildren at Aguablanca, a suburb of the city of Cali. The difference is significant ($P < .001$). Mean urinary iodine excretion in 43 samples from Aguablanca was 338 (126 to 617) µg per day, which is within the range of values that have been found in Candelaria.

Cross-sectional surveys were made in the general population of Candelaria in March 1960 and again in March 1967. Overall prevalence rates of goiter of 22 per cent were found on both occasions.

For correlation with the prevalence figures, various parameters of iodine metabolism were measured in goitrous and nongoitrous individuals representative of the general population of Candelaria during the survey of March 1967. No statistically significant differences existed between goitrous and nongoitrous subjects in urinary iodine excretion ($P > .50$), PBI concentrations ($P > .80$) and thyroidal ^{131}I uptakes ($P > .90$) (Table 2). PBI values were consistently within normal limits. Thyroidal ^{131}I uptakes were within the low normal range, correlating well with the high daily urinary iodine excretions.

Table 1. Comparison of various parameters of iodine metabolism in goitrous and nongoitrous children from the town of Candelaria, Cauca Valley, Colombia.

Date		Normal children				Goitrous children				
		% Thyroidal ^{131}I uptake 3 hrs.	24 hrs.	PBI µg %	U ^{127}I** µg /day	% Thyroidal ^{131}I uptake 3 hrs.	24 hrs.	PBI µg %	U ^{127}I µg /day	"t" test* (p values)
November 1962	n:	(10)		(10)	(10)	(10)		(10)	(10)	
	$\bar{x}$:	9.1	15.2	7.28	273	9.7	17.2	6.85	268	> 0.95
	r:	(7.0-10.0)	(8.0-19.0)	(6.1-8.6)	(104-504)	(5.0-12.0)	(8.0-23.0)	(5.1-8.7)	(154-446)	
April 1965	n:				(39)				(29)	
	$\bar{x}$:	-		-	301	-		-	336	> 0.80
	r:				(81-592)				(141-646)	
December 1965	n:				(37)				(34)	
	$\bar{x}$:	-		-	325	-		-	315	> 0.95
	r:				(96-575)				(153-745)	
October 1966	n:		(6)		(23)		(6)		(14)	
	$\bar{x}$:	-	14.6	-	178	-	13.2	-	223	> 0.20
	r:		(8.2-17.6)		(61-480)		(1.2-17.7)		(40-356)	
March 1967	n:		(8)	(5)	(10)		(11)	(6)	(25)	
	$\bar{x}$:	-	9.8	5.90	246	-	9.9	6.40	261	> 0.70
	r:		(6.1-15.9)	(5.4-6.6)	(123-412)		(5.3-15.9)	(4.8-8.3)	(130-462)	
November 1967	n:				(12)				(11)	
	$\bar{x}$:	-		-	285	-		-	409	> 0.10
	r:				(150-391)				(219-710)	

** *Urinary iodine excretion.*

* *Levels of statistical significance for urinary iodine excretions between normal and goitrous children.*

n Number of subjects.

$\bar{x}$ Mean values.

r Range.

*Table 2. Iodine metabolism in goitrous and nongoitrous subjects from the town of Candelaria.**

Subjects		% Thyroidal I^{131} uptake (24 hrs.)	PBI µg%	Urinary iodide excretion µg/day
Normal	n:	(40)	(25)	(141)
	$\bar{x}$:	10.7	6.06	455
	range:	(3.6-23.0)	(4.0-9.8)	(18-1492)
Goitrous	n:	(50)	(25)	(90)
	$\bar{x}$:	10.6	5.94	497
	range:	(1.3-26.7)	(4.1-8.3)	(130-1862)
	p:	> .90	> .80	> .50

* *March 1967.*

n Number of subjects.

$\bar{x}$ Mean values.

p Levels of statistical significance as compared to values in normals.

Urinary iodine excretion was also determined in samples collected immediately after their arrival in this country from 85 Peace Corps individuals in 1967, none of whom had enlarged thyroid glands. Mean and range values of 257 (61 to 963) µg per day were found. As these values were significantly lower than those from Candelaria and none of the Peace Corps subjects had goiter, there was direct evidence that the daily dietary iodine intake at Candelaria was more than adequate.

A comparison of the frequency distribution of goiter by age in the general population of Candelaria in March 1960 and March 1967 appears in Figure 3. There is a marked resemblance in the shape of the two curves and no major changes occurred in frequency distribution during this interval of seven years. On neither occasion was goiter observed below 5 years of age. Therefore, one must conclude that by 1967 some children in the age group 5 to 12 years had developed goiter despite an adequate iodine supplementation. Furthermore, a few children that were born while their mothers were on a sufficient iodine intake developed goiters by 1967.

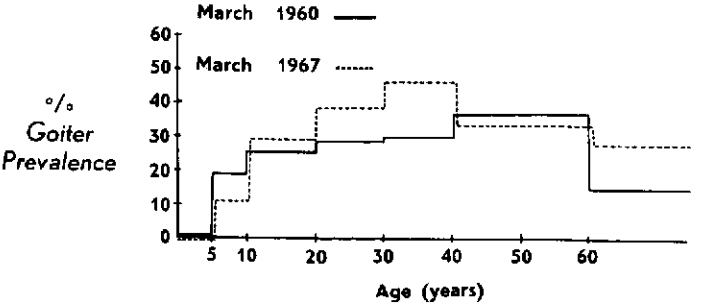

Figure 3. Percentage frequency distribution of goiter by age in the general population of the town of Candelaria in March 1960 and March 1967.

COMMENTS AND CONCLUSIONS ON THE ABOVE OBSERVATIONS

Before the iodization program began, statistically significant differences existed in the prevalence rates of goiter among towns located near one another. Once iodine prophylaxis was established, we found: (1) a marked decrease in the frequency of goiter in the towns with high rates, but no change in those with low rates; (2) constant and fixed prevalence rates of goiter of around 30 per cent in school and adult populations of Candelaria during a period of eight years when the population was receiving uniformly adequate iodine supplementation as documented by consistenly high urinary iodine excretions, low normal thyroidal ^{131}I uptakes, and sufficient iodine content of the salt; (3) an incidence or attack rate of goiter in schoolchildren of 12.8 per cent in an eight-month period; (4) the absence of goiter during the first five years of age and its subsequent appearance while taking iodine; (5) the development of goiter in children born while their mothers were on an appropriate iodine intake; and, finally (6) a statistically significant difference in the frequency of goiter between schoolchildren of similar age distribution, socioeconomic status and urinary iodine excretion, but living 20 miles away. These facts provide evidence to support the thesis that the goiter endemia which is still present at Candelaria is not responsive to an adequate iodine supplementation and therefore must be caused by factors other than a state of iodine deficiency. It seems possible that such an endemic might become more prominent and take on different functional characteristics whenever iodine deficiency coexists, and that once iodine is supplied the frequency of goiter is reduced to a 20 to 30 per cent residual rate. Hypothyroidism and cretinism are not associated with the Cauca Valley endemia but hyperthyroidism and carcinoma of the thyroid gland are common medical problems (12, 13, 47, 49).

Our situation is by no means unique. Endemic goiter has also been described in other areas where sufficient iodine was supplemented (4, 6, 15, 24, 41). A general characteristic of this situation is that prevalence rates of goiter are much lower than those observed when there is nutritional iodine deficiency. Usually, rates of 20 to 30 per cent have been found. The prolonged and longitudinal nature of our studies provide evidence that iodine prophylaxis has been as effective as possible in reducing prevalence rates of goiter in Candelaria. However, there is no question that iodine supplementation is a public health measure that effectively reduced the previous high rates and substantially diminished the risk of developing goiter in a large segment of the population. Therefore, the presence of iodine is an important conditioning factor in determining the magnitude of the Cauca Valley endemia.

The reciprocal situation also occurs. Severe iodine deficiency has been demonstrated in the absence of endemic goiter (5, 35). It has been reported recently by Delange et al. (9) that a striking difference in prevalence of goiter exists in two groups of natives of Idjwi Island, both showing marked iodine deficiency. These findings suggest that iodine deficiency is not the sole cause of endemic goiter. In north Idjwi, besides iodine deficiency, the participation of a goitrogenic factor was postulated. Thus it is necessary to consider other factors which may be involved in the pathogenesis of endemic goiter in the Cauca Valley.

FURTHER EPIDEMIOLOGICAL STUDIES

Schoolchildren were plotted on a map of Candelaria according to the address given at the time of the survey in April 1965. An uneven distribution of goiter was apparent. Therefore, the urban area was stratified as illustrated in Figure 4, and the frequency of goiter determined for each zone. Table 3 shows that the lowest percentage of goitrous children lived in Zone B. This was the only district in which frequency of goiter (11.5 per cent) was significantly different from that of the urban area as a whole (21.7 per cent) ($P < .05$). Thus, several factors that may have caused this distribution were analyzed.

1. The first factor considered was differences in iodine supplementation. Table 3 shows that there was no correlation between the uneven distribution of goiter and mean urinary iodine excretion rates, which were uniform and consistently high in all zones.

2. Since the highest frequency of goiter in schoolchildren occurred at 13 years of age (14) and therefore predominance of a certain age group could significantly alter prevalence rates of goiter, the frequency distribution by age of the school population in each zone was studied. Figure 5 shows that age distributions in Zones B and C and the urban area were the same, in contrast with that observed in children coming to school from rural areas. Thus, differences in frequency distribution by age of the school population in the urban area were not responsible for the observed uneven distribution of goiter.

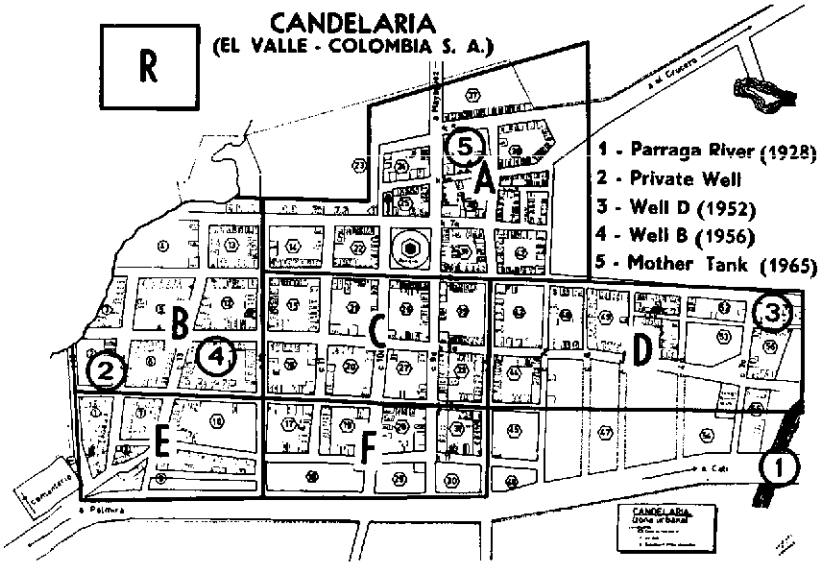

Figure 4. Map of the stratified urban area of Candelaria. It shows the water supply systems since 1928. R: Rural area.

Table 3. Distribution of goitrous schoolchildren, by zones, in the stratified urban area of Candelaria.

Zone	April 1965			December 1965		
	n	Goiter %	Urinary iodine µg/day (mean)	n	Goiter %	p values
Urban	397	21.7	316	443	26.8	NS
A	91	21.0	347	92	20.7	NS
B	52	11.5*	250	81	30.9	<.01
C	100	29.0	332	69	24.6	NS
D	44	25.0	350	71	28.2	NS
E	58	22.4	220	59	30.5	NS
F	52	15.4	333	51	29.4	NS
Rural (R)	94	27.7	328	69	26.1	NS

n - Number of schoolchildren.
p - Levels of statistical significance for each area and zone between % values of April and December.
NS - Non-significant at the 5% level.
** - p <.05, if tested against percentage in urban area (21.7%) as a whole.*

3. Another factor was sex. It has been reported that sex differences do not affect prevalence rates of goiter at school age (14). Furthermore, similar percentages of females (33 to 38 per cent) were living in all zones.

4. A socioeconomic survey in a 5 per cent randomized sample of households representative of the stratified urban area of Candelaria was conducted in 1966-1967. It included information about (a) family size and composition; (b) age and sex of each family member; (c) civil status; (d) education; (e) occupation; (f) income; (g) distribution of expenses; (h) housing conditions; and (i) migration. The results obtained neither explained the uneven distribution of goiter nor gave significant clues regarding factors that may be involved in the pathogenesis of endemic goiter.

5. A three-day dietary survey was also carried out in each of the households where the socioeconomic studies were conducted. Diet composition, food habits, caloric intake, and sources of food were similar among all zones of the urban area. Banana was the most common food element of the diet.

6. Information was also obtained about the water supply of the town. As shown in Figure 4 various water sources have been used and several changes have occurred since 1928 in the water supply system of the town. The first aqueduct was operated in 1928, the water being pumped out from the Párraga River. Otherwise, the water was obtained from shallow (≃ 12 ft deep) private wells, some of which are still operating. The Párraga River supply was discontinued in 1952, when a well 150 feet deep was sunk in Zone D (Well D). It supplied must of the town until 1956, when another well 138 feet deep was dug in Zone B (Well B). These two wells provided the water for the whole town through

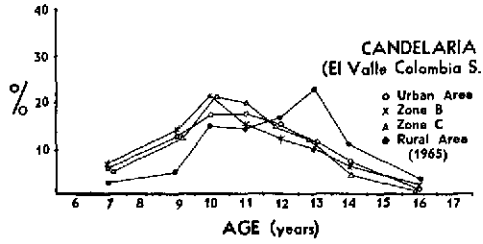

Figure 5. Frequency distribution by age of the school population in Zones B and C, urban area, and rural area of Candelaria.

separate tanks and independent pipe lines until early in 1965, when a new water system was installed. It consists of a tank with capacity for 75,000 gallons in Zone A (Mother Tank) that collects all water from Wells B and D plus the water of another well in Zone A, 150 feet deep and dug in 1965. Since then, water has been distributed from this tank through a completely new and common pipeline system to the whole town. This water does not receive any kind of treatment.

The school population was again plotted in the stratified urban area of Candelaria according to the address given during the survey of December 1965. The distribution of goiter was then compared with that observed eight months previously. Table 3 shows a homogeneous distribution of goiter at the time of the second survey. During this eight-month period an increase in the percentage of goiter from 11.5 to 30.9 occurred in Zone B ($P < .01$). No differences of significance were observed in any other zone.

At the time of the survey of April 1965 the new water supply system had been operating for no more than three months and at least 11 months had elapsed when the December survey was conducted. Thus, there arose the distinct possibility that the differences in water supply were responsible for the initial uneven distribution of goiter and its subsequent change.

IDENTIFICATION OF GOITROGENIC SUBSTANCES IN WATER

Since ancient times a relation between goiter and the quality of drinking waters has been suggested (45). Caldas in 1808 and Camacho in 1810 were the first to attribute the Colombian endemia to the quality of local drinking waters. Some of these are of exceptional hardness (40). In 1831 Boussingault (3) commented on the association of goiter with limestone formation. Stott (43) suggested that goiter in India was directly related to the high calcium content of the drinking water. Murray et al. (30), studying goiter or "Derby neck" as it is sometimes called in England, came to the conclusion that even in the presence of similar iodine content there was a greater incidence of visible thyroid glands in areas with "hard" waters, as in England, than in areas with "soft" waters, as in Scotland. Nevertheless, it was also mentioned that goiter may occur in places with "soft" waters like New Devon. In Indonesia in Tjibodas district there is a well called "the well of Gondok" (the well of goiter). By superstition, drinking from this well leads to the development of

goiter (28). As early as in 1911 Repin (32) induced goiter in rats by giving them waters from Saint-Pancrace, a highly endemic place. Calcium has been the element most commonly implicated as "goitrogenic" in hard waters (19, 44, 45).

Since the classical investigations of McCarrison (26) at the beginning of this century, water pollution has also been thought to cause goiter. Recently Vought et al. (46) have suggested that pollution of drinking waters may exert a goitrogenic effect in northern Virginia.

Because the degree of hardness and bacterial contamination of waters have been more persistenly suggested as responsible factors of their goitrogenic activity, water samples representative of the different water supply system used in Candelaria since 1928 (Figure 4) were taken for physicochemical and bacteriological analysis. Water from the city of Cali was also investigated. Table 4 summarizes the important findings of the physiochemical analysis carried out on four different occasions by standard methods for the examination of water as recommended by the American Public Health Association (42). A complete spectrum in quality of waters from "soft" to "very hard" was found (37). Water from Wells B and D and the Mother Tank, which are untreated and constitute the present supply in Candelaria, are "hard" waters (150 to 300 ppm) with high concentrations of calcium, magnesium, manganese, and sulfate. The water from the private well (rarely used) is "very hard" (above 500 ppm) with particularly high concentrations of calcium, magnesium, sulfate, chloride, and nitrate, but a concentration of manganese within the acceptable range. The water from the Párraga River once used by the people of Candelaria (1928) as drinking water is moderately hard (75 to 150 ppm). The water from Cali, which is treated, is a soft water (0 to 75 ppm). It has low concentrations of the various elements already mentioned. Table 5 shows the results of bacteriological analysis. Waters from the Párraga River and the private well are heavily contaminated with bacteria. Other waters from Candelaria show less contamination and the Cali water is free from bacteria.

With this information it was decided to test in the rat whether these water have "goitrogenic" activity.

Experiment No. 1

Female albino rats of the Charles River C.D. Strain (descendent of the Sprague-Dawley strain) weighing initially 150 to 170 gm were divided into two groups. One group was fed with Purina Laboratory Chow (Ralston Purina Co.) containing 2 mg ^{127}I per kg and the other with Remington low-iodine test diet (Nutritional Biochemical Corporation) containing 10 μg ^{127}I per kg. Each of the two groups was further divided into eight subgroups of four rats each. To each subgroup, one on the low-iodine diet (LID) and another on the Purina Laboratory Chow, a particular water was assigned for the complete period of the experiment lasting 65 days. It was thought that rats given "demineralized" water from Cali, which is "soft" (1 ppm) (Table 4) and without bacteria (Table 5), would be an appropriate control of the experiment, and rats on the same water but with added methimazole, 25-30 μg per day per rat, as an adequate standard for "goitrogenic" activity. The degree of this activity was determinated by six different parameters: (1) thyroid weight; (2) thyroid ^{131}I uptake (24 hour); (3) iodine (^{127}I) content of the thyroid gland; (4) histology; (5) intrathyroidal iodine metabolism ($\frac{MIT + DIT}{I^-}$): iodination of tyrosines (2),

*Table 4. Physicochemical characteristics of waters from the city of Cali and the town of Candelaria.**

Sample		Total hardness** ppm	Calcium ppm	Magnesium ppm	Manganese ppm	Sulfates ppm	Chlorides ppm	Nitrogen nitrates ppm	Fluorides ppm
Cali (demineralized)		1 Soft	- 0 -	0.2	0.01	1.8 (1.2-3.0)	2.1 (1.5-3.2)	0.02 (0.01-0.05)	0.05 (0.05-0.06)
Cali		43 (34-48) Soft	10.4 (8.0-12.8)	4.1 (3.4-4.9)	0.02	12.9 (10.7-16.0)	7.4 (6.7-8.6)	0.12 (0.07-0.18)	0.61 (0.50-0.70)
CANDELARIA	Párraga River	127 (114-142) Mod. hard	28.8 (24.8-33.6)	13.4 (12.6-14.1)	0.03 (0.03-0.04)	38.3 (25.0-57.0)	4.6 (3.4-5.2)	0.09 (0.04-0.15)	0.22 (0.18-0.30)
	Well B	247 (209-318) Hard	48.1 (40.8-54.4)	30.9 (20.9-52.5)	0.55 (0.50-0.60)	49.0 (45.0-57.0)	8.7 (5.2-12.0)	0.11 (0.02-0.24)	0.13 (0.10-0.17)
	Well D	238 (223-264) Hard	47.1 (45.6-71.2)	23.0 (13.6-28.7)	0.80 (0.70-0.90)	50.0 (40.0-55.0)	12.0 (10.8-13.6)	0.06 (0.02-0.10)	0.17 (0.10-0.21)
	Mother Tank (A+B)	235 (222-252) Hard	55.7 (54.0-58.4)	23.1 (20.9-27.0)	0.50 (0.45-0.55)	30.0 (22.0-38.0)	7.5 (6.8-8.6)	0.08 (0.02-0.14)	0.13 (0.10-0.20)
	Private well	514 (438-556) Very hard	70.2 (47.2-92.8)	82.0 (50.0-106.0)	0.04 (0.02-0.05)	72.0 (61.0-90.0)	110.0 (100.0-120.0)	13.9 (8.6-20.0)	0.23 (0.20-0.30)

** Mean and range values of analysis at four different times are given (March, July, October, and November 1967).*

*** Soft: 0-75 ppm.-Mod. hard: 75-150 ppm.-Hard: 150-300 ppm.-Very hard: > 300 ppm.*

Table 5. Bacteriological analysis of waters from the city of Cali and the town of Candelaria.

Water sample	E. coli* No. /100ml	EMB	BGB	Bacterial count** Colonies /ml
Cali (demineralized)	0	-	-	200
Cali	0	-	-	0
CANDELARIA Párraga River	240,000	+	+	10.500
CANDELARIA Well B	240	+	+	600
CANDELARIA Well D	240	+	+	875
CANDELARIA Mother Tank	- 0 -	-	-	1.000
CANDELARIA Private Well	940,000	+	+	3.500

* *Lactose broth.*
EMB Eosin-methylene blue agar.
BGB Brilliant green-bile salt agar.
** *Agar, 37° C/24 hours.*

MIT/DIT ratio and ^{131}I thyroxin concentration); and (6) PB^{131}I and Conversion Ratio (24 h). The mean thyroid weight (9.4 mg per 100 gm rat) of rats receiving l-thyroxin (T_4), 2.5 µg, and l-triiodothyronine (T_3), 0.6 µg per day per rat, was used as a baseline to judge thyroid enlargement. Half of this amount of hormone has been shown to prevent goiter in rats on a low iodine diet and methimazole (0.05 per cent) (52).

An inverse relation was found between thyroid weight and thyroid ^{131}I uptake and iodine (^{127}I) content. (Figure 6). The larger the gland, the lower were the ^{131}I uptake and concentration and total content of iodine. Thyroids of rats drinking waters from Cali and Well D of Candelaria were significantly larger than those of rats taking waters from Well B and the Párraga River ($P < .05$ to $< .001$). The enlargement and functional behavior of the glands of rats on methimazole were comparable to those of rats drinking tap water from Cali. Thus these waters appear to have very high goitrogenic activity. Demineralized water from Cali and tap water from Well D of Candelaria had high goitrogenic activity. While thyroid enlargement in rats drinking water from the Mother Tank was comparable to that found in those taking waters from the private well,

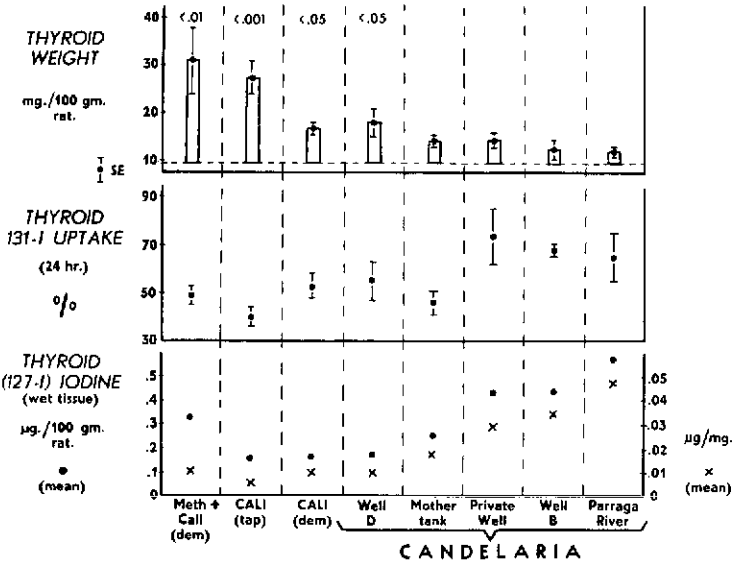

Figure 6. "Goitrogenic activity" in waters from the city of Cali and the town of Candelaria, October-December 1967. Meth: Methimazole, 25-30 µg /day/rat. <.05-<.001: levels of statistical significance when compared to Well B and Párraga River groups. dem: demineralized water.

Well B, and the Párraga River, the thyroid ^{131}I uptake and the concentration and total content of thyroid iodine were significantly lower. Thus this water seems to have "intermediate goitrogenic activity." Waters from the private well, Well B, and the Párraga River induced little thyroid enlargement but high thyroid ^{131}I uptakes and high concentrations and total content of thyroidal iodine. Therefore, these water have low goitrogenic activity. The distribution of radioactive (^{131}I) compounds in thyroid hydrolysates according to methods previously described (49) appears in Table 6. The results of a control group fed on Purina are also shown for comparison. It is apparent that in all groups on low-iodine diet there was marked and similar decrease in iodination of tyrosines (2). MIT/DIT ratios were high as expected, but diiodotyrosine (DIT) formation was most affected in the Cali water groups and least affected in the private well and the Párraga River groups. Thyroxin synthesis was impaired in rats on Cali waters but was unaffected in rats taking waters from the wells of Candelaria. Rats given the Párraga River water with low goitrogenic activity showed a lower percentage of ^{131}I thyroxin than rats given Well D water with high goitrogenic activity. Methimazole did not further exaggerate the defects observed in hormone synthesis induced by the Cali water.

Thus there was no correlation between the degree of hardness or bacterial contamination and the degrees of "goitrogenic activity." The private well, with the highest degree of hardness (> 500 ppm) and the most contaminated with bacteria, had low goitrogenic activity. Demineralized water from Cali with the least degree of hardness (1 ppm) and free from bacteria had a high goitrogenic activity. Therefore other factor(s) must account for the goitrogenicity of these waters.

Table 6. Conversion ratios (C.R.) in plasma and distribution of radioactive (^{131}I) compounds in thyroid hydrolysates of rats on low-iodine diet and waters from the city of Cali and the town of Candelaria (October-December 1967).

	CALI			CANDELARIA (TAP)					CONTROL
	(Dem.) + Meth.	(Tap)	(Dem.)	Well D	Mother Tank	Private well	Well B	Párraga River	Purina* diet
$\frac{DIT + MIT}{I^-}$	6.8	4.8	5.5	4.7	2.3	3.5	3.4	5.0	19.7
MIT/DIT	2.4	2.7	2.6	2.2	2.3	1.9	2.3	1.8	0.7
THYROXIN %	6.1	8.0	8.5	18.4	19.9	22.2	21.1	13.1	21.8
C.R., % 24 hrs. (Mean ± 150)	75.8 ±4.3	78.8 ±1.3	72.7 ±4.0	85.3 ±5.8	84.8 ±4.5	85.2 ±4.4	81.5 ±1.6	87.7 ±1.4	20.4 ±6.3

* *Ralston Purina Co. (≃ 2 mg. ^{127}I/kg).*

(Dem) Demineralized water.

(Tap) Tap waters.

Meth Methimazole, 30 µg/day/rat.

MIT Monoiodotyrosine.

DIT Diiodotyrosine $\frac{DIT + MIT}{I^-}$*: Iodination of tyrosines.*

In contrast with the results on low iodine diet, no appreciable differences were observed in any parameter among the groups fed with Purina Laboratory Chow. Even the action of methimazole was completely masked by the Purina diet. Rats on this diet had smaller thyroids (6.0 mg per 100 gm rat), lower thyroidal ^{131}I uptakes (13.2 per cent), MIT/DIT ratios (0.7) and conversion ratios (32.7 per cent), and higher thyroidal (^{127}I) iodine content (0.97 µg per mg and 5.9 µg per thyroid weight per 100 gm rat), proportion of ^{131}I thyroxin (23.3 per cent) and iodination of tyrosines (18.6) than animals on the low iodine diet.

Experiment No. 2

Waters from Cali and Well B and Well D of Candelaria were used for this experiment. The specific aims were: (1) to test the consistency of results by replicating the first experiment with a larger number of animals, and (2) to determine goitrogenic activity in distillates and residues of the three waters. Approximately two-thirds of an original volume of tap water were distilled ("distillate") and the remaining one-third of concentrated water was used as the "residue." Experimental conditions and parameters for measuring goitrogenic activity were the same as those used during the first experiment. At this time rats were younger, with initial weights of 120 to 150 gm. Only the low iodine diet was used. The experiment lasted 65 days.

A comparison of thyroid weights between the first and second experiments are shown in Figure 7. The consistency of results is clear. Here again waters from Cali and Well D showed higher goitrogenic activity than the Well B water.

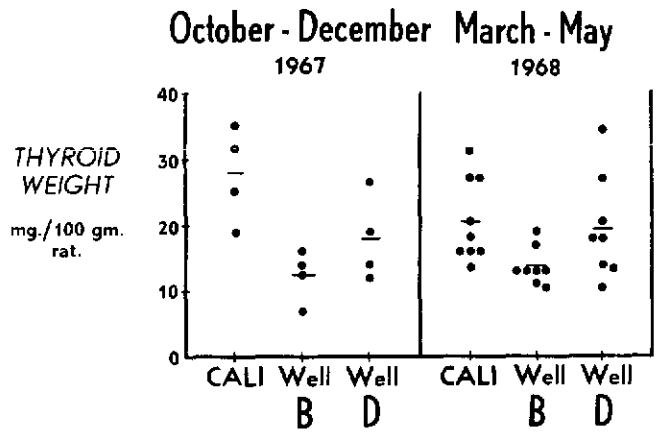

Figure 7. Comparison of thyroid weights between experiments in October-December 1967 and March-May 1968.

The differences are statistically significant when data from both observations are pooled ($P < .001$ to $P < .05$).

The thyroid glands of rats drinking waters from Well B and Cali are shown in Figures 8 and 9.

The results obtained for weights, ^{131}I uptakes, and iodine (^{127}I) content of thyroid glands from rats put on tap, distilled, and residual waters of Cali and Well B and Well D are summarized in Figure 10. Goitrogenic activity was consistently greater in distillates than in tap waters of the three sources, but the differences in activity observed among tap waters remained present in distillates. Rats drinking distilled water had larger glands, lower uptakes, and lower concentrations and total iodine content of the thyroid than rats on tap waters. The three distillates were "soft" (< 10 ppm) and acid (pH 5.5). Water residues from Wells D and B were devoid of goitrogenic activity; enlargement of thyroid glands was negligible, and thyroid ^{131}I uptakes were elevated, as were also the total content and concentrations of iodine in the glands. These changes were more prominent in rats taking the Well B water residue. This demonstrates again the lower goitrogenic activity of the Well B water. In contrast the goitrogenic activity of the Cali water residue was almost as potent as that of the tap water. This may be related to the different physicochemical characteristics of residual waters from Cali and Wells B and D. While in the untreated waters from the wells the solid phase forms insoluble salts which are precipitated by boiling, in the treated water from Cali the solid phase concentrate in ionized form and therefore raises its total "hardness" (stable hardness). Thus, the Wells B and D water residues had a total hardness of less than 6 ppm, while the Cali water residue had 175 ppm. The three residues were alkaline (pH≈9.0).

The results of studies on thyroidal hormone synthesis appear in Table 7. Again, impaired DIT formation and thyroxin synthesis were observed with the three waters. These defects were more apparent with distillates than with tap waters. However, Well B distillate induced lower MIT/DIT ratios and a higher proportion of radioactive T_4. This indicated lesser thyroid depressant action

Figure 8. Thyroid gland of a rat drinking water from Well B of Candelaria.

Figure 9. Thyroid gland of a rat drinking water from the city of Cali.

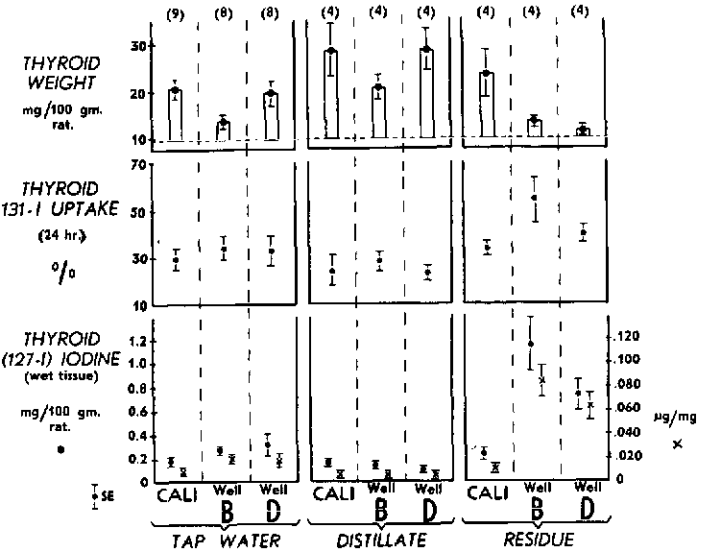

Figure 10. "Goitrogenic activity" in tap waters and in distillates and residues of waters from the city of Cali and the town of Candelaria. March-May 1968. ( ): number of rats.

than the Cali or Well D. distillates. Wells B and D water residues significantly decreased MIT/DIT ratios but only the Well B water residue improved thyroxin sunthesis. This confirms that residual waters from Wells B and D have little or no goitrogenic activity, but also strengthens the fact that the Well B water has lesser antithyroid activity than the Well D water. The rate of hormone synthesis in thyroid glands from rats on Well B water residue was similar to that observed in rats taking combined T_4, 2.5 µg, and T_3, 0.6 µg per day per rat, a program which prevented the development of goiter in rats drinking Cali water distillates. As in the first experiment, the addition of 90 µg per day per rat of methimazole to Cali water distillates did not further exaggerate its antithyroid activity.

These findings indicate that the goitrogenic activity concentrated in the distillate from these waters, while it disappeared from the water residues. Furthermore, the activity was not affected by boiling but was inhibited by administration of T_4 and T_3.

COMMENTS AND CONCLUSIONS

Trials to induce goiter in rats with low-iodine diets (LID) have given varying results, as is shown in Table 8. While Levine et al. (23), Remington (31), McCarthy et al. (27), and Greer et al. (18) were able to induce goiters in rats on LID in 35 to 84 days; Money et al. (29) and Ekpechi et al. (11) failed to produce them with LID in periods as long as 206 days. At least four factors may explain these different results. (1) Iodine content of the diet. In the case of Money et al. (29) this must have been very low since a significant increase of the thyroidal ^{131}I uptake occurred as early as the second day on their LID, and 24-hour uptakes as high as 46 per cent remained constant

Table 7. Distribution of radioactive (^{131}I) compounds in thyroid hydrolysates of rats on low-iodine diet and waters from the city of Cali and the town of Candelaria (March-May 1968).

Water source	Tap waters			Distillates			Residues		
	DIT + MIT / I^-	MIT/DIT	T_4%	DIT + MIT / I^-	MIT/DIT	T_4%	DIT + DIT / I^-	MIT/DIT	T_4%
Cali	4.3	2.1	9.8	3.4	2.6	6.9	4.6	2.1	7.7
Well B	3.5	1.8	11.4	4.1	2.1	10.1	5.9	1.2	16.1
Well D	5.2	1.8	10.9	4.1	2.9	6.8	3.8	1.2	9.5
Cali + Methimazole 90 µg/day/rat				4.6	1.9	8.9			
Cali + l-T_4, 2.5 l-T_3, 0.6 µg/day/rat				6.7	1.2	17.2			

MIT - *Monoiodotyrosine.*
DIT - *Diiodotyrosine.*
$\frac{DIT + MIT}{I^-}$ - *Iodination of tyrosines.*
l-T_4 - *Thyroxin.*
l-T_3 - *Triiodothyronine.*

*Table 8. Trials to induce goiter in the rat by prolonged feeding with low-iodine diets (LID).**

Days	Sex	Body wt. gm (initial)	Thyroid wt. mg/100 gm rat	^{131}I uptake %	Thyroid ^{127}I µg/mg	Author and year
35	-	60	53.2	-	.015	Levine et al., 1933
35	M & F	60	32.3**	-	.017	Remington, R.E., 1937
84	M	100	27.9	-	-	Mc Carthy et al., 1959
42	M	100 - 120	18.9	-	-	Greer et al., 1967
56			23.4	-	-	
70			33.1	-	-	
77			21.3	-	-	
84			58.4	-	-	
270			87.4	-	-	
34	M	310+	5.6	45.8	.728	Money et al., 1952
106		279+	9.1	36.4	.208	
237		377+	16.5	40.5	.049	
206	F	170 - 200	10-11	26.0	.100	Ekpechi et al., 1965

* *Thyroid weights of 4.7-12.6 mg/100 gm rat, have been reported on Purina Laboratory Chow (≃2 mg ^{127}I kg) or GPI diet (0.4 mg ^{127}I kg, Levine et al. (23).*
** *Drinking distilled water.*
\+ *Final weight.*

during the whole experiment lasting 237 days. Concomitantly, a progressive decrease of thyroidal iodine (^{127}I) concentration was observed. (2) Age and previous diet. These two factors determine iodine stores of the thyroid gland which are important because a reciprocal relation between thyroidal iodine content and degree of thyroid enlargement has been consistently found (11, 23, 29, 31), and was also observed in our experiments. (3) Susceptibility of the strain. (4) Presence of goitrogenic or antigoitrogenic substances in food or water.

The same factors must be considered in order to give proper interpretation to our results. Because all animals were of similar age and strain and were fed with the same lot of LID, the first three factors and those concerned with goitrogenic or antigoitrogenic (1) substances in the diet can be ruled out. Thus one is left with the presence of a goitrogenic or an antigoitrogenic factor(s) in some of the waters. The most important antigoitrogenic factor to be considered is iodine. The possibility exists that some groups of rats were obtaining an excess of iodine from sources other than the LID. An analysis of such sources is summarized in Table 9. A daily iodine intake of 0.2 μg per rat from the LID was calculated; the daily consumption of food was 18 to 20 gm per rat and the LID contained 10 μg of ^{127}I per kg. The daily intake of iodine was the same for all groups of rats, since they had similar increments in weight at the end of experiments. Considering the iodine content of the different waters, the total daily iodine intake of our rats ranged between 0.21 μg and 0.34 μg. According to Levine et al. (23), rats at this level of daily iodine have thyroid glands that weigh 21.4 to 28.6 mg per 100 gm body weight. These values are within the range of what we have called "high" goitrogenic activity (Cali and Well D waters). To maintain the weight of thyroid glands at the level of what we have called "low" goitrogenic activity (Párraga River and Well B waters) an additional 1.5 to 3.5 μg of iodine per rat must be supplemented daily. This means that our rats should have consumed waters containing 60 to 100 μg of ^{127}I per kg. None of the waters in our experiments contained such high concentrations. In Candelaria the Well D water, which consistently had the highest iodine concentration (5.4 μg per kg), also showed the highest goitrogenic activity. In contrast, Párraga River and Well B waters, which consistently had the lowest iodine concentrations (2.9 and 3.7 μg per kg), also showed the lowest goitrogenic activity. Furthermore, water distillates with no iodine did not contribute significantly to the daily iodine ingestion. Nevertheless, differences in goitrogenic activity among groups were still found. Thus, we may conclude that there was no correlation between the extra amounts of iodine and the degrees of goitrogenic activity.

The fact that lower conversion ratios and $PB^{131}I$ values were observed in rats on Cali water than in rats on Párraga River water is against the hypothesis that enlargement of the thyroid gland occurred in some groups because of a lower ingestion of iodine (Table 6). Furthermore, thyroidal ^{131}I uptakes were measured at 2, 5, 10, and 24 hours in rats placed on LID and Cali water distillates. As is shown in Figure 11, the highest uptake values were encountered at 24 hours. Similar results on LID have been reported by Leblond and Mann (22) and Money et al. (29). Therefore we may conclude that in rats with enlarged thyroid glands of our experiments, the ^{131}I uptakes were really depressed.

Once an excess of iodine ingestion by some of the groups has been ruled out, the presence of a goitrogenic factor(s) in Cali and Well D waters must

*Table 9. Analysis of factors that may influence the iodine intake of rats in experiments on the thyroidal effects of waters from the city of Cali and the town of Candelaria.**

Water source	Iodine in waters µg/kg Tap	Distillate	Residue	% Body wt.** (mean)	Water intake ml/day/rat (mean)	Iodine intake (tap waters) µg/day/rat
Cali	0.2+	0.07	0.6	64	28	.006
Candelaria: Well D	5.4+ (4.6-6.0)	0.43	12.6	65	26	.140
Candelaria: Mother Tank	4.3	-	-	66	30	.129
Candelaria: Private well	3.9	-	-	61	30	.117
Candelaria: Well B	3.7+ (3.2-4.2)	0.13	12.3	63	26	.096
Candelaria: Párraga River	2.9	-	-	57	24	.070

* *Rats were fed throughout experiments with the same lot of Remington low-iodine test diet (Nutritional Biochemicals Corp., ≃10µg ^{127}I kg).*

** *Per cent increments in body weight.*

\+ *Mean and range values of three determinations.*

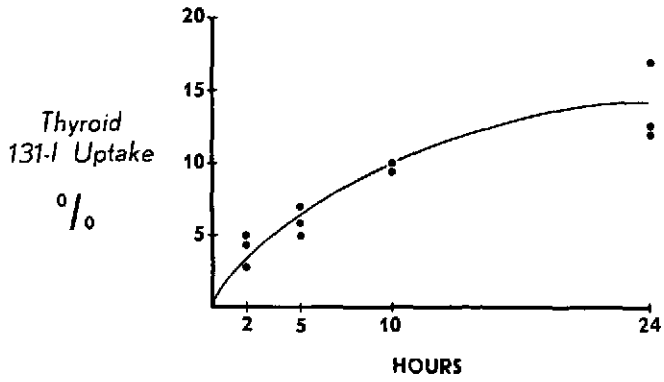

Figure 11. Thyroid ^{131}I uptakes at 2, 5, 10, and 24 hours in rats on LID and distilled water from the city of Cali.

be postulated. The consistency of the results and the correlation between epidemiological observations and experimental findings further support this hypothesis.

We have already ruled out the possibility that "goitrogenic activity" in these waters is due to bacterial contamination or degree of "hardness." Ca^{++}, Mg^{++}, Mn^{++}, sulfates, nitrates, and chlorides are not goitrogenic factor(s) in the concentrations found in "very hard" waters. Furthermore, the goitrogenic factor(s) concentrates in the distillate and is not destroyed by boiling. The possibility that an organic volatile compound with goitrogenic activity is present in these waters has been considered.

The effects of this factor on the thyroid gland may be summarized as follows: (1) impairment of thyroxine synthesis; (2) decrease of diiodotyrosine (DIT) formation; (3) depletion of iodine stores; (4) suppression of thyroid ^{131}I uptake; and (5) induction of goiter. Thyroid hormones (T_4 and T_3) reverse these alterations. These effects are similar to those produced by propylthiouracil (PTU) and PTU-like antithyroid agents (2, 20, 25, 33, 34, 38, 39).

SUMMARY

A high incidence of endemic goiter has been known for many years in the Cauca Valley of Colombia. Prevalence rates as high as 80 per cent have been reported in the past but the prevalence has decreased since iodized salt was introduced in 1955. Nevertheless, endemic goiter has not disappeared from the childhood population of this region in spite of ample iodine intake, as demonstrated by the adequate amounts of iodine found in 24-hour collections of urine.

The possibility of positive goitrogenic factors has been entertained. Studies have been done on rats given water from various sources in the district and from the city of Cali.

The results up to the present time suggest the possibility that there are volatile components in some of the drinking waters which may have a goitrogenic effect. Studies to identify these possible goitrogenic agents are now in progress.

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CHAPTER 25

THYROID FUNCTION IN ADOLESCENTS FROM THE GOITER ENDEMIC OF THE CAUCA VALLEY, COLOMBIA, SOUTH AMERICA

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It is generally accepted that iodine deficiency is the dominant factor in the development of endemic goiter (27). Therapeutic and preventive measures are therefore directed toward an adequate iodine supply for the affected population. This, however, may not be true in all instances. Endemic goiter has been described in areas with adequate iodine supplies (8), and even in the presence of unusually high iodine intake (28). Further, the possibility has been suggested that marked iodine deficiency might be found in populations which do not exhibit any increase in the occurrence of goiter (7, 11, 26). The experience of four decades of iodine prophylaxis has shown a persistent residual incidence of goiter in spite of many years of adequate iodine intake. The effects of varying amounts of stable iodide itself on the function of the human thyroid gland have been studied and a susceptible population group has been defined in which iodine alone leads to inhibition of thyroid function (13, 23).

In the endemic goiter area of the Cauca Valley, chronic nutritional iodine deficiency has existed in the past (17, 31). Since the introduction of iodized salt in 1952 a marked reduction of goiter incidence has been demonstrated (16). However, eight years of repeated well-controlled epidemiological studies of goiter prevalence in Candelaria, a town in the valley not far from Cali, have shown a persistence of goiter among schoolchildren of around 30 per cent. This has been associated with an apparently adequate iodine intake as measured by urinary iodine excretion (mean 328 µg day, range 150-570 µg day) (14). These facts cast doubt on the concept that iodine deficiency is a major factor in the residual and persistent goiter found in the Cauca Valley. It might have been a permissive factor in the past.

The present investigation of kinetics of iodine metabolism was undertaken to define further the role of iodine as a causative factor for goiter in children of Candelaria. The possibility that there exists a faulty utilization of the ingested iodine was investigated and iodine itself as a cause of goiter evaluated. To establish unequivocally the fact whether or not there is iodine deficiency seems important for future planning of preventive measures since prophylaxis using depots of iodized oil can assure an adequate level of iodine for a patient in an iodine deficiency state and is now easily available where needed (18, 22). Yet this form of therapy does not seem likely to be of benefit to the children of Candelaria.

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MATERIALS AND METHODS

Iodine metabolism was studied in three males and four females from Candelaria, a rural village in the endemic goiter area of the Cauca Valley in southwestern Colombia. Pathological, epidemiological, and biochemical studies have been performed in this area over the years 1960 to 1968 (9, 14, 31, 32). Two patients had normal thyroid glands by palpation and served as nongoitrous euthyroid controls from the area under study. Five patients had palpable grade I or II diffuse goiter (WHO scale (24)), characteristic for the adolescent age group. One patient (#3) was treated with Methimazole, 60 mg daily, from the fifth day of the study. All subjects were hospitalized from eight to 16 days, the period of the study.

Pre-study examination included urinalysis, complete blood counts, and examination of feces for intestinal parasites and occult blood. With the exception of one patient (#7) who was under treatment for protein malnutrition and hookworm-induced anemia, all patients were healthy. Pertinent data are given in Table 1. All except patient #7 had a family history of goiter. The diet and food habits at home were known from a previous diet survey (14). Similar diets were given during the study. No drugs were allowed except to patient #7, who received ferrous sulfate. None of the patients had taken iodine or other medication for goiter in the past. All were using commercially available salt, which is iodized (1 part iodine to 25,000 parts salt). The daily estimated salt intake for this population is 15 gm (20).

Seventy-five to 150 μc of carrier-free radioactive iodide (^{131}I) supplied by Abbott Laboratories was given intravenously. Accumulation of ^{131}I in the region of the thyroid was measured using a NaI(th) scintillation detector, fitted to a spectrometer. Collimation was achieved by using a flat field collimator with specifications as recommended by the IAEC expert commission (3). A standard No. 2 lead filter was used. The mean ratio of measured to true thyroid uptakes using six different volumes and levels of activity (μc) was 1.027 (15). Plasma radioactivity was determined in 2 ml samples using a two-channel automatic gamma counter (Nuclear-Chicago). Blood samples were taken at 10-minute intervals during the first 30 minutes, then every half hour for three hours and at two-hour intervals for the rest of the day, followed by daily blood samples. Urine was collected for brief time periods during the first day of the study and then daily for determination of radioactivity, creatinine excretion, and stable iodine. Protein-bound ^{131}I ($PB^{131}I$) and conversion ratios were determined daily from the second day of the study. Protein bound ^{127}I- iodine (PBI) concentration was determined by a modification (1) of the alkaline-ash incineration method of Barker and Humphrey (2), using the reduction of ceric ammonium sulfate in the presence of arsenious acid. Iodotrol-R was used as internal standard during the whole procedure to correct for loss of iodide. The same method was used to measure iodide in urine and homogenates of thyroid tissue. When urinary iodide was determined hemoglobin was added as protein carrier (27). Daily urinary iodine excretion was calculated from the iodine/creatinine ratio (29). Creatinine in urine specimens was determined within two days of collection by the method of Bonsnes and Taussky (6).

Calculations were based on a three-compartment model of iodine metabolism (Figure 1). The results were analyzed according to formulae derived by Riggs (25), Berson and Yalow (5), and by Ermans et al. (12). Mathematical

Table 1. Clinical data on seven patients in thyroid function study.

Case	Age (yrs.)	Sex	Weight (kg)	Height (m)	Days of study	Diagnosis
			Group 1: Euthyroid without goiter			
1	27	F	56.5	1.60	14	Healthy volunteer
2	20	F	57.0	1.57	12	Healthy volunteer
			Group 2: Euthyroid with diffuse goiter			
3	12	F	37.5	1.43	11	Grade 1 goiter, known 5 yrs (25 gm)
4	20	F	62.5	1.61	13	Grade 1 goiter, since childhood (25 gm)
5	12	M	37.5	1.41	12	Grade 1 goiter, known 3 yrs (20 gm)
6	14	M	37.0	1.60	10	Grade 2 goiter, about 8 yrs (25 gm)
7	19	M	37.5	1.59	18	Grade 1 goiter, 10 yrs., protein malnutrition, anemia (20 gm)

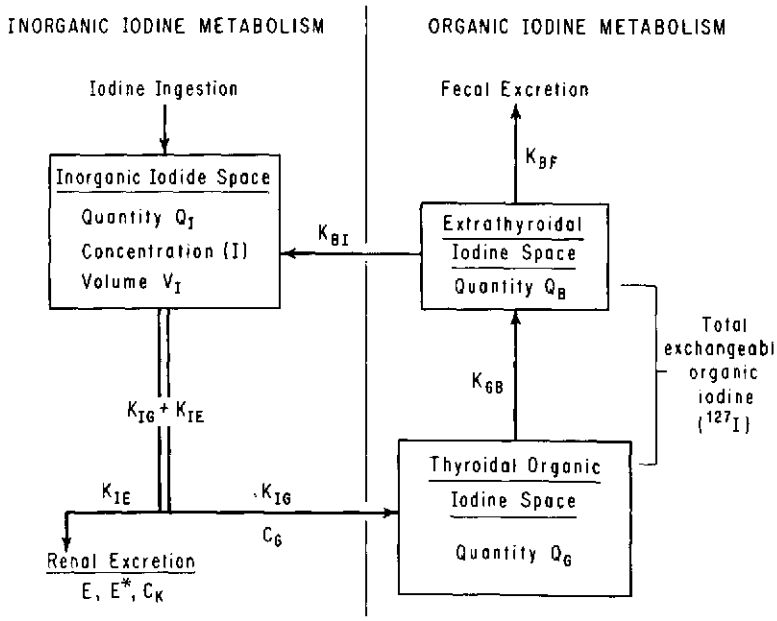

Figure 1. The three-compartment model of iodine metabolism used in the analysis of kinetic data.

symbols and general procedures of calculation were used as summarized by DeGroot (10) with only minor variations. Results of the kinetics were calculated by two or more different approaches. Only a short summary of symbols and formulae used is listed in Table 2. An example of the graphic evaluation of data is given in Figure 2.

RESULTS

The results can be explained in terms of the three-compartment model and fit within the established pattern of iodide metabolism. There was good agreement of data when calculated by different techniques.

Commonly used thyroid function tests are listed in Table 3. All patients were euthyroid by these criteria. Urinary excretion of stable iodine was 541 μg per day (mean) in nongoitrous patients and 568 μg per day in subjects with diffuse goiter. There was no change in iodide excretion during the period of the study. Consistent with these findings, plasma inorganic iodide ranged from 11 to 22 μg per l in both groups. The iodide pool when expressed in liters (Table 4) was within the normal range, but total exchangeable iodine was elevated (Table 5).

Radioactive iodide uptake, measured three and 24 hours after the intravenous administration of the dose, was 9.0 per cent and 17.0 per cent (mean), respectively, in nongoitrous patients, and 9.9 per cent and 17.9 per cent in patients with diffuse goiter. Thyroid ^{131}I clearance was low normal. Renal ^{131}I clearance was 29,3 ml per min and 23.1 ml per min respectively. Rates of movement of iodine between the various compartments are listed in Table 6. Total exchangeable iodine was high in both groups: 34.9 mg in patients without goiter and 20 mg in patients with diffuse goiter. Extrathyroidal exchangeable iodine was calculated on the basis of a previously measured thyroxine distribution space of 14.8 per cent of body weight (30) and was within the normal range. The thyroidal exchangeable iodine pool (Q_3) was 17.5 mg in the control group and 15.3 mg in patients with diffuse goiter (Table 5). The amount of exchangeable iodine present per gram tissue was 0.8 mg and 0.5 mg respectively. Chemically determined iodine content of the thyroid tissue had shown similar results (32). This indicates that all iodine present in the gland was exchangeable homogeneously.

The $PB^{131}I$ was 0.037 per cent per liter in both groups when measured 72 hours after the application of the dose. Constant values were reached between the tenth and twelfth day. There was no evidence for significant iodide escape from the gland. Calculated secretion rates of hormonal iodine and absolute uptakes showed only a minor discrepancy (Table 7). The observed urinary ^{131}I excretion was higher by a factor of 2 to 5 than the excretion predicted on the basis of hormone released (Table 8). Specific activity in the urine was lower than specific activity in the plasma at equilibrium state. The urinary ^{131}I excretion curve did not parallel the $PB^{131}I$ curve at the end of the study, but rather, followed the thyroid ^{131}I release curve.

Hormonal iodine secretion from the gland was within normal limits in both groups of patients studied (Table 7).

Table 2. Some formulas and symbols used in the calculations of thyroid function.

Symbol	Dimension	Remarks
$PB^{127}I$	µg/liter	Protein-bound iodine
$PB^{131}I$	% dose/liter	Protein-bound ^{131}I
U_t	% dose	Thyroidal ^{131}I uptake at time t
E^*_t	% dose	Cumulative urinary excretion of ^{131}I from beginning to time t
E	ug	Average ^{127}I content in 24-hour urine collections
I^*_t	% dose/liter	Plasma iodide ^{131}I concentration at time t
C_k	ml/min	Renal iodide clearance $C_k = \frac{E^*_{t_2} - E^*_{t_1} \times 1000}{\text{average } I^* \times t_2 - t_1}$ $t_2 - t_1$ = 180-30 min, 180-300 min
I^*_0	% dose/liter	Plasma ^{131}I at time zero; extrapolation of plasma curve to zero time (t = 1-8 hours)
I	µg/liter	Plasma inorganic iodide concentration $I = \frac{E}{C_k \times 1.44}$
$V_I^{\#1}$	liter	Iodide space $V_I^{\#1} = \frac{100}{I^*_0}$
$V_I^{\#2}$	liter	$V_I^{\#2} = \frac{100 - (U_t + E^*_t)}{I^*_t}$
C_G	ml/min	Thyroidal iodide clearance $C_G = \frac{(U_{t_2} - U_{t_1}) \times 1000}{\text{average } I^* \times t_2 - t_1}$
Umax	% dose	Theoretic maximal thyroid uptake
$K_{IG+IE}^{\#1}$	fraction (fr) of dose per hour	Plasma ^{131}I disappearance rate (4-8 hours)
$K_{IG+IE}^{\#2}$	fr/hr	Determined from semilogarithmic plot of Umax - U_t (t = 0-24 hours)
K_{IG}	fr/hr	Portion of above due to thyroid uptake $K_{IG} = U \times K_{IG+IE}$
K_{IE}	fr/hr	$K_{IE} = K_{IG+IE} - K_{IG}$

Table 2. Some formulas and symbols used in the calculations of thyroid function (continued).

Symbol	Dimension	Remarks
$AIU^{\#1}$	μg/day	Thyroid stable iodine uptake (absolute uptake) $AIU = \frac{UE}{1-U}$
$AIU^{\#2}$	μg/day	$K_{IG} \times Q_I^{\#1} \times 24$ $(Q_I = I \times V_I^{\#1})$
$AIU^{\#3}$	μg/day	$C_G \times (I) \times 1.44$
$K_G^{\#1}$	fr/day	^{127}I release constant (calculated from U_t)
$K_G^{\#2}$	fr/day	Calculated from urinary iodide excretion $K_G = \frac{\text{Average E*/day}}{\text{Average U/day}}$ (during the last days of the study)
$K_{GB}^{\#1}$	fr/day	Thyroidal iodine secretion rate constant $K_{GB}^{\#1} = \frac{K_G^{\#1}}{1-Umax}$
$K_{GB}^{\#2}$	fr/day	Calculated as above, using $K_G^{\#2}$
$K_{GB}^{\#3\ \#4}$	fr/day	Estimation according to method of Berson and Yalow (5)
^{127}I	μg	Exchangeable iodine (Berson and Yalow (5)) $^{127}I = \frac{\text{PBI/liter}}{PB^{131}I \text{ fraction retained/liter}}$ $PB^{131}I$ is expressed here in fraction of dose retained
$Q_B^{\#1}$	μg	Exchangeable extrathyroidal iodine $Q_B^{\#1}$ = PBI x body wt x distribution space of T4 (fraction/body wt)
$Q_B^{\#2}$	μg	= $PB^{131}I$ fraction retained/liter x body wt x T4 space x ^{127}I
$Q_G^{\#1}$	μg	Exchangeable iodine in thyroid $^{127}I = (Q_G + Q_B)$ $Q_G = {}^{127}I - Q_B^{\#1}$
$Q_G^{\#2}$	μg	$Q_G = {}^{127}I - Q_B^{\#2}$
$Q_G^{\#3}$	μg	$Q_G = \frac{Ut}{\text{SA serum PBI}}$, at final period of study
$H^{\#1-4}$	μg/day	Thyroidal iodine secretion $H = K_{GB}^{\#1-4} \times Q_G^{\#3}$
SA_S	% dose/μg I	Specific activity of serum PBI SA (PBI) = $\frac{\%\ \text{dose}\ PB^{131}I/\text{liter}}{PB^{127}I\ \mu g/\text{liter}}$

Table 2. Some formulas and symbols used in the calculations of thyroid function (continued).

Symbol	Dimension	Remarks
SA_U	% dose/μg I	Specific activity of urine SA (urine) = $\frac{\%\ \text{dose}\ E^*/\text{day}}{E\ \mu g/\text{day}}$
Predicted urinary iodine excretion (%/day) = body weight (kg) x 0.148 x $PB^{131}I$ (%/liter) x K_{BI} x (1-U*).		
K_{BI} is turnover rate of endogenously labelled thyroid hormone, assumed to be 0.1/day (fraction of dose).		

DISCUSSION

For the purpose of our study the three-compartment model proved adequate. Limitations of this model have been discussed (4) and multi-compartment models using computer programs for analysis have been proposed (cf. Chapter 4). However, these limitations do not alter the specific results aimed at in the present study.

A large number of transfer rate constants, pool sizes and spaces have been evaluated and are listed in the tables in order to permit comparison of the subjects from Candelaria with healthy subjects from nonendemic goiter areas. In the majority of the measured parameters of iodine metabolism there were no significant differences, which illustrates that an effective adaptation mechanism guarantees normal hormone production. Compared with subjects from nonendemic goiter areas (5, 10, 12, 25) patients from Candelaria had a high urinary iodide excretion. This was about three times higher than values reported from the United States and more than ten times higher than those reported from areas with known dietary iodine deficiency. The high urinary iodine excretion, high plasma inorganic iodide, and high total exchangeable iodide are reflections of a more than adequate dietary iodine intake in the patients of Candelaria.

All patients studied had a high total exchangeable iodine due to a large intrathyroidal iodine pool. In the presence of normal hormonal iodine secretion rate and absence of significant iodide leakage from the gland, the low $PB^{131}I$ indicates a homogeneous mixing of the administered ^{131}I in a large pool. Chemical measurements of the total thyroidal iodine were of the same order of magnitude as the calculated intrathyroidal exchangeable iodine. This is evidence that only one compartment of iodine existed in the thyroid gland.

There was some iodide escape from the gland, but this was within the order of magnitude seen in normal glands (10).

The increased size of the goitrous gland does not seem to be related to the dietary iodine intake, which was equal in both groups. However, on the same level of available iodine the normal gland was able to maintain a higher iodine concentration per gram thyroid weight. It seems that the inhibiting

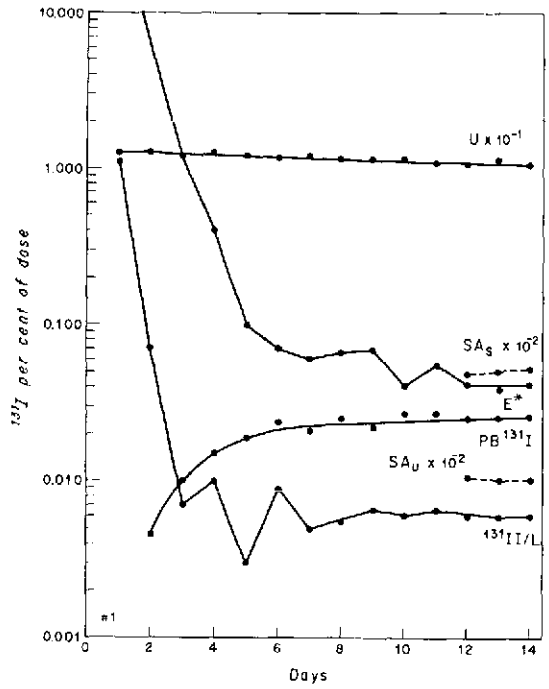

Figure 2. An example of the graphic evaluation of data according to the three-compartment model.

Table 3. Commonly used thyroid function tests and data on seven patients in study.

Case	$PB^{127}I$ µg/L	Uptake of ^{131}I 3 hr	Uptake of ^{131}I 24 hr	$PB^{131}I$ (72 hr), % dose/L	Iodide clearance, ml/min Thyroidal	Iodide clearance, ml/min Renal	Plasma inorganic iodine, µg/L	Urinary iodine excretion, µg/day
				Group 1: Euthyroid without goiter				
1	51.5	7.0	12.5	0.019	3.2	27.9	14.6	586
2	61.2	11.1	20.4	0.053	7.9	30.8	11.2	496
Mean	56.1	9.0	16.5	0.036	5.1	29.3	12.9	541
				Group 2: Euthyroid with diffuse goiter				
3	54.0	11.5	20.8	0.019	4.5	14.5	16.8	581
4	52.3	6.3	11.9	0.019	2.6	24.0	17.7	613
5	59.8	11.3	18.1	0.041	2.4	20.1	22.8	661
6	46.5	5.7	13.7	0.028	2.3	29.0	12.3	516
7	53.0	14.6	25.4	0.078	10.4	27.7	11.8	469
Mean±	53.1±	9.9±	17.9±	0.037±	4.4±	23.1±	16.3±	568±
SE	2.1	1.7	2.4	0.01	1.5	2.6	2.0	34.2

Table 4. Kinetics of iodine metabolism and data on seven patients in thyroid function study.

Reference	Iodide space, L		Iodide space (% body wt)		Maximal theoretic uptake (fraction dose)	Initial theoretic plasma ^{131}I concentration (% dose), I_0
	$V_I^{\#1}$	$V_I^{\#2}$	$V_I^{\#1}$	$V_I^{\#2}$	U_{max}	
Riggs (25)	25				0.33	
Hickey & Brownell (19)	24		26		0.34	
Berson & Yalow (5)					0.21-0.65	
DeGroot (10)	23		38.6		0.29	4.8
	Group 1: Euthyroid without goiter					
Case						
1	17.9	22.2	31.7	39.3	0.12	5.6
2	19.6	21.6	34.4	37.8	0.21	5.1
Mean	18.7	21.9	33.5	38.5	0.16	5.3
	Group 2: Euthyroid with diffuse goiter					
3	12.0	13.0	32.1	34.7	0.24	8.3
4	16.4	20.8	26.3	33.3	0.12	6.3
5	10.0	13.9	26.7	27.2	0.20	10.0
6	20.8	20.7	56.2	55.9	0.16	4.8
7	13.5	25.0	25.0	36.2	0.26	11.8
Mean±	14.5±	18.7±	33.3±	37.5±	0.19±	8.2±
SE	1.9	2.2	5.9	4.9	0.02	1.2

Table 5. Exchangeable iodine and data on seven patients in thyroid function study.

Reference	Total (^{127}I) μg	Extrathyroidal (Q_B), μg		Thyroidal (Q_G), μg			
		#1	#2	#1	#2	#3	Estimated ^{127}I, mg/gm wet wt
DeGroot (10)	11253	563	585	10690	10668	10400	
	Group 1: Euthyroid without goiter						
Case							
1	49519	404	412	49115	49107	16793	0.839
2	20400	488	488	19912	19912	18137	0.907
Mean	34959	446	450	34513	34509	17465	0.873
	Group 2: Euthyroid with diffuse goiter						
3	38571	283	279	38288	38292	26592	0.886
4	20472	469	434	20003	20038	13712	0.457
5	16685	310	302	16216	16383	18625	0.621
6	15500	242	241	15358	15259	12606	0.420
7	8745	274	284	8471	8461	5090	0.170
Mean±	20000±	316±	308±	19700±	19700±	15300±	0.511±
SE	5020	40	33	5010	5010	3550	0.118

Table 6. Kinetics of iodine metabolism and data on seven patients in thyroid function study.

Reference	K_{IE+IG}, fr hr		K_{IG}, fr hr		K_{IE}, fr hr		K_G, fr day		K_{GB}, fr day			
	#1	#2	#1	#2	#1	#2	#1	#2	#1	#2	#3	#4
Riggs (25)	.119		.039		.08			.0003		.0004		
Hickey & Brownell (19)	.098									.0032		
DeGroot (10)	.124	.099	.037		.087			.0089	.012	.0128		
					Group 1: Euthyroid without goiter							
Case												
1	.076	.101	.012	.009	.063	.092	.015	.005	.017	.006	.004	.004
2	.085	.052	.018	.012	.067	.041	.007	.007	.009	.008	.005	.004
Mean	.081	.077	.015	.006	.066	.066	.011	.006	.013	.007	.005	.004
					Group 2: Euthyroid with diffuse goiter							
3	.079	.069	.019	.017	.059	.053	.024	.015	.032	.020	.022	.022
4	.113	.117	.014	.014	.100	.099	.011	.005	.012	.005	.005	.005
5	.091	.104	.018	.020	.072	.084	.015	.046	.019	.006	.005	.005
6	.064	.065	.010	.010	.054	.054	.007	.009	.009	.010	.004	.004
7	.106	.157	.027	.040	.078	.078	.019	.006	.026	.008	.006	.006
Mean±	.091±	.102±	.017±	.020±	.073±	.074±	.015±	.016±	.019±	.009±	.008±	.008±
SE	.009	.04	.003	.005	.008	.009	.003	.007	.004	.002	.003	.003

Table 7. Kinetics of iodine metabolism.

Reference	Thyroid absolute iodide uptake (AIU) μg per day			Thyroid hormone ^{127}I secretion (H) μg per day			
	1	2	3	1	2	3	4
DeGroot (10)	54	71	52	138	119	107	112
Berson & Yalow (5)							83-175
Hickey & Brownell (19)	113				134		
	Group 1: Euthyroid without goiter						
Case							
1	84	69	68	285	101	67	67
2	136	96	127	163	145	91	72
Mean	110	82	97	224	123	80	70
	Group 2: Euthyroid with diffuse goiter						
3	53	41	43	850*	532*	585*	585*
4	88	62	65	164	69	69	69
5	67	77	77	354	112	93	93
6	91	62	41	113	126	50	50
7	163	156	176	132	41	30	30
Mean± SE	92±19	79±19	80±25	191±55	87±20	61±13	61±13

* *Number not included in calculation of mean and SE. Patient was treated with Methimazole from the fifth day through the rest of the study.*

Table 8. Kinetics of iodine metabolism and data on seven patients in thyroid function study.

Reference	SA serum ($\times 10^{-2}$), % dose / μg	SA urine ($\times 10^{-2}$), % dose / μg	Ratio, SA_U / SA_S	Urinary ^{131}I excretion, % dose: Observed	Urinary ^{131}I excretion, % dose: Predicted	Ratio, observed / Predicted	Recovery (48 hr), % dose
DeGroot (10)	.27	.20	.8	.22	.10	2.3	96
	Group 1: Euthyroid without goiter						
Case							
1	.0524	.0107	.2	.0433	.0187	2.3	91
2	.0816	.0232	.2	.0995	.0682	1.4	92
Mean	.0670	.0169	.2	.0714	.0434	1.8	91
	Group 2: Euthyroid with diffuse goiter						
3	.0722	.0504	.6	.200	.0157	12.5	90
4	.0491	.0783	.1	.048	.0187	2.6	98
5	.0902	.0116	.1	.077	.0226	3.4	92
6	.1182	.0338	.2	.174	.0241	7.2	86
7	.3300	.0241	.1	.113	.0682	1.9	87
Mean± SE	.1319±	.0396±	.3	.112±	.0299±	5.5±	90±

factor is sufficiently compensated by the diffuse enlargement of the gland, since hormonal iodine secretion is adequate. None of the above-noted abnormalities, including the constantly elevated urinary iodine excretion, are compatible with dietary iodine deficiency. The relative high iodine intake can also be dismissed as a possible cause of the goiter (assuming the presence of a susceptible population group). Thiocyanate does not induce discharge of ^{131}I from the gland, and hypothyroidism is not a feature of the goiter endemic (14). The mechanism underlying the frequent enlargement of the thyroid during adolescence and puberty has been studied (21), and the presence of an increased renal iodine clearance and hyperactivity of the thyroid was documented. This suggests a completely different mechanism as seen in the patients under study. In the absence of nutritional iodine deficiency, a goitrogen seems to be the most likely cause of the residual and persistent goiter in the children of Candelaria. Its effect could be an inhibition of the organification mechanism. The presence of an elevated TSH level in the blood is under study.

SUMMARY

Fifteen years after the introduction of iodine prophylaxis there is a persistent residual prevalence of goiter (30 per cent) among schoolchildren of Candelaria, a village in the center of the goiter endemic in the Cauca Valley, Colombia. Five years of surveys have shown a constantly elevated level of urinary iodine excretion regardless of the presence of goiter.

Kinetic studies of iodine metabolism were performed in four female and three male adolescents from this area. Two nongoitrous subjects were used as controls. Studies revealed a high urinary iodine excretion, an expanded thyroidal iodine pool, and high plasma inorganic iodide levels in all patients regardless of the existence of goiter. Hormonal iodine secretion rate was normal. Consistent with these findings was a low ^{131}I uptake, low $PB^{131}I$, and a low specific activity in the plasma at equilibrium. There was only insignificant iodine leakage from the gland in both groups.

The main difference between patients with and without goiter was a higher concentration of iodine per unit of weight in the nongoitrous glands.

The data support the concept of a homogeneous mixing of the administered iodide (^{131}I) in a large uni-compartmented iodine pool and a slow secretion pattern. There was no evidence for dietary iodine deficiency. Iodine excess in a susceptible population or the so-called adolescent goiter can be excluded as a causative factor. A goitrogen with inhibition of organification is suspected.

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SECTION VII

ENDEMIC GOITER IN ECUADOR

CHAPTER 26

IODIZED OIL IN THE PREVENTION OF ENDEMIC GOITER AND ASSOCIATED DEFECTS IN THE ANDEAN REGION OF ECUADOR

I. PROGRAM DESIGN, EFFECTS ON GOITER PREVALENCE, THYROID FUNCTION, AND IODINE EXCRETION[1]

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INTRODUCTION

In South American countries which made up the Inca Empire, the Quechua word coto is synonymous with goiter. In Quechua, coto means mound or protuberance, and the Indians used coto in reference to tumor in the neck (10, 11, 20, 22). Goiter was frequent among the Indians of the Andes at the time of arrival of the Spaniards (27). The recent discovery of a pre-Colombian figure with a prominent goiter testifies to the commonplace nature of the affliction (Figure 1).

The Spanish conquest of the Inca Empire caused a rapid disintegration of the economy. In 1543 Agustín de Zárate (37) had referred to the great number of llamas which existed in the Province of Quito, and Cieza de León, in 1547, stated that "the people are peaceful, and there is an abundance of bread and grains." (9) By 1596 Father Cobo was already worried about the state of poverty and degeneration of the Indians. Thus the socioeconomic patterns which the Europeans imposed upon this continent placed a great stress upon the normal biologic and cultural development of the American natives. In the eighteenth and nineteenth centuries, Jorge Juan and Antonio de Ulloa (26), Humboldt (25), Caldas (6), Boussingault (5), Orton (31), and Wolf (36) noted the extreme poverty which reigned among the Indians and half-breeds of the rural zones of the Andes. They remarked upon the great number of goitrous, deafmute, and defective persons in this region.

The Spanish and their direct descendents also had goiter on an important scale, and travelers of that time refer to this condition. The small sculptured pieces of the eighteenth century support this observation (Figure 2), especially since the Quitenian images of that time were almost entirely of a religious nature.

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2/ From the Radioisotopes Department, National Polytechnic Institute, and the Central University Medical School, Quito, Ecuador.

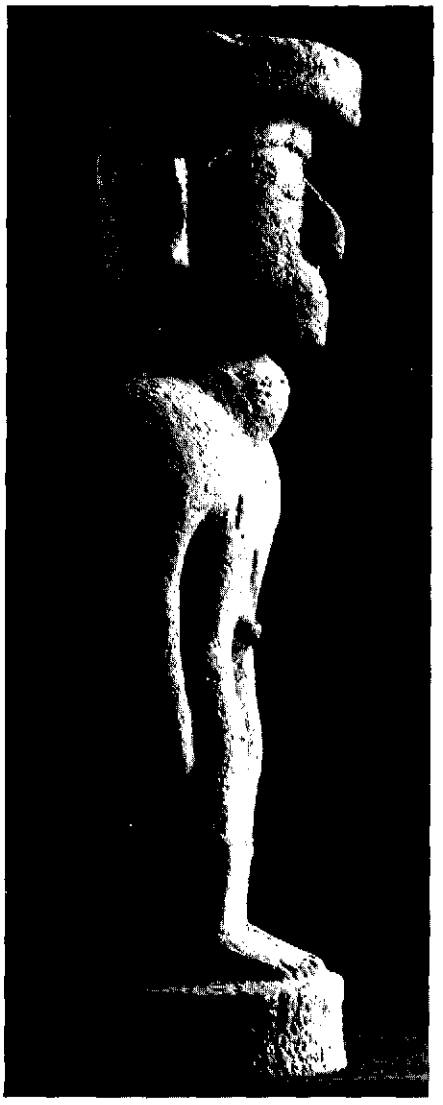

Figure 1. Pre-Colombian sculpture (50 cm high), showing the "Colorado" people who once resided in the Andean region of Ecuador, in the basin of the Guaillabamba River. From there they migrated to the coast. The age has been calculated at 800 years. Now in the Anthropologic Museum of Quito.

Figure 2. Small sculpture (15 cm high) from Quito in the last of the eighteenth century. Now in the Convent "El Carmen Alto," Quito.

In 1958 the Ecuadorean Institute of Nutrition conducted a survey (18) and found endemic goiter to be a grave problem in the Andean Region (Figure 3). It was observed that the prevalence of goiter varied between neighboring villages, that the size of goiter diminished in villages located at more than 3,200 meters above sea level, and that the mentally deficient persons, deaf-mutes, mutes, and deaf who were observed during the survey did not present the characteristics aspects of hypothyroidism. Studies of iodine metabolism indicated iodine deficiency as the principal causative factor (13, 16). The results were similar to the findings of Stanbury et al. (34) in endemic goiter in the Andean Region of Mendoza, Argentina.

Ecologic, ethnic, and socioeconomic studies in eight rural villages of the Ecuadorean Andean region in 1965 (19, 20) supported the following conclusions: (1) For endemic goiter to exist in a community, man must live under

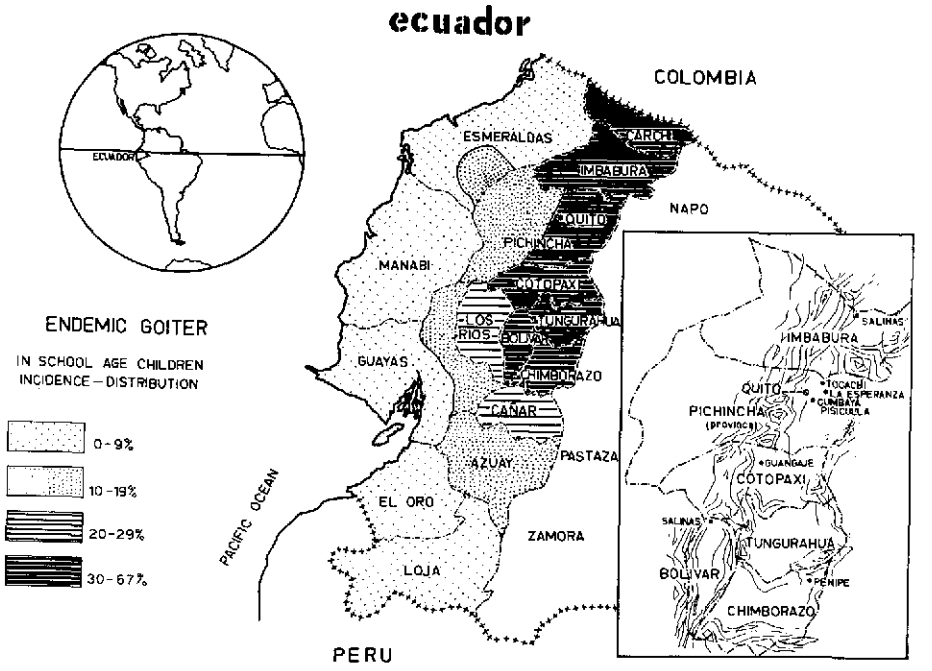

Figure 3. Geographic location of the Republic of Ecuador, and partial map of its territorial divisions by provinces. In the Province of Pichincha is included Quito, the capital of the country. In the frame is shown the location of the eight rural villages, including Tocachi and La Esperanza. Inventory of the total population of each one was made one year before the start of the iodized oil prophylactic program.

chronic iodine deficiency. (2) Severity of the endemic is related to the magnitude of iodine deficiency, but intercurrent socioeconomic and biologic factors in the community may modify the incidence of goiter. (3) These intercurrent factors are of themselves incapable of causing the endemic. (4) The prevalence of endemic cretinism, deafmutism, and motor abnormalities is highly correlated with the intensity of endemic goiter.

There are, unfortunately, many regions of the world where iodination of salt is not feasible at present. A number of years ago a program of goiter prevention by the injection of iodized oil was begun in New Guinea. Information available from those surveys (24, 28) indicated that prophylactic programs were effective in reducing the prevalence of goiter and that the procedure was practical and safe. The results obtained in New Guinea, however, failed to provide entirely satisfactory information regarding the effectiveness of this form of prophylaxis in reducing the incidence of goiter and gave practically no information regarding prevention of those disabilities which have been demonstrated to be in epidemiologic association with severe endemic goiter, such as endemic cretinism, endemic deafmutism, deafness, mutism, bone deformities, and endemic mental retardation.

Iodization of common salt has not been implemented in Ecuador. The socioeconomic conditions of the Andean people, basically composed of Indians

and half-breeds, have varied little since the Spanish colonial times or have changed at a very slow pace. Thus, everything has pointed to a virgin endemic of an ancient date, the primary causative factor of which has been a severe chronic deficiency of iodine. On the basis of these facts, we elected in March 1966 to carry out a program for prevention of endemic goiter and associated defects in two isolated villages of the Ecuadorean Andean region by means of the intramuscular administration of iodized oil.

MATERIALS AND METHODS

A socioeconomic inventory of the total population of eight rural villages in the Andean Provinces most affected by goiter (Figure 3) was made one year before administration of the iodized oil. The inventory was performed by one medical doctor and three medical students. Accompanying them was a representative of the Ecuadorean National Planning Board. He studied the economic status of each community by means of a survey on complete families taken as a random sample.

Two previously studied villages were chosen for the prophylactic program, Tocachi and La Esperanza (Figure 3). They were selected because of their remoteness and because in both of them endemic goiter is severe and cretinism is commonplace. They are situated about 70 kilometers north of Quito. They are six miles apart and are ethnically, socially, and medically entirely comparable. They are remote from any medical facility. The villagers have unusually limited social mobility and contact with the outside world. These villages share many characteristics with hundreds of others in the South American Andean region.

We elected to give iodine to the Tocachi population, since the population concentration was ideal. La Esperanza remained as the control village. The program was started in March 1966. The iodized oil was Ethiodol (37 per cent iodized poppy seed oil, each ml containing 475 mg of iodine, from E. Fougera, Inc., Hicksville, L.I., New York). Disposable plastic syringes were used to avoid problems of sterilization at high altitudes. The following dosage schedule was used:

Up to two years	0.2 ml of iodized oil
2 - 6 years	0.5 ml of iodized oil
6 - 12 years	1.0 ml of iodized oil
12 years old and up	2.0 ml of iodized oil

The oil was administered intramuscularly in the gluteal region in small children and in the deltoid region in adults. Drawback was practiced to ensure that oil was not injected intravenously. Merthiolate was used for skin sterilization.

The studies were done just before or at the same time as the iodized oil administration. A nutritional survey was conducted by sampling. Fifty families from Tocachi and 75 from La Esperanza were chosen.

Goiter prevalence was determined by five teams (each comprised of one medical doctor, two medical students, and one local leader). Four of the five teams were in charge of the four sections into which each village was divided.

Precise data had been prepared on each section regarding house location, families in each house, names and ages of all members of a family, etc. The fifth team was stationed at the priest's house, where subjects previously chosen for special studies were examined. The attitude and cooperation of the people were excellent in both villages.

Evaluation of the thyroid size was in accordance with the classification of Pérez et al. 1961, and modified by us. This is:

Grade 0_a	-	not palpable;
Grade 0_b	-	palpable, but not visible with the head raised;
Grade I	-	easily palpable with head in normal position and visible with the head raised; Those glands presenting easily palpable nodules with head in normal position but not visible with head raised were included in this grade.
Grade II	-	easily visible with head in normal position;
Grade III	-	visible at a distance;
Grade IV	-	monstrous goiters.

For epidemiologic purposes, glands were considered abnormal when Grade I or larger. These glands were recorded as diffuse or nodular. A series of trials were needed for the responsible team leaders to come to agreement about grade and type (diffuse or nodular). The five teams administered the iodized oil to the Tocachi population at the same time as the epidemiologic survey. Ninety per cent (960) of the Tocachi villagers were injected. The other 10 per cent refused the injection. There were no cases of local reaction to the iodine, except for one of transitory erythema.

For evaluation of cretinism, the fundamental fact taken into account was the mental deficiency of the subject. The mental deficiency should be obvious in the opinion of the surveyer, and confirmed by the manner in which the subject lived in relation to the rest of the community. The subject would be considered by his family incapable of conducting the normal activities of the average inhabitants of the village (agricultural tasks, small crafts, etc.). This criterion was employed because many of the inhabitants of these communities were characterized by a certain degree of simplicity in comparison to residents of an urban area.

Examination of selected children included a PA x-ray of the left hand, as well as observation for gingival emergence of the deciduous and permanent teeth. Hand x-rays were taken using Kodak no-screen Ready Pack Medical x-ray films, at a tube-to-film distance of 91.5 cm (36 in) with a Bucky field-portable x-ray unit. Anthropometric measurements were also made in all these children.

Duplicate samples of water were taken, as well as samples of the crude sea-salt which is consumed in both villages. Samples of soil were also taken. Samples of first morning urine before breakfast were obtained. Half of these samples were sent to Boston Medical Laboratories (Boston, Massachusetts) in order to determine the stable iodine content. The other samples were examined in Quito. The samples of urine were used to determine iodine and creatinine according to the method of Bosnes and Taussky (4).

Blood samples were also taken for TI, PBI, T_4I, BEI, and BII determinations according to the methods of Benotti and Benotti (1, 3) and of Murphy (30). Resin uptake of ^{131}I-labeled triiodothyronine was according to Mitchell et al. (29).

Volunteers from both villages were brought to Quito. The ^{131}I thyroid uptake, conversion ratio, $PB^{131}I$ per cent per liter, salivary iodide clearance, saliva/plasma ratio, and saliva/PBI ratio tests were done after a dose of 50 microcuries of ^{131}I.

Shortly after the completion of the injection program a physician and a midwife were assigned fulltime to Tocachi and La Esperanza. Small dispensaries were established for administering to the general medical needs of the communities, but particularly to keep continuous close observation on the effects of the prophylactic program and on the progress and results of pregnancies as they might occur. All pregnancies in both villages have been followed and neuromotor development and physical growth of all of the children born in both villages have been studied since March 1966.

Epidemiological surveys on goiter prevalence and incidence were conducted at 6, 12, 20, and 25 months after the injection program. Thyroid functional studies and determinations of iodine and creatinine in urine were done at the same times.

After 25 months of iodinization in April 1968, exactly the same studies were made as in March 1966. These included nutritional surveys, x-ray studies, etc. Most of these were made on the same subjects. In April 1968 we also conducted intelligence performance tests, using the Goodenough Method modified in recent years by Harris (23), on school-age children.

RESULTS AND DISCUSSION

Both in Tocachi and La Esperanza the socioeconomic situation is precarious (Table 1).* This was noted by Luis León (27), who wrote, 28 years ago, that these villages "tend toward degeneration and extinction." At present the situation has changed but little. Both in Tocachi and La Esperanza there is a high percentage of infant mortality, a high incidence of illegitimacy, a low level of literacy, little exposure to culture outside the villages, and great poverty reflected by the small percentage of artisans and the low income. Chronic iodine deficiency is severe in both villages (Table 1). Prevalence of goiter and associated defects is high (Table 2).

* All tables appear at the end of this article.

The mentally defective persons were divided into two types: Type I, with mental deficiency and severe impairment in hearing and speech; and Type II, with mental deficiency, impaired hearing and speech, short stature, and motor abnormalities including spastic paresis of the lower extremities. Both types are believed to be examples of endemic cretinism. The syndrome of cretinism in both villages is much like the endemic cretinism occurring in the Mulia Valley in western New Guinea (8). In Mulia iodine deficiency is equally severe and the incidence of cretinism is strikingly similar to that in Tocachi. In Tocachi, La Esperanza, and in Mulia, the clinical syndrome of cretinism is distinct from that associated with endemic goiter in the Belgian Congo, where hypothyroidism and dwarfing typically occur (12).

Numerous reports on the clinical features of cretinism in relation to endemic goiter have been reviewed by Choufoer (8) and need not be extensively reconsidered here. Studies on thyroid function and skeletal muscle structure in cretins from both Tocachi and La Esperanza (19, 35) have outlined two important facts. Thyroid function was similar in cretins and "normal" subjects in both villages, and there was no evident disorder in skeletal muscle structure. Thus there was no evidence of skeletal muscle involvement as in myxedema or in untreated congenital hypothyroidism, such as myotonia , increased muscle bulk, or delayed relaxation of stretch reflexes. We believe that intrauterine hypothyroidism leads to the neural abnormalities, and that these persist after birth despite the subsequent course of thyroid function. If the thyroid is able to compensate through any mechanism to prevent continued hypothyroidism, a deafmute, mentally defective individual of normal proportions may result. Otherwise the person may develop as a typical dwarfed cretin, who may be hypothyroid even in adult life.

Nutritional surveys done in March 1966 and in June 1968 outlined the following facts: caloric consumption was low, as were protein, fat, and vitamin A, especially of animal origin. The daily diet in these communities is fundamentally based upon barley, corn, and potatoes and other tubers. There has been no noteworthy improvement in diet during these two years (Table 3).

The height of adult subjects from Tocachi and those from La Esperanza was not significantly different (Table 4). Mean values for weight differed noticeably ($P < 0.04$ for males and < 0.03 for females). This is presumably attributable to a better caloric intake in La Esperanza.

A fall in the prevalence of goiter, both in the total population and in men and women, was regular and consistent for 20 months after Ethiodol administration. After the 20th month, also in the total population and in men and women, the decrease stopped and prevalence began to rise. The ratio of goiter in women and men increased while the prevalence of goiter in the total population decreased (Figure 4, Tables 5, 7, 9, 11, 14).

The prevalence of diffuse and nodular goiter, at the time of the survey conducted six months after iodization, presented the following picture (Figure 5): nodular goiter noticeably increased, while diffuse goiter decreased. In the following surveys, and up to the 20th month, nodularity steadily decreased. At the survey on the 25th month nodularity started a new increase. The nodular-diffuse goiter ratio increased, while the prevalence of goiter in the total population increased (20) (Tables 5, 7, 9, 11, 13).

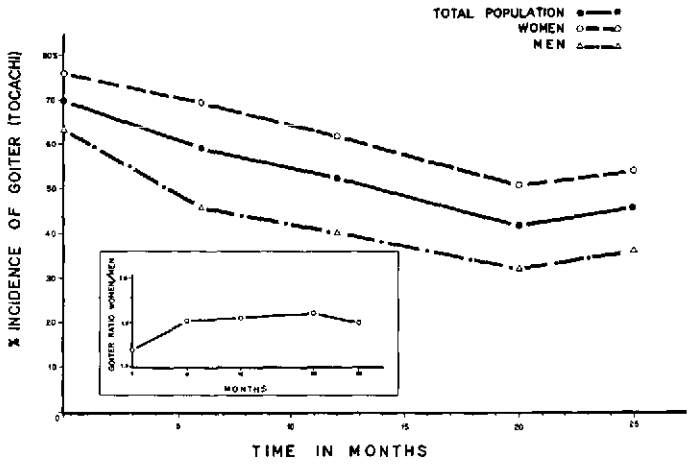

Figure 4. Total goiter prevalence, and prevalence in men and women from Tocachi, at the time of iodized oil administration and in the surveys at 6, 12, 20, and 25 months after administration. Female-male goiter ratio for the same periods is shown in the frame (Tables 5, 7, 9, 11, 13).

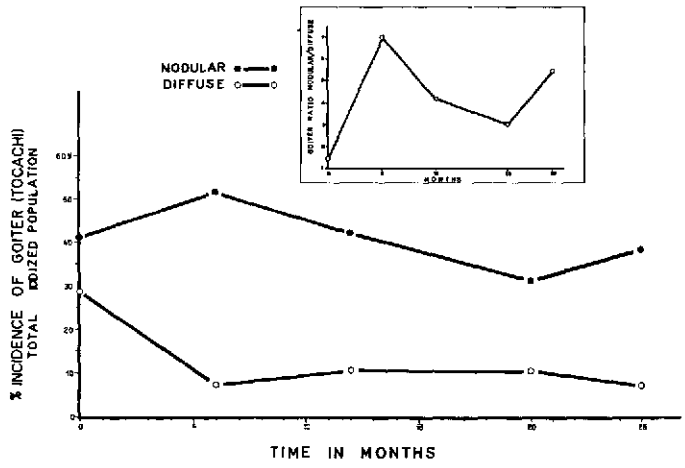

Figure 5. Prevalence of nodular and diffuse goiter for the total treated population from Tocachi at the time of iodized oil administration and in the surveys at 6, 12, 20, and 25 months after administration. Nodular/ diffuse goiter ratio for the same periods is shown in the frame (Tables 5, 7, 9, 11, 13).

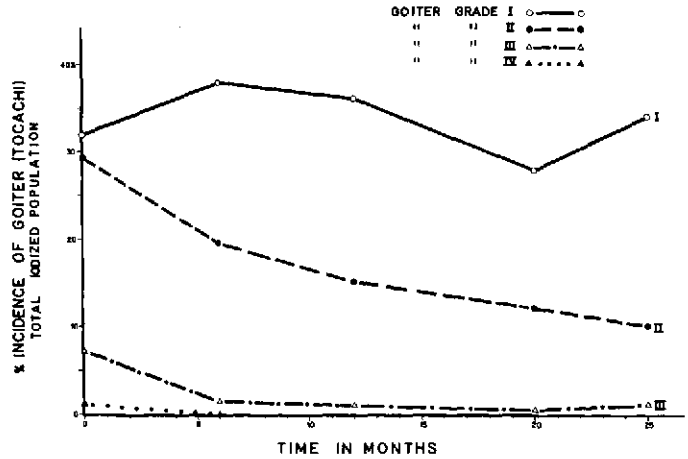

Figure 6. Goiter prevalence according to size for the total injected population from Tocachi at the time of iodized oil administration and in the surveys at 6, 12, 20, and 25 months after administration (Tables 5, 7, 9, 11, 13).

As to goiter size, it was evident that administration of Ethiodol produced a distinct decrease of the incidence of Grade II goiters and larger, and this decrease continued into the survey at 25 months after iodization. Although the decrease in size of large goiters was achieved at the expense of an increase in the incidence of Grade I goiters, after the survey at 12 months Grade I goiters also tended to diminish. This trend persisted after 20 months. After the survey at 20 months, however, this type of goiter (but not those of larger size) tended to increase (Figure 6, Tables 5, 7, 9, 11, 13).

A linear study of the evolution of different grades and types of glands was made (Tables 15, 16, 17, 18, 19, 20, 21). There was a steady and progressive reduction of large goiters until the 20th month after iodization. Thus a Grade III goiter (Figure 7, Table 21), for instance, first became Grade II or I, and then these became Ob and Oa thyroids, as time passed, up to the 20th month. From the 20th month a considerable number of Grade I and II goiters became Ob thyroids, most of the Grade II goiters became Grade I, and the few Grade III goiters became Grade II. This has been the epidemiological picture which has been evident at the 25th month after iodization. As to linear evolution of nodular goiters (Figure 8, Table 20), we took as an example what happens to nodular goiter Grade II. More than half of the Grade II nodular goiters became nodular Grade I during the first six months. A good number of nodular Grade I progressively evolved and became Ob or Oa thyroids. At the same time nodular Grade II goiters became nodular Grade I. After the 20th month a significant number of nodular Grade II goiters became Nodular I, Oa thyroids became Ob, and some of these became Grade I. We must explain in this way the sharp increase of Grade I goiter prevalence 25 months after administration of Ethiodol, and also the beginning of an increase of goiter prevalence in the total population at that time. It seems unquestionable that the sharp increase of nodular goiter found six months after iodization is a result of involution of hyperplastic thyroid tissue resulting from the action of Ethiodol.

Regarding the effect of Ethiodol on goiter prevalence by age (Tables 6, 8, 10, 12, 14), the reduction diminished as age increased. The maximum reduction was seen during the first 18 years of life. There was minimal reduction after 40 years of age. Reduction at 25 months after iodization was 36 per cent for the total population. There was not a single instance of a palpable thyroid in children born in Tocachi to iodized mothers. Eighteen per cent of the children of La Esperanza up to 2 years of age, who were examined at the time of the April 1968 survey, presented palpable glands. As to the goiter prevalence in La Esperanza from March 1966 to April 1968, results indicate that there has been a significant increase (Tables 22, 23).

Six months after iodization, uptake of ^{131}I by the thyroid proved to be clearly depressed. A restoration toward normality was observed in successive control surveys, including the control conducted 25 months after injection (Table 24, Figure 9). The $PB^{131}I$ was low in a considerable number of cases in all control surveys (Table 25).

The deposit oil was not extractable with butanol. BEI has remained practically within normal limits 25 months after iodization (Table 26). Thus even during the first months the glands retained a normal capacity for transforming iodide into T_4 and T_3. PBI's were high until two years after iodization. This indicates that PBI determinations are not a valid index of thyroid function when iodized oil is used. Similar results have been reported by Carter, who studied the effects of oral Lipiodol on PBI concentrations (7).

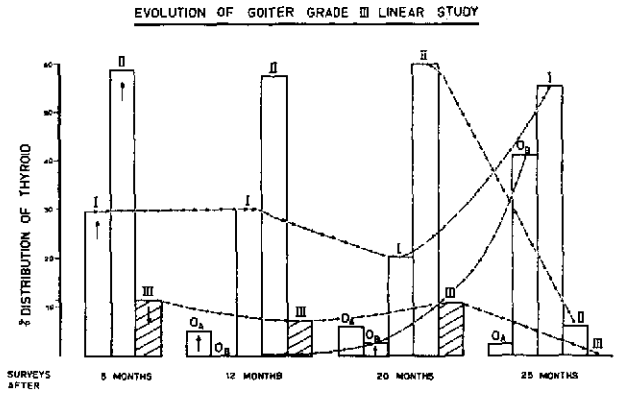

Figure 7. Goiter Grade III evolution, linear study. Surveys made in Tocachi at 6, 12, 20, and 25 months after iodized oil administration (Table 21). The number of subjects who presented Grade III goiter during the survey done just before injection was 70.

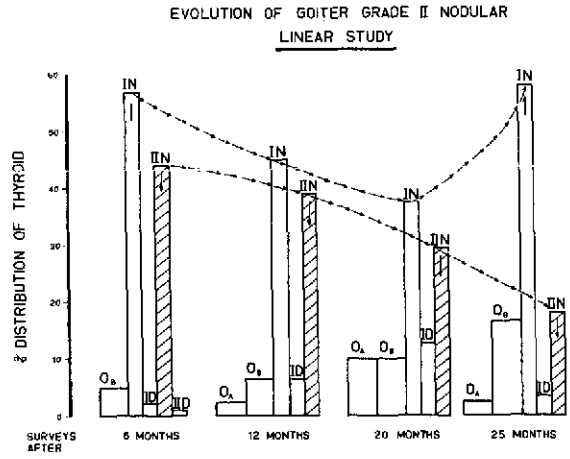

Figure 8. Evolution of Grade II nodular goiter, linear study. Surveys made in Tocachi at 6, 12, 20, and 25 months after iodized oil administration (Table 20). The number of subjects who presented Grade II nodular goiter during the survey done just before injection was 200.

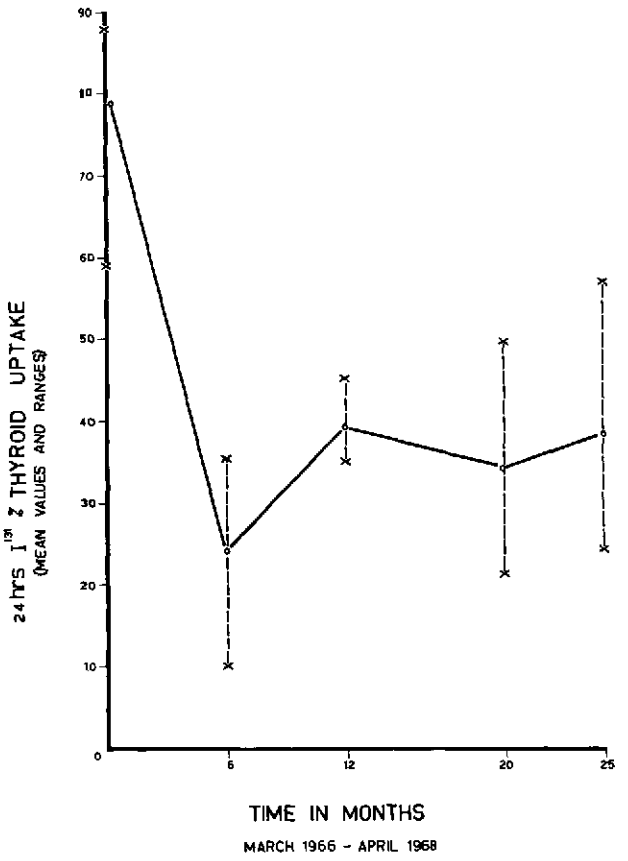

Figure 9. Mean values and ranges for the 24-hour ^{131}I o/o thyroid uptake, in Tocachi volunteers examined just before the injection of Ethiodol, and after 6, 12, 20, and 25 months.

Six months after iodization the Achilles reflex relaxation time was not statistically different between the inhabitants from Tocachi and those from La Esperanza (Tocachi: number of subjects = 195; mean value = 292 milliseconds; S.D. = 43. La Esperanza: number of subjects = 195; mean value = 293; S.D. = 32. t = 0.36). There were almost no subjects with values suggesting hypometabolism in either village.

The urinary excretion of iodine (UEI) (Table 27, Figure 10) followed an exponential pattern. The excretion pattern could be expressed by $UEI_t = UEI_0 \cdot e^{-0.126 \cdot t}$. Since the subjects on whom we did UEI determinations were adults who had received 2 ml of Ethiodol (950 mg), elimination of those 950 mg would be virtually accomplished at the 40th month after injection. These results imply that an Ethiodol dose equal to half of that used would be eliminated in no more than thirty-five months.

Three subjects from Tocachi who refused injection in March 1966 were examined in April 1968. Their ^{131}I and ^{127}I tests and urinary iodine excretion were superimposable on those found in the same village before the prophilaxis program was started.

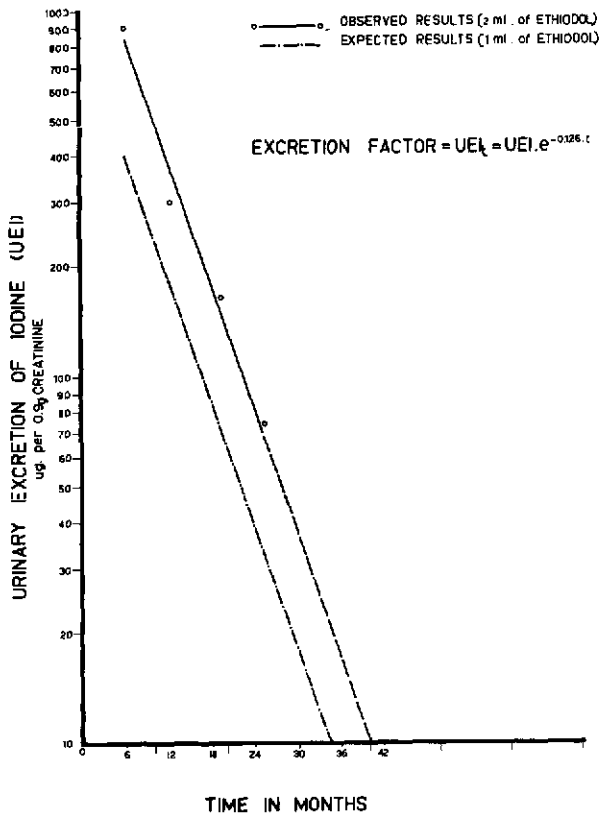

Figure 10. Evolution of urinary excretion of iodine (µg per 0.9 g creatinine). Mean values at 6, 12, 20, and 25 months after iodization. The UEI mean value in Tocachi was 10.4 µg daily before injection.

In the survey six months after administration of Ethiodol, there were a few subjects in Tocachi who presented a clinical picture suggesting hyperthyroidism. They were taken back to Quito for hospitalization and careful examination. The disease was confirmed in three women, all elderly and with large nodular goiters (Table 28). The laboratory tests useful for their diagnosis were the BMR, the BEI and T_3- ^{131}I resin uptake, since the other tests were exactly the same as for other iodized subjects who were not suspected of having hyperthyroidism. It is interesting to note that the suppression test with KSCN in these three patients performed according to the method of Sánchez-Martin (33) was the same as described by him for normal subjects, and also by Fierro-Benítez and Garcés (15) for subjects with endemic goiter without autonomous nodules.

Tapazol administration at a dose of 45 mg a day for two or three months was enough to change the picture in two (cases 1 and 2) of the three women. One of them was ready for an incidental gastrectomy three months from the beginning of treatment.

The third woman presented hyperthyroidism one year after injection. Among laboratory tests done in March 1967, the results that were different from those of the other iodized subjects from Tocachi who had the same tests at the same time were the BEI, serum thyroxine, and resin uptake of ^{131}I-T_3. These were high. All these tests were within normal limits for other iodized subjects. In April 1968 this third woman continued to present clinical hyperthyroidism in spite of Tapazol administration at a dose of 75 mg per day (taken irregularly). Laboratory data were: ^{131}I thyroid uptake: 8 hr = 30 per cent; 24 hr = 38 per cent; 96 hr = 24 per cent; 24 hr conversion ratio = 56 per cent; 24 hr PB^{131}I per cent dose per liter = 0,24; BEI = 9.0 µg per cent; BII = 4 µg per cent. She was hospitalized by the end of April 1968 and became clinically euthyroid after six weeks of daily administration of 75 mg of Tapazol.

SUMMARY

Intramuscular injections of iodized oil (Ethiodol) have produced no local or general reactions of iodine intolerance. They may be administered by technical personnel under medical supervision.

Goiter in an isolated rural district of the Ecuadorean Andes has had a 36 per cent reduction two years after administration of iodized oil. Reduction has been small in the age group over 40 years.

Reduction of goiter incidence was regular and progressive until the period between the 20th and the 25th months after iodization, after which time prevalence of goiter began to increase. This was primarily an increase in the number of Grade I goiters. Larger goiters at the same period of time continued to decrease in size, and Grade I goiters did not increase in size. At the 25th month after the injection program there was a sharp reduction in the prevalence of large goiters.

The increased incidence of small nodular goiters, which was so noticeable at the 6th month survey, should be attributed to a better delineation of these nodules as a result of involution of previously hyperplastic thyroid tissue.

Administration of iodized oil to mothers before delivery has prevented the appearance of goiter in their children, at least for the first two years. Administration of iodized oil has produced a restoration of thyroid function to normal.

Urinary excretion of iodine following administration of iodized oil follows an exponential pattern. At doses used in the present program (2 ml of Ethiodol for adults), the urinary excretion of iodine would be over basal figures until 40 months after injection. If the dose would be reduced to 1 ml, the increased excretion would remain until 35 months after injection.

Among 960 subjects injected, three developed hyperthyroidism. They were women more than 45 years old with large nodular goiters. Laboratory tests useful for diagnosis were: BMR, BEI, T_4, and resin uptake of ^{131}I-labeled T_3. Tapazol was effective in curing the disease. One of the three hyperthyroid required hospitalization.

The present study indicates that the use of intramuscularly injected iodized oil is a useful means for combating endemic goiter and cretinism in rural areas where the endemic is severe. The method is cheap, long-acting, relatively free of side effects, and can be easily applied through modest local health services.

Using 1 ml of Ethiodol as a dose, results should be similar to those reported in this paper. Accordingly, we recommend that 1 ml of Ethiodol be used for adults in future programs, and proportionally smaller doses for children. The entire population of the goitrous area from 0 to 45 years of age of both sexes should be injected. There is no need to exclude persons with nodular goiter, but they may be given a smaller dose.

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Table 1. Ethnic and socioeconomic aspects of Tocachi and La Esperanza and data on iodine content of salt, water, soil, and urine.

	TOCACHI	LA ESPERANZA
Population	1,100	2,500
Altitude (meters above sea level)	2,952	2,883
% half-breeds	59.43	45.24
% Indians	38.80	50.08
% subjects born in the village	97.00	91.00
% infant mortality	43.00	29.00
% natural abortion	2.80	4.20
% unwed mothers	26.00	14.00
% illiterate subjects	31.00	34.00
% subjects who have visited the coast region	5.00	9.00
% artisans	5.00	5.00
Annual income per person (U.S. dollars)	90	85
Iodine content of salt, water, soil, and urine		
Iodine in salt (μg/g)	0.24	0.24
Iodine in water (μg/l)	1.00	0.85
Iodine in soil (μg/kg)	7.00	23.00
I^{127} urinary excretion (μg per 0.9 g creatinine)	10.4	17.7

Table 2. Prevalence of goiter and of neural and motor abnormalities, per cent of total population (March 1966).

	TOCACHI	LA ESPERANZA
General goiter	69.7	52.8
Nodular goiter	41.1	23.4
Diffuse goiter	28.6	29.3
ENDEMIC CRETINISM TYPE I: Mental deficiency and severe impairment in hearing and speech	7.4	5.5
ENDEMIC CRETINISM TYPE II: Mental deficiency and severe impairment in hearing and speech and short stature and motor abnormalities	0.8	0.5
DEAFMUTISM Severe impairment in hearing and speech	4.5	2.5
DEAFNESS Severe impairment in hearing	1.6	0.0
MUTISM Severe deficit in speech	0.4	0.4
MOTOR ABNORMALITIES	1.0	0.8
OTHER CONGENITAL MALFORMATIONS	0.4	1.2

Table 3. Average consumption of calories and nutritive elements per person per day. Tocachi and La Esperanza, March 1966 and June 1968. Children who were less than one year old were not taken into account (Ecuadorean National Institute of Nutrition).

	TOCACHI		LA ESPERANZA	
	March 1966	June 1968	March 1966	June 1968
Calories	1,577.64	1,604.49	1,699.58	1,860.00
Total protein (g)	38.66	39.15	39.03	48.31
Animal protein (g)	5.85	2.77	4.06	4.48
Vegetable protein (g)	32.81	36.38	34.97	43.83
Total fat (g)	22.32	25.17	26.30	24.16
Animal fat (g)	13.56	2.21	11.05	0.31
Vegetable fat (g)	8.76	22.96	15.25	23.85
Carbohydrate (g)	280.54	312.30	327.79	362.40
Calcium (g)	0.20	0.16	0.22	0.29
Iron (mg)	20.45	19.72	23.47	32.60
Total vitamin A (I.U.)	588.65	823.71	1,340.76	1,000.00
Animal vitamin A (I.U.)	114.81	40.79	75.82	152.00
Vegetable vitamin A (I.U.)	473.84	782.92	1,264.94	848.00
Thiamine (mg)	0.92	1.30	1.18	1.43
Niacin (mg)	17.86	27.68	25.09	29.32
Riboflavin (mg)	0.50	0.55	0.54	0.83
Vitamin C (mg)	56.32	119.94	93.81	108.61

Table 4. Height and weight of the subjects from 19 to 50 years old. Tocachi and La Esperanza, May 1966.

			Number	Mean value	Standard deviation	Probability
HEIGHT (cm)	MEN	Tocachi	113	152	8.3	0.7
		La Esperanza	80	156	6.7	
	WOMEN	Tocachi	206	142	6.7	0.6
		La Esperanza	220	145	6.4	
WEIGHT (kg)	MEN	Tocachi	113	53.4	6.8	0.04
		La Esperanza	80	57.0	7.0	
	WOMEN	Tocachi	213	45.6	7.0	0.03
		La Esperanza	217	50.0	6.6	

Table 5. Prevalence and distribution of goiter by type and size in the survey conducted just before injection. Total subjects surveyed: 960. Tocachi, March 1966.

		DIFFUSE GOITER							NODULAR GOITER					TOTAL
		Oa	Ob	Oa+Ob	DI	DII	DIII	Total D	NI	NII	NIII	NIV	Total N	GOITER
MALE	No.	137	35	172	100	37	1	138	60	72	22	5	159	297
	%	29.2	7.4	36.6	21.3	7.8	0.2	29.4	12.7	15.3	4.6	1.0	33.9	63.3
FEMALE	No.	89	29	118	91	44	2	137	57	128	45	6	236	373
	%	18.1	5.9	24.0	18.5	8.9	0.4	27.9	11.6	26.0	9.1	1.2	50.3	75.9
M + F	No.	226	64	290	191	81	3	275	117	200	67	11	395	670
	%	23.5	6.6	30.2	19.8	8.4	0.3	28.6	12.1	20.8	6.0	1.1	41.1	69.7

Table 6. Prevalence and distribution of goiter by age in the survey conducted just before injection. Total subjects surveyed: 960. Tocachi, March 1966.

AGE PERIOD YEARS	NUMBER		DIFFUSE GOITER						NODULAR GOITER					TOTAL
			Oa	Ob	Oa+Ob	DI	DII	DIII	Total D	NI	NII	NIII	NIV	Total N	GOITER
0-5	178	No.	98	18	116	45	15		60		2			2	54
		%	55.0	10.1	65.1	25.2	8.4		33.7		1.1			1.1	34.9
6-12	160	No.	37	12	49	68	30		98	5	8			13	111
		%	23.1	7.5	30.6	42.5	18.7		61.2	3.1	5.0			8.1	69.3
13-18	85	No.	11	8	19	23	7		30	9	27			36	66
		%	12.9	9.4	22.3	27.0	8.2		35.2	10.5	31.7			42.3	77.6
19-40	292	No.	54	12	66	40	13	3	56	60	82	24	4	170	226
		%	18.4	4.1	22.6	13.6	4.4	1.0	19.1	20.5	28.0	8.2	1.3	58.2	77.3
41-+	245	No.	26	14	40	15	16		31	43	81	43	7	174	205
		%	10.6	5.7	16.3	6.1	6.5		12.5	17.5	33.0	17.5	2.8	71.0	83.6

Table 7. Prevalence and distribution of goiter by type and size in the survey conducted six months after injection. Total subjects surveyed: 490. Tocachi, September 1966.

		DIFFUSE GOITER						NODULAR GOITER					TOTAL
		Oa	Ob	Oa+Ob	DI	DII	DIII Total D	NI	NII	NIII	NIV	Total N	GOITER
MALE	No.	54	63	117	8	2	10	68	20	1		89	99
	%	25.0	29.1	54.1	3.7	0.9	4.6	31.4	9.2	0.4		41.2	45.8
FEMALE	No.	46	38	84	21	5	26	88	70	6		164	190
	%	16.7	13.8	30.6	7.6	1.8	9.4	32.1	25.5	2.1		59.8	69.3
M + F	No.	100	101	201	29	7	36	156	90	7		253	289
	%	20.4	20.6	41.0	5.9	1.4	7.3	31.8	18.3	1.4		51.6	58.9

Table 8. Prevalence and distribution of goiter by age in the survey conducted six months after injection. Total subjects surveyed: 490. Tocachi, September 1966.

AGE PERIOD YEARS	NUMBER		DIFFUSE GOITER						NODULAR GOITER					TOTAL
			Oa	Ob	Oa+Ob	DI	DII	DIII Total D	NI	NII	NIII	NIV	Total N	GOITER
0-5	135	No.	79	37	116	6	1	7	11	1			12	19
		%	58.5	27.4	85.9	4.4	0.7	5.1	8.1	0.7			18.8	14.0
6-12	76	No.	6	27	33	13	2	15	22	6			28	43
		%	7.8	35.5	43.4	17.1	2.6	19.7	28.9	7.8			36.8	56.5
13-18	45	No.	2	14	16	4		4	18	7			25	29
		%	4.4	31.1	35.5	8.8		8.8	40.0	15.5			55.5	55.1
19-40	129	No.	8	18	26	6	4	10	66	26	1		93	103
		%	6.2	13.9	20.1	4.6	3.1	7.7	51.1	20.1	0.7		72.0	79.8
41-+	105	No.	5	5	10				39	50	6		95	95
		%	4.7	4.7	9.5				37.1	47.6	5.7		90.4	90.4

Table 9. Prevalence and distribution of goiter by type and size in the survey conducted 12 months after injection. Total subjects surveyed: 584. Tocachi, March 1967.

		DIFFUSE GOITER							NODULAR GOITER					TOTAL
		Oa	Ob	Oa+Ob	DI	DII	DIII	Total D	NI	NII	NIII	NIV	Total N	GOITER
MALE	No.	88	63	151	21	2		23	56	18	3		77	100
	%	35.0	25.0	60.1	8.3	0.7		9.1	22.3	7.1	1.1		30.6	39.8
FEMALE	No.	71	56	127	36	2		38	98	67	3		168	206
	%	21.3	16.8	38.1	10.8	0.6		11.4	29.4	20.1	0.9		50.4	61.8
M + F	No.	159	119	278	57	4		61	154	85	6		245	306
	%	27.2	20.3	47.6	9.7	0.6		10.4	26.3	14.5	1.0		41.9	52.3

Table 10. Prevalence and distribution of goiter by age in the survey conducted 12 months after injection. Total subjects surveyed: 584. Tocachi, March 1967.

AGE PERIOD YEARS	NUMBER		DIFFUSE GOITER						NODULAR GOITER					TOTAL
			Oa	Ob	Oa+Ob	DI	DII	DIII	Total D	NI	NII	NIII	NIV	Total N	GOITER
0-5	127	No.	94	22	106	6			6	5				5	11
		%	74.0	17.3	91.3	4.7			4.7	3.9				3.9	8.6
6-12	115	No.	22	41	63	29			29	22	1			23	52
		%	19.1	35.6	54.7	25.2			25.2	19.1	0.8			20.0	45.2
13-18	58	No.	8	19	27	11	1		12	16	3			19	31
		%	13.7	32.7	46.5	18.9	1.7		20.6	27.5	5.1			32.7	53.4
19-40	141	No.	20	28	48	8	3		11	51	29	2		82	93
		%	14.1	19.8	34.0	5.6	2.1		7.8	36.1	20.5	1.4		58.1	65.9
41-+	143	No.	15	9	24	3			3	60	52	4		116	119
		%	10.4	6.2	16.7	2.0			2.0	41.9	36.3	2.7		81.1	83.2

Table 11. Prevalence and distribution of goiter by type and size in the survey conducted 20 months after injection. Total subjects surveyed: 560. Tocachi, November 1967.

		DIFFUSE GOITER						NODULAR GOITER					TOTAL
		Oa	Ob	Oa+Ob	DI	DII	DIII Total D	NI	NII	NIII	NIV	Total N	GOITER
MALE	No.	118	51	169	15	1	16	45	16	2		63	79
	%	47.5	20.5	68.5	6.0	0.4	6.4	18.1	6.4	0.8		25.4	14.1
FEMALE	No.	99	50	149	37	5	42	59	46	6		111	153
	%	32.7	16.5	49.3	12.2	1.6	13.9	19.5	15.2	1.9		36.7	27.3
M + F	No.	217	101	318	52	6	58	104	62	8		174	232
	%	38.7	18.0	56.7	9.2	1.0	10.3	18.5	11.0	1.4		31.0	41.4

Table 12. Prevalence and distribution of goiter by age in the survey conducted 20 months after injection. Total subjects surveyed: 560. Tocachi, November 1967.

AGE PERIOD YEARS	NUMBER		DIFFUSE GOITER						NODULAR GOITER					TOTAL
			Oa	Ob	Oa+Ob	DI	DII	DIII Total D	NI	NII	NIII	NIV	Total N	GOITER
0-5	156	No.	121	12	133	7		7	8	2			10	17
		%	77.5	7.6	85.2	4.4		4.4	5.1	1.2			6.4	10.8
6-12	95	No.	18	34	52	23	4	27	14	2			16	43
		%	18.9	35.7	54.7	24.2	4.2	28.4	14.7	2.1			16.8	45.2
13-18	43	No.	10	17	27	8		8	8				8	16
		%	23.2	39.5	62.7	18.6		18.6	18.6				18.6	37.2
19-40	134	No.	39	24	63	11	2	13	32	25	1		58	71
		%	29.1	17.9	47.0	8.2	1.4	9.7	23.8	18.6	0.7		43.2	52.9
41-+	138	No.	29	14	43	3		3	42	43	7		92	95
		%	21.0	10.1	31.1	2.1		2.1	30.4	31.1	5.0		66.6	68.8

Table 13. Prevalence and distribution of goiter by type and size in the survey conducted 25 months after injection. Total subjects surveyed: 758. Tocachi, April 1968.

		DIFFUSE GOITER							NODULAR GOITER					TOTAL
		Oa	Ob	Oa+Ob	DI	DII	DIII	Total D	NI	NII	NIII	NIV	Total N	GOITER
MALE	No.	129	100	229	14			14	94	18	2		114	128
	%	36.1	28.0	64.1	3.9			3.9	26.3	5.0	0.5		31.9	35.8
FEMALE	No.	96	90	186	39	1		40	111	58	6		175	215
	%	23.9	22.4	46.3	9.7	0.2		9.9	17.6	14.4	1.4		43.6	53.6
M + F	No.	225	190	415	53	1		54	205	76	8		289	343
	%	29.7	25.1	54.8	7.0	0.1		7.1	27.1	10.0	1.0		38.2	45.3

Table 14. Prevalence and distribution of goiter by age in the survey conducted 25 months after injection. Total subjects surveyed: 758. Tocachi, April 1968.

AGE PERIOD YEARS	NUMBER		DIFFUSE GOITER							NODULAR GOITER					TOTAL
			Oa	Ob	Oa+Ob	DI	DII	DIII	Total D	NI	NII	NIII	NIV	Total N	GOITER
0-5	166	No.	130	21	115	11			11	1	3			4	15
		%	78.3	12.6	90.9	6.6			6.6	0.6	1.8			2.4	9.0
6-12	139	No.	20	87	107	25	1		26	5	1			6	32
		%	14.3	62.5	76.9	17.9	0.7		18.7	3.5	0.7			4.3	23.1
13-18	64	No.	14	32	46	11			11	7				7	18
		%	21.8	50.0	71.8	17.1	17.1		17.1	10.9				10.9	28.2
19-40	192	No.	31	45	76	5			5	88	19	4		111	116
		%	16.1	23.4	39.5	2.6			2.6	45.8	9.8	2.0		57.8	60.5
41-+	197	No.	30	5	35	1			1	104	53	4		161	162
		%	15.2	2.5	17.7	0.5			0.5	52.7	26.9	2.0		81.7	82.3

Table 15. Evolution of thyroids of grade Oa, linear study. Number of subjects of this group surveyed in March 1966: 226.

		TOCACHI											
		SURVEY AFTER 6 MONTHS			SURVEY AFTER 12 MONTHS			SURVEY AFTER 20 MONTHS			SURVEY AFTER 25 MONTHS		
THYROID		MALE No. 59	FEMALE No. 40	M+F No. 99	MALE No. 71	FEMALE No. 42	M+F No. 113	MALE No. 59	FEMALE No. 34	M+F No. 93	MALE No. 88	FEMALE No. 54	M+F No. 142
Oa	No.	34	22	56	48	24	72	44	23	67	55	22	77
	%	57.6	55.0	56.5	67.6	57.1	63.7	74.5	67.6	72.0	62.5	40.7	54.2
Ob	No.	12	8	20	16	11	27	13	8	21	21	21	42
	%	20.3	20.0	22.2	22.5	26.1	23.8	22.0	23.5	22.5	23.8	38.8	29.5
IN	No.	8	5	13	5	3	8	2	2	4	9	3	12
	%	13.5	12.5	13.1	7.0	7.1	7.0	3.3	5.8	4.3	10.2	5.5	8.4
ID	No.	3	4	7	1	4	5	-	1	1	1	6	7
	%	5.0	10.0	7.0	1.4	9.5	4.4	-	2.9	1.0	1.1	11.1	4.9
IIN	No.	2	-	2	-	-	-				2	2	4
	%	3.3	-	2.0	-	-	-				2.2	3.7	2.8
IID	No.	-	1	1	1	-	1						
	%	-	2.5	1.0	1.4	-	0.8						

Table 16. Evolution of thyroids Ob, linear study. Number of subjects of this group surveyed in March 1966: 64.

		T O C A C H I											
		SURVEY AFTER 6 MONTHS			SURVEY AFTER 12 MONTHS			SURVEY AFTER 20 MONTHS			SURVEY AFTER 25 MONTHS		
THYROID		MALE No. 17	FEMALE No. 13	M+F No. 30	MALE No. 20	FEMALE No. 16	M+F No. 36	MALE No. 20	FEMALE No. 14	M+F No. 34	MALE No. 27	FEMALE No. 18	M+F No. 45
Oa	No.	3	2	5	6	2	8	7	6	13	13	3	16
	%	17.6	15.3	16.6	30.0	12.5	22.2	35.0	42.8	38.2	48.1	16.6	35.5
Ob	No.	9	7	16	7	11	18	9	4	13	8	9	17
	%	52.9	53.8	53.6	35.0	68.7	50.0	45.0	28.5	38.2	29.6	50.0	37.7
IN	No.	5	1	6	4	3	7	3	3	6	4	2	6
	%	29.4	7.6	20.0	20.0	18.7	19.4	15.0	21.4	17.6	14.8	11.1	13.3
ID	No.	-	2	2	2	-	2	1	1	2	2	4	6
	%	-	15.3	6.6	10.0	-	5.5	5.0	7.1	5.8	7.4	22.2	13.3
IIN	No.				1	-	1						
	%				5.0		2.7						
IID	No.	-	1	1									
	%	-	7.6	3.3									

Table 17. Evolution of goiters diffuse I, linear study. Number of subjects of this group surveyed in March 1966: 191.*

		TOCACHI											
		SURVEY AFTER 6 MONTHS			SURVEY AFTER 12 MONTHS			SURVEY AFTER 20 MONTHS			SURVEY AFTER 25 MONTHS		
THYROID		MALE No. 58	FEMALE No. 48	M+F No. 106	MALE No. 50	FEMALE No. 64	M+F No. 114	MALE No. 55	FEMALE No. 51	M+F No. 106	MALE No. 73	FEMALE No. 64	M+F No. 137
Oa	No.	7	8	15	10	13	23	20	16	36	14	14	28
	%	12.0	16.6	14.1	20.0	20.3	20.1	36.3	31.3	33.9	19.1	21.8	20.4
Ob	No.	27	14	41	21	19	40	16	17	33	42	26	68
	%	46.5	29.1	38.6	42.0	29.6	35.0	29.0	33.3	31.1	57.5	40.6	49.6
IN	No.	17	15	32	10	15	25	7	7	14	9	9	18
	%	29.3	31.2	30.1	20.0	23.4	21.9	12.7	13.7	13.2	12.3	14.0	13.1
ID	No.	5	6	11	8	16	24	10	10	20	8	13	21
	%	8.6	12.5	10.3	16.0	25.0	21.0	18.1	19.6	18.8	10.9	20.3	15.3
IIN	No.	2	3	5	1	-	1	2	1	3	-	1	1
	%	3.4	6.2	4.7	2.0	-	0.8	3.6	1.9	2.8	-	1.5	0.7
IID	No.	-	2	2	-	1	1				-	1	1
	%	-	4.1	1.8	-	1.5	0.8				-	1.5	0.7

Table 18. Evolution of goiters nodular I, linear study. Number of subjects of this group surveyed in March 1966: 117.

		T O C A C H I											
		SURVEY AFTER 6 MONTHS			SURVEY AFTER 12 MONTHS			SURVEY AFTER 20 MONTHS			SURVEY AFTER 25 MONTHS		
THYROID		MALE No. 22	FEMALE No. 34	M+F No. 56	MALE No. 35	FEMALE No. 35	M+F No. 70	MALE No. 33	FEMALE No. 28	M+F No. 61	MALE No. 41	FEMALE No. 35	M+F No. 76
Oa	No.		1	1	6	1	7	10	6	16	5	3	8
	%		2.9	1.7	17.1	2.8	10.0	30.3	21.4	26.2	12.1	8.5	10.5
Ob	No.	2	5	7	14	8	22	7	5	12	6	6	12
	%	9.0	14.7	12.5	40	22.8	31.4	21.2	17.8	19.6	14.6	17.1	15.7
IN	No.	14	16	30	14	18	32	15	8	23	27	20	47
	%	63.6	47.0	53.5	40.0	51.4	45.7	45.4	28.5	37.7	65.8	57.1	61.8
ID	No.	2	3	5	-	5	5	-	5	5	1	1	2
	%	9.0	8.8	8.9	-	14.2	7.1	-	17.8	8.1	2.4	2.8	2.6
IIN	No.	4	9	13	1	3	4	1	4	5	2	5	7
	%	18.0	26.4	23.2	2.8	8.5	5.7	3.0	14.2	8.1	4.8	14.2	9.2
IID													

Table 19. Evolution of goiters diffuse II, linear study. Number of subjects of this group surveyed in March 1966: 81.

		TOCACHI											
		SURVEY AFTER 6 MONTHS			SURVEY AFTER 12 MONTHS			SURVEY AFTER 20 MONTHS			SURVEY AFTER 25 MONTHS		
THYROID		MALE No. 19	FEMALE No. 24	M+F No. 43	MALE No. 27	FEMALE No. 30	M+F No. 57	MALE No. 23	FEMALE No. 27	M+F No. 50	MALE No. 30	FEMALE No. 32	M+F No. 62
Oa	No.	-	2	2	1	2	3	5	4	9	3	3	6
	%	-	8.3	4.6	3.7	6.6	5.2	21.7	14.8	18.0	10.0	9.3	9.6
Ob	No.	8	2	10	7	3	10	4	4	8	15	7	22
	%	42.1	8.3	23.2	25.9	10.0	17.5	17.3	14.8	16.0	50.0	21.8	35.4
IN	No.	6	11	17	10	10	20	7	3	10	-	-	-
	%	31.5	45.8	39.5	37.0	33.3	35.0	30.4	11.1	20.0	-	-	-
ID	No.	1	3	4	8	8	16	3	7	10	4	8	12
	%	5.4	12.5	9.3	29.6	26.6	28.0	13.0	25.9	20.0	13.3	25.0	19.3
IIN	No.	2	6	8	1	6	7	3	5	8	7	9	16
	%	10.8	25.0	18.6	3.7	20.0	12.2	13.0	18.5	16.0	23.3	28.1	25.8
IID	No.	2	-	2	-	1	1	1	4	5	1	5	6
	%	10.8	-	4.6	-	3.3	1.7	4.3	14.8	10.0	3.3	15.6	9.6

Table 20. Evolution of goiters nodular II, linear study. Number of subjects of this group surveyed in March 1966: 200.

		TOCACHI											
		SURVEY AFTER 6 MONTHS			SURVEY AFTER 12 MONTHS			SURVEY AFTER 20 MONTHS			SURVEY AFTER 25 MONTHS		
THYROID		MALE No. 24	FEMALE No. 73	M+F No. 97	MALE No. 34	FEMALE No. 91	M+F No. 126	MALE No. 27	FEMALE No. 78	M+F No. 105	MALE No. 48	FEMALE No. 101	M+F No. 149
Oa	No.	-	-	-	2	1	3	5	6	11	.2	2	4
	%	-	-	-	5.8	1.0	2.3	18.5	7.6	10.4	4.1	1.9	2.6
Ob	No.	4	1	5	3	5	8	4	6	10	8	16	24
	%	16.6	1.3	5.1	8.8	5.4	6.3	14.8	7.6	10.4	16.6	15.8	16.1
IN	No.	12	34	46	17	39	56	12	28	40	29	58	87
	%	50.0	46.5	57.7	50.0	42.8	44.4	44.4	35.8	38.0	60.4	57.4	58.3
ID	No.	-	2	2	3	5	8	1	12	13	1	4	5
	%	-	2.6	2.0	8.8	5.4	6.3	3.7	15.3	12.3	2.0	3.9	3.3
IIN	No.	8	35	43	9	40	49	5	26	31	7	20	27
	%	33.3	47.9	44.3	26.4	43.9	38.8	18.5	33.3	29.5	14.5	19.8	18.1
IID	No.	-	1	1									
	%	-	1.3	1.0									

Table 21. Evolution of goiters nodular III, linear study. Number of subjects of this group surveyed in March 1966: 67.

		TOCACHI											
		SURVEY AFTER 6 MONTHS			SURVEY AFTER 12 MONTHS			SURVEY AFTER 20 MONTHS			SURVEY AFTER 25 MONTHS		
THYROID		MALE No. 6	FEMALE No. 27	M+F No. 33	MALE No. 10	FEMALE No. 27	M+F No. 37	MALE No. 6	FEMALE No. 27	M+F No. 33	MALE No. 12	FEMALE No. 34	M+F No. 46
Oa	No.				1	1	2	1	1	2	-	1	1
	%				10.0	3.7	5.4	16.6	3.7	6.0	-	2.9	2.1
Ob	No.												
	%												
IN	No.	2	8	10	2	7	9	-	6	6	8	9	17
	%	33.3	29.6	30.3	20.0	25.9	24.3	-	22.2	18.1	66.6	26.4	36.9
ID	No.				-	2	2	-	1	1			
	%				-	7.4	5.4	-	3.7	3.0			
IIN	No.	4	15	19	5	16	21	5	15	20	4	21	25
	%	66.6	55.4	57.5	50.0	59.2	56.7	83.3	55.5	60.6	33.3	61.7	54.3
IIIN	No.	-	4	4	2	1	3	-	4	4	-	3	3
	%	-	14.8	12.1	20.0	3.7	8.1	-	14.8	12.0	-	8.8	6.5

Table 22. Prevalence and distribution of goiter by type and size. La Esperanza, March 1966.

		DIFFUSE GOITER							NODULAR GOITER					TOTAL
		Oa	Ob	Oa+Ob	DI	DII	DIII	Total D	NI	NII	NIII	NIV	Total N	GOITER
MALE	No.	218	34	252	106	23	1	130	32	20	9		61	191
	%	50.3	7.8	58.1	24.4	5.3	0.2	30.0	7.3	4.6	2.0		14.0	44.0
FEMALE	No.	179	34	213	107	42	5	154	59	77	27	3	166	320
	%	33.5	6.3	39.9	20.0	7.8	0.9	28.8	11.0	14.4	5.0	0.5	31.1	60.0
M + F	No.	397	68	465	203	65	6	284	91	97	36	3	227	511
	%	41.0	7.0	48.1	22.0	6.7	0.6	29.3	9.4	10.0	3.7	0.3	23.4	52.8

Table 23. Prevalence and distribution of goiter by type and size. La Esperanza, April 1968.

		DIFFUSE GOITER							NODULAR GOITER					TOTAL
		Oa	Ob	Oa+Ob	DI	DII	DIII	Total D	NI	NII	NIII	NIV	Total N	GOITER
MALE	No.	116	162	278	98	15		113	143	44	9		196	309
	%	19.7	27.6	47.4	16.7	2.5		19.2	24.4	7.5	1.5		33.4	52.7
FEMALE	No.	77	122	199	107	31	2	140	137	141	25	1	304	444
	%	11.9	18.9	30.9	16.6	4.8	0.3	21.7	21.3	21.9	3.8	0.1	47.2	69.0
M + F	No.	193	284	477	205	46	2	253	280	185	34	1	500	753
	%	15.7	23.1	38.8	16.6	3.7	0.1	20.5	22.7	15.0	2.7	0.08	40.6	61.2

Table 24. Evolution of ^{131}I thyroid uptake in iodized subjects from Tocachi.

	2 hr. Mean value range	24 hr. Mean value range	96 hr. Mean value range
Before iodization No. 18	60 45-70	79 59-88	75 61-86
After 6 months No. 10	10 7-18	23 10-38	22 8-38
After 12 months No. 7	14 9-18	39 35-45	38 33-45
After 20 months No. 7	12 8-17	34 21-49	31 17-45
After 25 months No. 10	12 6-18	38 24-56	34 20-53
Non-iodized subjects (1 April 1968) No. 3	43 38-48	72 70-74	61 54-68

Table 25. Evolution of ^{131}I thyroid function in iodized subjects from Tocachi.

	Conversion ratio per cent, 24 hr. Mean value range	$PB^{131}I$ per cent 24 hr. Mean value range
Before iodization	86 55-92	0,29 0.10-0.34
After 6 months	6 2-15	0,019 0.008-0.04
After 12 months	14 8-21	0.04 0.02-0.07
After 20 months	31 15-51	0.21 0.06-0.42
After 25 months	36 11-57	0,14 0.02-0.24

Table 26. Evolution of ^{127}I blood tests in iodized subjects from Tocachi.

	TI µg% Mean value range	PBI µg% Mean value range	BEI µg% Mean value range	BII µg% Mean value range
Before iodization No. 18	2.84 1.2-5.6	2.76 1.2-5.0	2.08 0.8-5.0	0.60 0.4-0.8
After 6 months No. 10	20 9- 20	20 8- 20	4.40 2.0-7.0	10.00 6.0-15.4
After 12 months No. 14	11.16 8.0-15.2	11.14 7.8-14.4	5.85 4.6-6.8	5.62 2.2-10.2
After 20 months No. 5	9.13 7.3-10.8	8.01 7.1-9.5	5.50 4.1-6.5	2.20 0.9-3.2
After 25 months No. 14	7.72 2.0-12.2	7.15 1.5-11.0	5.69 1.4-7.4	1.57 0.4-4.5

Table 27. Evolution of urinary excretion of iodine in iodized subjects from Tocachi.

	UEI µg/100 ml.		CREATININE mg/100 ml.		UEI µg per 0.9 g creatinine	
	Mean value	Range	Mean value	Range	Mean value	Range
Before iodization No. 27	0.37	0.2-0.8	32.8	21-69	10.4	7.4-25.2
After 6 months No. 10	32.50	9.2-10.3	31.8	19-59	920.0	482-1,575
After 12 months No. 35	9.90	2.4-16.0	31.0	13-44	292.6	142-415
After 20 months No. 13	5.45	1.3-10.2	30.6	15-50	160.4	80-173
After 25 months No. 43	2.89	0.5-8.9	33.2	18-58	78.4	30-135
Non-iodized subjects (April 1968) No. 18	0.53	0.2-1.4	32.8	17-54	14.5	11.5-24.7

Table 28. Data obtained in the thyrotoxic women from Tocachi (after six months of administration of iodized oil).

	Patient No. 1	Patient No. 2	Patient No. 3
Age	45	67	60
Goiter March 1966	N-II	N-III	N-II
Goiter October 1966	N-I	N-III	N-I
BMR, per cent	+24	+53	+82
Cholesterol mg per 100 ml	212	190	178
^{131}I thyroid uptake			
8 hours	16	23	24
24 hours	22	32	33
96 hours	20	31	30
PB^{131}I per liter at 24 hours	0.02	0.008	0.09
KSCN Suppression test - per cent dose	1	4	0
BEI, µg per cent	7.8	12.0	8.0
BII, µg per cent	14.0	10.5	13.5
T_3-^{131}I resin uptake	40	50	52

CHAPTER 27

IODIZED OIL IN THE PREVENTION OF ENDEMIC GOITER AND ASSOCIATED DEFECTS IN THE ANDEAN REGION OF ECUADOR[1]

II. EFFECTS ON NEURO-MOTOR DEVELOPMENT AND SOMATIC GROWTH IN CHILDREN BEFORE TWO YEARS

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A program of prophylaxis of endemic goiter and cretinism by means of intramuscular administration of iodized oil was started in the Ecuadorian villages of Tocachi and La Esperanza in March 1966. Neuro-motor maturation and somatic growth have been studied in children born since that time. We would like to emphasize that these two villages are similar in incidence of endemic goiter, incidence of cretinism, and in socioeconomic, ecological and ethnic factors, and both have a similar degree of isolation (5, 6). Inhabitants of Tocachi were given iodized oil, whereas those of La Esperanza served as control subjects.

MATERIALS AND METHODS

All children born after March 1966 were studied chronologically at the following times: 0-15 days, four to six weeks, three to four months, six months, nine to ten months, 12-14 months, 18 months, and 24 months (a few cases). These children were born to mothers who were carefully followed during pregnancy. Between 60 and 70 per cent of deliveries were assisted either by a midwife (La Esperanza) or physician (Tocachi). All children were delivered at home. The delivery date was given by the Deliveries Office at each village for those children who were not born under our care.

The study included information on family background, names of parents, age, thyroid examination, goiter type according to the classification used for this study, and whether or not they received iodized oil. Information on the existence of abnormalities and on constitutional patterns was collected from each of the family members. Particular attention was given to the prenatal period when studying the personal background of each child. Any abnormalities occurring during pregnancy, infections, gynecological and obstetrical problems, and whether the mother was or was not iodized, were recorded. Delivery type, condition of the newborn, breathing, and early progress were also registered.

1/ From the Radioisotopes Department, National Polytechnic Institute, and the Central University Medical School, Quito, Ecuador.

Usually the first examination took place at the home. Subsequently these were done at the village dispensary. Initially there were a number of refusals by parents because of many factors, including mental deficiency or misunderstanding of the purpose of the study, but cooperation improved during the course of the study.

During each evaluation anthropometric growth, neuro-motor maturation, and dental and skeletal development were assessed. A general clinical examination was done with emphasis on pathology, nutritional status, and thyroid function.

The following measurements of somatic growth were made (15): weight, height, head, abdominal and thoracic circumference, length of inferior segment from the superior section of the symphysis pubis to the heel, superior segment (by subtracting the inferior segment from the total height), and the S/I ratio.

We have employed Gesell's Scales (8, 9) as standards for measuring neuromotor development, and the Gareiso and Escardo studies (7) as a guide for neurological control. Visual, auditory, social, and language development were noted, as well as motor and intellectual development and evolution of reflex activity.

Skeletal development was assessed by x-rays of the left hand taken at age one to four months and at 12-14 months. These were taken with Kodak Non-Screen Ready Pack medical x-ray film at a tube-to-film distance of 36 inches with a Bucky field-portable x-ray unit.

RESULTS

This report is concerned primarily with the problems of development and maturation of the infant population born in Tocachi and La Esperanza after March 1966, the time when the Tocachi group was given iodized oil. Ninety children were born in Tocachi from March 1966 to April 1968; sixteen (17 per cent) died and two (2 per cent) went away. One hundred and seventy-seven children were born in La Esperanza by April 1968; seventeen (9.6 per cent) died and five (2.7 per cent) went away. Children born in Tocachi were born to mothers who received deposit iodine; children born in La Esperanza belonged to a population which did not receive prophylaxis.

The children subsist in a generally poor environment; there is high infant morbidity and mortality, and many of the mothers are unwed (Tables 1, 2, and 3). Commonly encountered were gastrointestinal syndromes (gastroenteritis, enteritis, colitis, etc.), respiratory diseases (bronchitis, tonsillitis, etc.), diseases of the sensory organs (blepharitis, conjunctivitis, otitis), and congenital malformations (hernias, preauricular nodules, supernumerary nipples). Children of Tocachi presented a greater number of these diseases (Table 2). The children of Tocachi also showed a higher degree of undernutrition, at all surveys (Table 3).

From observations on the mothers we concluded that they did not present, at the time of their pregnancies, any kind of infectious or gyneco-obstetrical disorders which could be indicted as a cause for the impaired development of their children.

Table 1. Biological and social factors in relation to the children studied.

Village	Children born March '66-April '68	Children died March '66-April '68	% Unwed mothers	Maternal age
Tocachi	90	16-17 %	35.5%	23.3 Mean 20-48 Range
La Esperanza	117	17-9.6%	16.0%	27.81 Mean 17-47 Range

Two of the Tocachi mothers were deafmute cretins, and two were mentally deficient with low hearing and dyslalia. Their children have developed normally.

Three of the mothers of La Esperanza were deafmute cretins. One of them had a male child who is a cretin, another had a mentally deficient child, and only one had a baby who is developing "normally." One of the mothers in La Esperanza is oligophrenic. Her daughter, born during the period of our study, is an imbecile. Two mothers, one in Tocachi and one in La Esperanza, had already produced mentally deficient children, but their children born during the time of our study are normal.

One child in La Esperanza has a father who has agenesis of a distal phalanx of one of his thumbs. The child has no malformation and has developed normally.

There is a greater incidence of goiter in La Esperanza and a significant percentage of nodular goiters (Table 4). This is concluded from thyroid evaluation of the mothers after delivery.

The somatometric results from children from Tocachi and La Esperanza are shown by superimposed curves constituted on the basis of mean values obtained for each age group studied. We compared averages of each one of these anthropometric measurements from both Tocachi and La Esperanza, with data from a private clinic in Quito (high income group) (2), and with one from the outpatients at the "Isidro Ayora" Maternity Hospital (low income group) (13) in Quito. This was done in order to obtain differential data regarding growth for Ecuadorian children from rural and urban areas. For comparative purposes we also plotted curves for the American averages given by Nelson (15) and by the Fels Research Institute (4), Nellhaus (14), and Wilkins (17).

Weight (Figure 1)

Body weight is probably the best indication of growth and nutritional status, because it is a product of all the elements that contribute to growth.

Height (Figure 2)

Somatic growth in children from Tocachi and La Esperanza was generally similar. It is lower in both villages when compared with the low income infant group from Quito, and also even lower when compared with American standards.

Table 2. Pathology. Percentage incidence of more frequent diseases in the children studied.

Age in months	1.25		3.5		6		9.5		13		18	
Village	TOC.	ESP.	TOC.	ESP.	TOC.	ESP.	TOC.	ESP.	TOC.	ESP.	TOC.	ESP.
Groups of diseases:												
Gastrointestinal syndrome	8.8%	5.0%	7.5%	8.7%	10.0%	6.0%	23.5%	11.32%	22.2%	5.3%	47.6%	11.5%
Respiratory disease	19.1%	17.0%	28.8%	19.7%	2.8%	2.5%	11.8%	12.3%	13.3%	12.8%	28.6%	9.0%
Disease of the sensory organs	4.4%	4.0%	4.5%	2.4%	0.0%	4.3%	3.9%	5.6%	2.2%	4.2%	0.0%	0.0%
Congenital malformation	8.8%	15.0%	16.7%	6.3%	14.0%	6.0%	10.0%	6.0%	4.4%	0.0%	4.8%	3.3%
Diseases of skin	1.5%	7.0%	0.0%	0.0%	2.0%	1.0%	0.0%	0.0%	0.0%	1.0%	0.0%	0.0%

Table 3. Nutritional status.

Age in months	Satisfactory		Deficient	
	Tocachi	La Esperanza	Tocachi	La Esperanza
1.25	64.7%	86%	35.3%	14%
3.5	81%	97%	19%	3%
6	75.5%	88%	24.5%	12%
9.5	68%	86%	32%	14%
13	39%	56%	61%	44%
18	30%	69%	70%	31%

Table 4. Evaluation of maternal thyroid at the time of first examination after delivery.

Thyroid	Tocachi* (per cent)	La Esperanza (per cent)
$O_a + O_b$	50.7	20.9
DI + DII	17.3	13.1
NI + NII + NIII	31.8	65.8
D + N	49.2	78.9

N - Nodular.
D - Diffuse.
** - Subjects in Tocachi had received iodized oil.*

There is a fall-off in averages around six months of age. This presumably indicates the poor environment in which the children develop. Weight and height gain deficiency is increased at this age (Table 5). The percentage increase in height after delivery was 37.5 per cent in Tocachi at 12 months of age, and 40.5 per cent in La Esperanza. Thus in Tocachi there is the same kind of handicap in height as is found in adults. Also, the expected normal increment for height is, at this age, 50 per cent.

The weight and height deceleration is an obvious sign of undernutrition and high incidence of disease determined by deficient socioeconomic factors so common in developing countries.

Cephalic, thoracic, and abdominal circumferences appear in Figures 3, 4, and 5. The increase in head circumference in both villages and for each group is lower than the American standard (14). However, it is interesting to note that averages in Tocachi are slightly better than those in La Esperanza in all age groups (Tables 5 and 6). From study of the body segments (Figure 6, 7, and 8) it appeared that, although the children of Tocachi and La Esperanza tend to be smaller when compared with general standards, their growth is proportional.

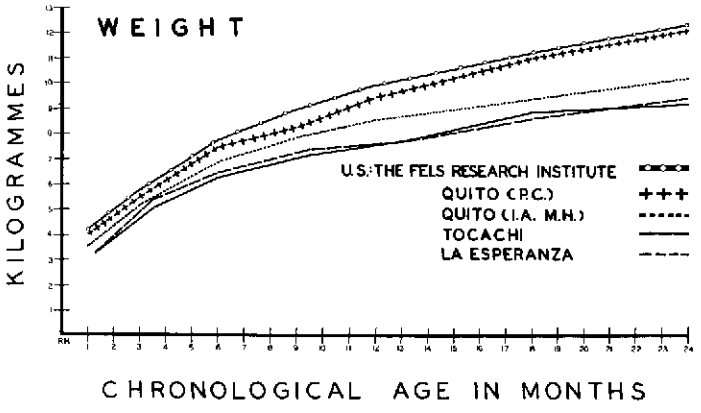

Figure 1. Weight, mean values. Tocachi, La Esperanza, Quito (private clinic and "Isidro Ayora" Maternity Hospital) and the United States (Fels Research Institute, Ohio).

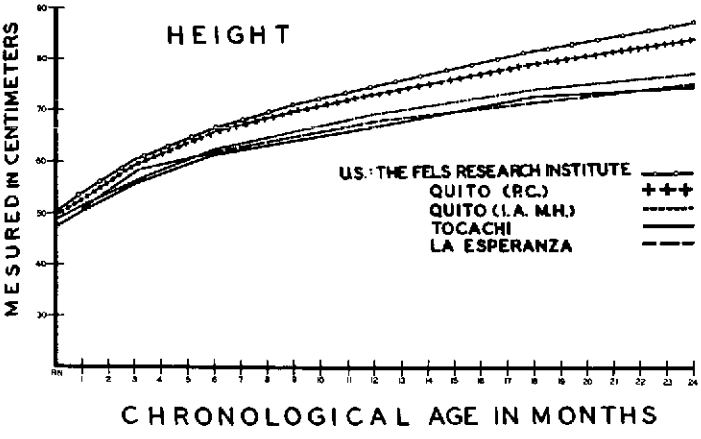

Figure 2. Height, mean values. Tocachi, La Esperanza, Quito (private clinic and "Isidro Ayora" Maternity Hospital) and the United States (Fels Research Institute, Ohio).

Table 5. Weight, height, and head circumference. Growth increments, mean values, at the time of each survey period.

Age in months	Height (cm)		Weight (kg)		Head circumference (cm)	
	Tocachi	La Esperanza	Tocachi	La Esperanza	Tocachi	La Esperanza
1.25	2.22		1.16		1.46	
3.5	4.92	5.84	2.90	3.15	2.57	2.58
6	3.92	4.13	2.61	2.38	2.36	1.95
9.5	2.57	3.35	1.25	1.54	1.20	1.23
13	2.56	2.59	1.13	0.95	0.83	0.78
18	3.66	3.27	1.88	2.43	1.27	0.96

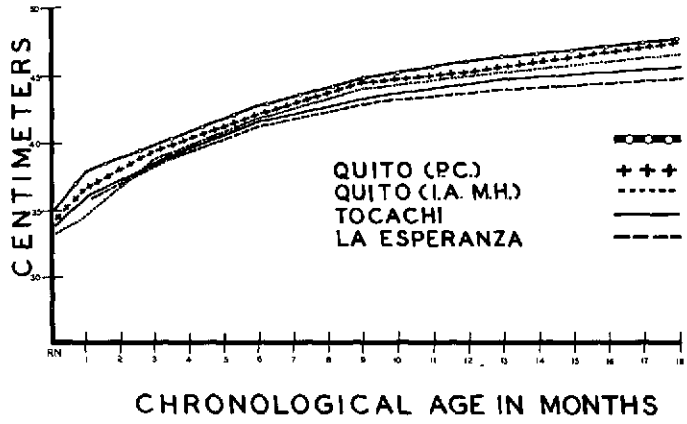

Figure 3. Head circumference, mean values. Tocachi, La Esperanza, Quito (private clinic and "Isidro Ayora" Maternity Hospital) and the United States (Nellhaus, Denver (14)).

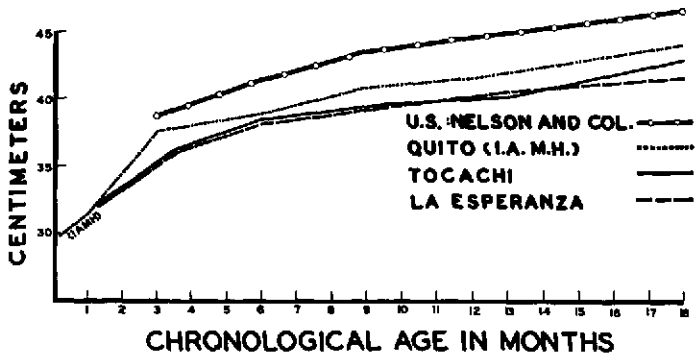

Figure 4. Abdominal circumference, mean values. Tocachi, La Esperanza, Quito ("Isidro Ayora" Maternity Hospital) and the United States (Nelson (15)).

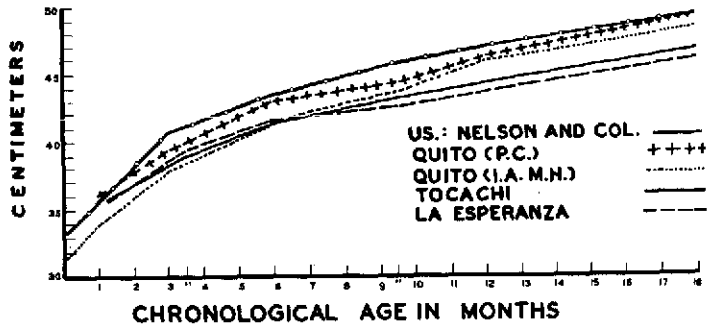

Figure 5. Thoracic circumference, mean values. Tocachi, La Esperanza, Quito (private clinic and "Isidro Ayora" Maternity Hospital) and the United States (Nelson (15)).

Table 6. Head circumference at each control period, percentage increase. Mean values: Tocachi, La Esperanza, Quito ("Isidro Ayora," Maternity Hospital) and U. S. (Nelson and col.).

Locality	Age in months					
	1	3	6	9	12	18
Tocachi	5.68%	13.98%	23.74%	26.68%	28.52%	33.54%
La Esperanza	5.63%	13.75%	22.73%	26.41%	27.71%	32.52%
Quito (Maternity Hospital)	5.3%	14.3%	23.2%	28.8%	32.5%	37.6%
U.S. (Nelson and col.)	10.0%	15.5%	23.8%	29.4%	33.0%	37.1%

A large number of children were found with O_b thyroids (Table 7), and 4 per cent had Grade I diffuse goiter in La Esperanza. No case of thyroid enlargement was found in Tocachi.

One patient was found in La Esperanza with a teratoma and another with a congenital malformation (fifth metacarpal disgenesis). None was found in Tocachi.

We have used Gesell's scales as standards for measuring neuro-motor development. They have helped to recognize and interpret the levels of maturity and the condition of the central nervous system. The conditions and objectives of the neuro-motor tests are so designed that typical reactions are expected for each stage of development.

Levels of neurologic maturation were reached in both villages at older ages than is considered normal (3, 7, 12, and 15).

There was progressive behavioral retardation in both villages as tested by manipulative tasks, perceptual, linguistic, and gross motor tasks (Figures 9-15). Average developmental quotient in Tocachi was 92.77 per cent (normal). It was 89 per cent in La Esperanza (low normal (Table 8).

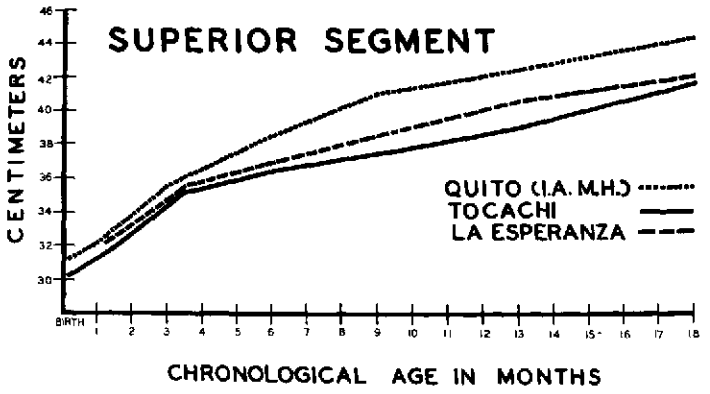

Figure 6. Superior segment, mean values. Tocachi, La Esperanza, and Quito ("Isidro Ayora" Maternity Hospital).

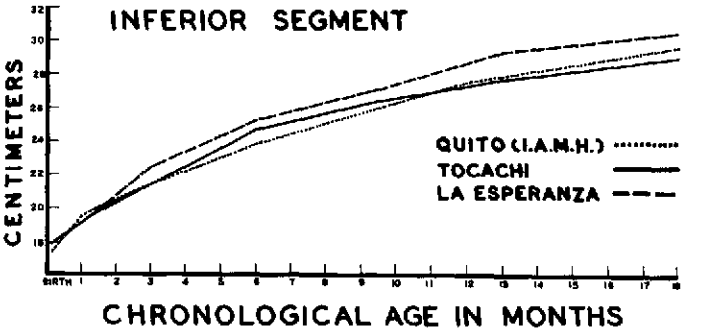

Figure 7. Inferior segment, mean values. Tocachi, La Esperanza, and Quito ("Isidro Ayora" Maternity Hospital).

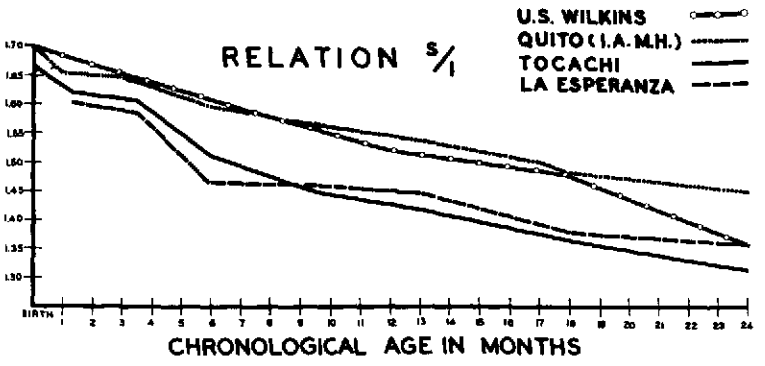

Figure 8. Ratio of superior to inferior segment (S/I), mean values. Tocachi, La Esperanza, Quito ("Isidro Ayora" Maternity Hospital) and the United States (Wilkins (17)).

Table 7. Linear study of thyroid size in children born in La Esperanza, after March 1966. No children in Tocachi had thyroids other than O_a.

Age in months	1.25	3.5	6	9.5	13	18	24
Thyroid O_b	0.0%	0.0%	3.2%	5.0%	9.6%	24.0%	46.0%
Thyroid I diffuse	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	4.0%

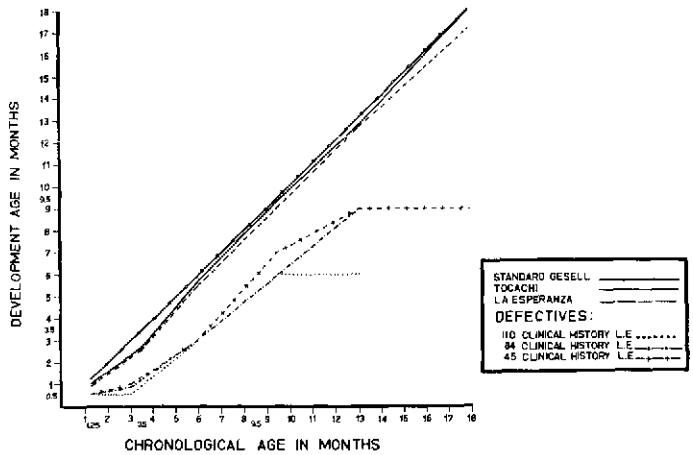

Figure 9. Developmental age by visual testing, mean values. Tocachi, La Esperanza, and mental defectives from La Esperanza. Gesell Standard (8).

So far three cases of severe mental deficiency have appeared in La Esperanza. One is a typical cretin (Figure 16). The mother, an Indian by race and 24 years old, is a deafmute cretin with motor abnormalities and Grade III nodular goiter. Pregnancy and delivery were normal. At present the child is 13 months old, has a Developmental Quotient of 30.7 per cent, has not started dentition yet, is undernourished, and responds to neuro-motor development tests as follows:

1) Looks at big objects for short moments and does not follow their movement.

2) Does not answer to noise.

3) (a) Raises the head slightly.
   (b) Moves head and tends to keep it extended when seated with hands held.
   (c) Does not grope for objects.

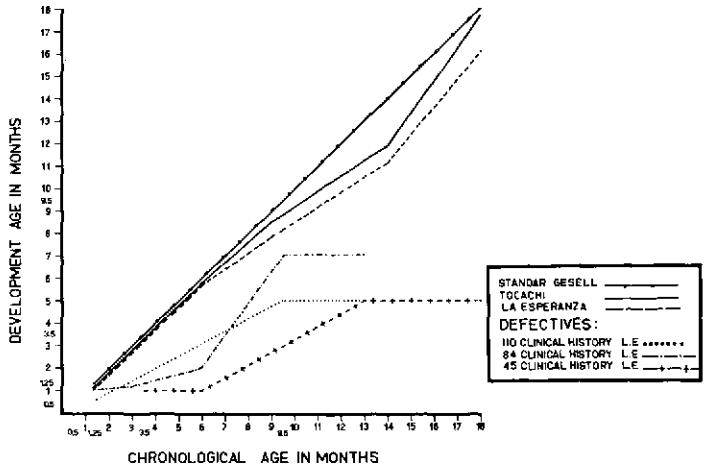

Figure 10. Developmental age by auditory testing, mean values. Tocachi, La Esperanza, and mental defectives from La Esperanza. Gesell Standard (8).

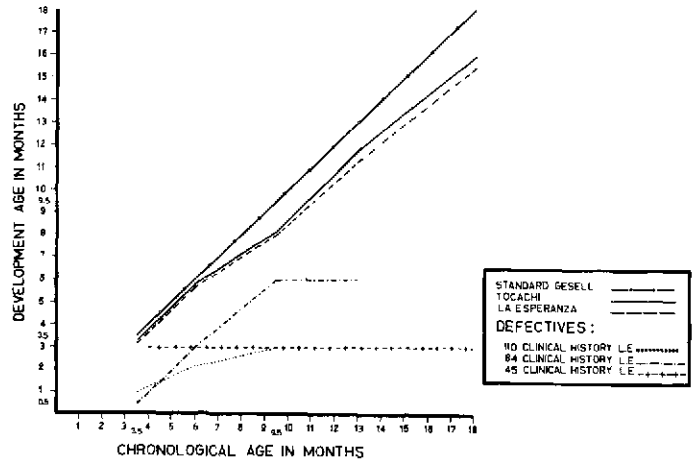

Figure 11. Social and linguistic development, mean values. Tocachi, La Esperanza, and mental defectives from La Esperanza. Gesell Standard (8).

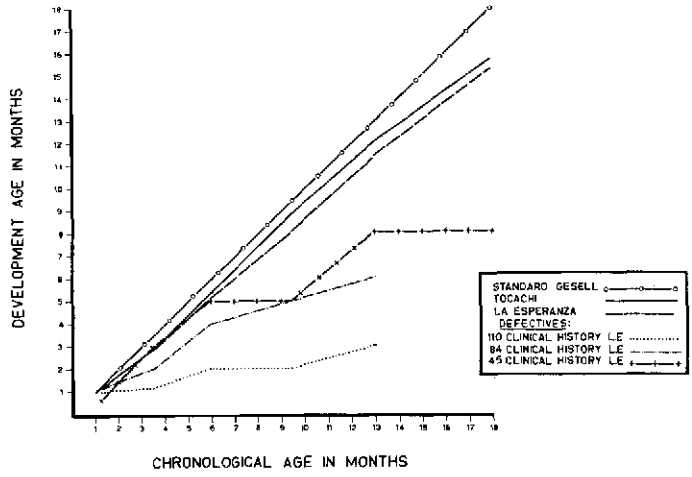

Figure 12. Motor development, mean values. Tocachi, La Esperanza, and mental defectives from La Esperanza. Gesell Standard (8).

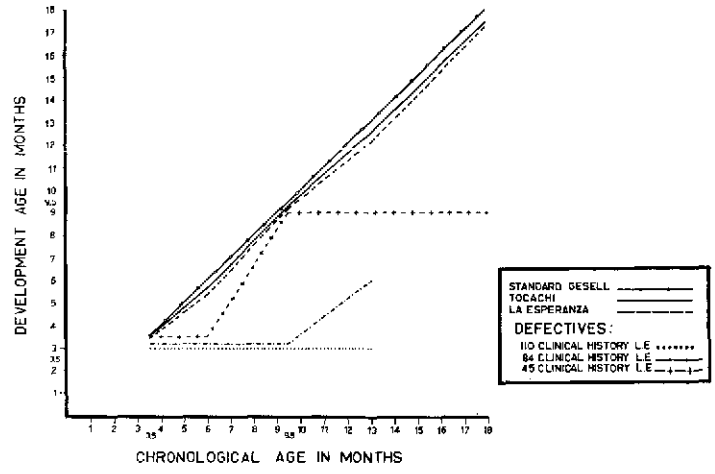

Figure 13. Evolution of reflex activity, mean values. Tocachi, La Esperanza, and mental defectives from La Esperanza. Gesell Standard (8).

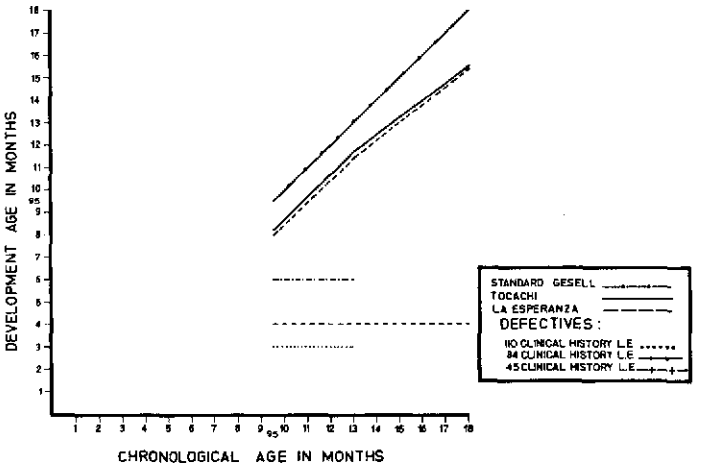

Figure 14. Maturation of intellectual function, mean values. Tocachi, La Esperanza, and mental defectives from La Esperanza. Gesell Standard (8).

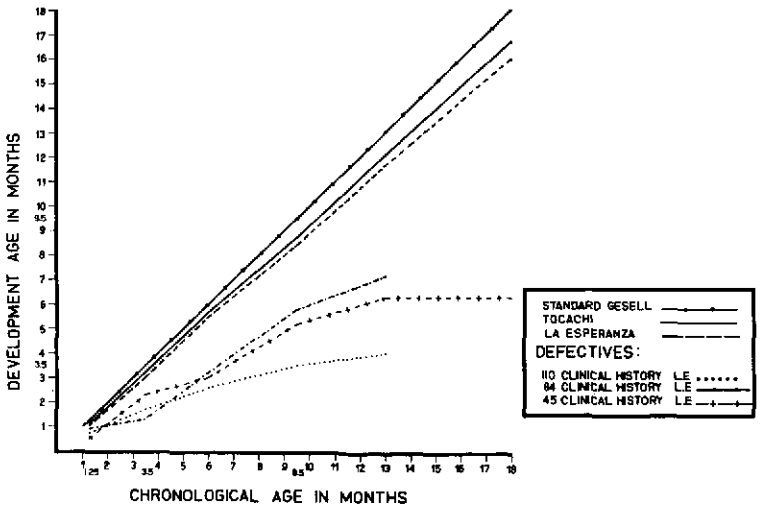

Figure 15. Neuro-motor maturation, evolution of all developmental aspects. Tocachi, La Esperanza, and mental defectives from La Esperanza. Gesell Standard (8).

Table 8. Neuro-motor Development Quotient. Mean values for Tocachi and La Esperanza children and for the three mentally deficient children from La Esperanza.

Village	Age in months		
	9.5	13	18
Tocachi	92.21%	93.21%	92.77%
La Esperanza	89 00%	90.00%	89.00%
Defective (#110)	38.52%	30.76%	-
Defective (#45)	54.31%	48.69%	35.16%
Defective (#84)	61.36%	55.00%	-

Figure 16. #110 Clinical history: male, 13 months old; height 66 cm ; weight 16 pounds; inferior segment 25 cm, without deciduous tooth; O_a thyroid; deafmute and cretin (30.76 per cent D.Q.).

(d) Limited hip abduction; tendency to keep legs crossed; continuous grasping of hand.

4) Slightly perceptible Moro's reflex; Magnus Klein reflex not present; grasping reflex present in feet and hands (pithecoid position of thumbs); symmetric patellar reflex.

5) Immediate withdrawal when pinched.

6) Highly irritable; laughs at times or smiles slightly; does not produce sounds.

Blood samples were taken in April 1968 (Table 9).

We may conclude from these results that serum thyroxine values are significantly lower than normal, or than in adult cretins from Tocachi and La Esperanza (5, 6).

Table 9. Serum values for cretinous child from La Esperanza and his relatives.

Subjects	TI µg %	T_4 µg %	T_4I µg %	I.R.-H.G.R. µg ml
14-month male cretin	2.1	0.7	0.5	2.0
24-year-old mother (cretin, Grade III nodular goiter)	12.8	0.4	0.3	4.0
58-year-old grandmother (Grade II nodular goiter)	2.3	0.8	0.5	0.68
62-year-old grandfather (Grade I nodular goiter)	3.7	2.7	1.8	3.0

I.R.-H.G.R. - Immunoreactive human growth hormone (by Dr. S. Refetoff, Clinical Research Center, Massachusetts Institute of Technology).

There was another mentally deficient child in La Esperanza. The mother is a 25-year-old deafmute cretin with no motor disturbances and Grade III nodular goiter. Pregnancy was normal and delivery was without medical attention. The child died when he was 13 months old. Developmental Quotient was 55 per cent. There was no dentition. Nutritional state was good. Neuro-motor test presented the following:

1) Follows only movements of large objects with some interest; looks momentarily at small objects but does not react to lateral objects.

2) Turns at rattle noise; does not follow voice.

3) (a) Extends head in prone position.
   (b) Keeps head firm when seated with hands held; does not sit with holder.
   (c) Holds two blocks at a time; takes objects to mouth.
   (d) Picks marbles with thumb and index movements.
   (e) Does not creep, does not crawl.
   (f) Somewhat limited hip abduction.
   (g) Symmetric tone.

4) No Moro or Magnus Klein reflexes; grasping reflex present in feet; symmetric patellar reflex present; no clonus; plantar reaction on left when in extended position and on right foot when in flexion.

5) Withdrawal and cry when pinched.

6) Does not produce sounds or make faces; does not imitate or look for hidden object; smiles dully.

A third child has a neuro-motor deficit corresponding to imbecility. Her mother is a 34-year-old retardate with a Grade III nodular goiter. This girl was found to be mentally deficient at one month of age and has had mild epileptic symptoms from three months. At 14 months the first tooth erupted, and she sat alone. The nutritional state was generally good. At 18 months of age she had ten teeth. Development Quotient was 35.16 per cent. Neuro-motor test gave the following:

1) Follows large objects with no interest; does not pay attention and does not respond to small objects.

2) Does not respond to rattle.

3) (a) Walks by herself.
(b) Slow thumb and index movements.
(c) Takes objects to mouth.
(d) Holds two blocks at a time.
(e) Easy hip abduction.
(f) Symmetric tone.
(g) Constantly grasps hands; pithecoid position of thumbs.

4) No Moro or Magnus Klein reflexes; persistent grasping reflex in feet; plantar reaction when bilaterally extended; symmetric patellar reflex; no clonus.

5) Does not make sounds; shouts a-a-a- when crying; does not follow simple orders; no facial expression; does not imitate; smiles dully.

Thus there is relative retardation when judged by the standard measures used in Tocachi. There is no case in Tocachi as far from normal as the ones found in La Esperanza. There is one case of mongolism in La Esperanza. Thus the studies on neuro-motor development, when considered as a whole, suggest that there is some advance in neurological development in children of Tocachi, as compared with children of La Esperanza.

Dental development showed significant retardation in both villages (Table 10).

DISCUSSION AND CONCLUSIONS

The principal task of fetus, infant, and child is to grow and develop. Growth produces structural changes and transformations closely correlated with physiological functions. Growth and development result from an intricate pattern of genetic, nutritional, social, and cultural forces which dynamically affect the child from the moment of conception. Although the pattern is the same in general for each child, there are important individual differences in each case, all of them within the broad limits of what is called "normality." If one wants reasonably acceptable answers to assessment of neural development, the child must have at least a relatively normal development of the basic sensory channels, especially sight and hearing. He also needs some kind of

Table 10. Tooth eruption percentage according to age.

Age in months	6		9.5		13		18	
Village	Tocachi	La Esperanza	Tocachi	La Esperanza	Tocachi	La Esperanza	Tocachi	La Esperanza
Different dental pieces:								
1-1	6.66%	15.9%	50.93%	41.7%	48.8%	47.7%	4.34%	18.33%
2-2	-	0.81%	16.98%	24.3%	55.6%	47.7%	39 %	20 %
4-4	1.66%	-	7.5%	11.6%	35.5%	33 %	69.5%	53.3%
3-3	-	0.81%	13.2%	13.6%	51.1%	46.5%	47.8%	38.3%
7-7	-	-	-	-	-	-	17.4%	23.3%
8-8	-	-	-	6.9%	2.2%	-	17.4%	26.6%
5-5	-	-	-	-	-	2.2%	78.26%	63.3%
6-6	-	-	-	-	-	1.13%	52.17%	58.3%
9-9	-	-	-	-	-	-		-
10-10	-	-	-	-	-	-		-

perceptive machinery, a reasonable intellectual level, and a motivation to meet the tasks assigned. These considerations define the difficulties of appraisal of neuromuscular development in backward and deprived areas.

The high percentage of undernutrition (higher in Tocachi) is a result of neglect, poor alimentary habits, and a high degree of concomitant morbidity. In contrast with Clements (1), we are of the opinion that people with endemic goiter have more risk of having children with problems of neuro-motor development. Prophylactic measures have not thus far increased somatic growth, but up to the present they perhaps have contributed to neuro-motor development and possibly have prevented cretinism.

SUMMARY

All children born in a region of severe endemic goiter in rural Ecuador have been examined and followed in order to assess the effects of iodized oil as a prophylactic measure for preventing endemic cretinism and other possibly related defects.

Preliminary evidence is consistent with this measure as a preventive of cretinism, but there is no evidence yet that the iodized oil promotes somatic growth, at least during the first 18 months of life.

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CHAPTER 28

IODINE THERAPY FOR ENDEMIC GOITER AND ITS EFFECT UPON SKELETAL DEVELOPMENT OF THE CHILD

Harry Israel, III,[1] Rodrigo Fierro-Benítez,[2] and Juan Garcés[3]

The interplay of nutrition, childhood disease, emotional well-being, and genetics are clearly recognized for their role upon physical growth of the child. While all have received considerable attention, special emphasis is now being placed on nutrition since such a large proportion of the world's population is inadequately nourished. The mounting information regarding poor diet and lagging growth further amplifies this. An inordinate number of studies on growth, especially from tropical areas, involves protein-calorie malnutrition. Few have dealt critically with the problem of iodine malnutrition and endemic goiter. This investigation, part of the iodine prophylaxis program in Ecuador, deals with one parameter of physical development, the skeleton. The other systems have been dealt with elsewhere (13).

In order to assess the effects of the iodized oil prophylactic program in Ecuador on the skeletal system, a sample of village children from iodine-treated Tocachi and the control village, La Esperanza, were surveyed.

Study design of the iodine therapy program, having been described previously (cf. Chapter 26) will not be again reviewed except to explain that the left hand of children from both villages was x-rayed at the time of iodine injection (March 1966) and repeated two years later (April 1968). This was accomplished using a generator-driven portable Bucky x-ray unit and Kodak non-screen medical x-ray film. The x-ray unit, transported to each village, was carefully positioned to take films at a fixed tube to film distance of 36 inches (90 cm). Initially some 300 x-rays of persons between 1 and 13 years of age were exposed, and two years later over 400 films were taken. It was possible during the interim two-year period to collect a sizeable radiographic sample of children 3 months to 2 years of age. Included in the entire group was a total of 29 Tocachi males and 28 females and 23 La Esperanza boys and 17 girls that were sampled longitudinally. The hand radiographs, the most widely used reference in skeletal development, were assessed for ossification status, bone width (metacarpal II), and bone length (metacarpal II). Skeletal maturity as judged from western standards has not been completed.

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OSSIFICATION STATUS BEFORE AND AFTER IODINE THERAPY

Delayed ossification has been implicated in malnutrition generally, and more specifically in the protein-calorie type. This long held hypothesis has recently been questioned (4) by findings in Latin America. Ossification as used here refers to the total number of hand-wrist centers (5). This simply involves counting the number present at a given age up to the maximum of 28. In well-nourished populations all are present around the age of 6 and in the Ecuadorians by the age of 8 or 9.

The villages of Tocachi and La Esperanza, as dealt with elsewhere, were first tested separately and no difference could be detected on the basis of ossification, or any other standards; therefore the villages were pooled (9).

As expected, pretreatment ossification status among the Ecuadorians fell considerably behind the United States (Fels) standards (Figure 1) (9). When matched against an available group of Guatemalan villagers, the Ecuadorians were randomly distributed above and below the sex specific trend lines. The chi square test was used to confirm that there was no significant difference from chance. Since there were insufficient numbers of Ecuadorians to develop pretreatment trend lines for ossification, and they so evenly segregated themselves about sampled Guatemalan villagers, the assumption was made that the children of Ecuador closely resembled the Guatemalans in ossification status. Accordingly, the trend line could safely be interchanged, one for the other.

The two-year follow-up of Tocachi and La Esperanza, separately matched against the male and female trend lines of Guatemala, revealed that among Tocachi boys from one to six, 28 were above the trend and 12 below ($X^2 = 5.62$; $0.05 > P$); whereas among La Esperanza boys there were 17 above and 15 below ($X^2 = 0.03$) (Figure 2).

Finally with the girls from Tocachi, 15 were above and 17 were below ($X^2 = .03$) and in La Esperanza 14 above and 18 below ($X^2 = 0.3$) (Figure 3). Only the Tocachi boys were significantly above the trend; the other three groups were randomly distributed.

The evidence suggesting change over a two-year period is hardly convincing (Tocachi boys) but then again, the use of Guatemalan norms for comparative purposes may not give a clear picture for detecting any beneficial effects. Possibly one of the indices of skeletal maturity such as that of Greulich and Pyle when applied will shed additional light on the ossification status of the children. The best known skeletal maturity predictors use Western standards, but they may prove useful among Ecuadorians, as they have when applied to populations in other developing countries.

Considering only the information available at this moment, there is little evidence that any real change has occurred.

BONE WIDTH

Alteration in the transverse diameter of the hand bones, most reliably the second metacarpal (6), has been shown to be a sensitive indicator of skeletal change in growth and aging (4, 6), and in health and disease (1, 7, 10,

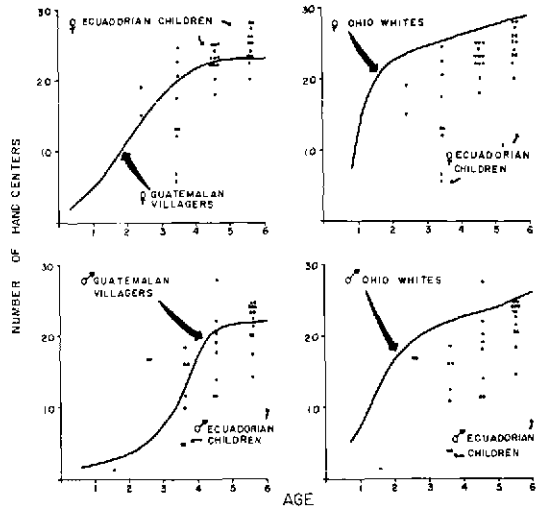

Figure 1. Individual pretreatment values of Ecuadorian children (1966) for appearance of ossification centers in the hand. It is shown that on an individual basis Ecuadorian children fall below the trend line for well-nourished Ohio whites while they randomly distribute themselves about the trend for Guatemalan villagers.

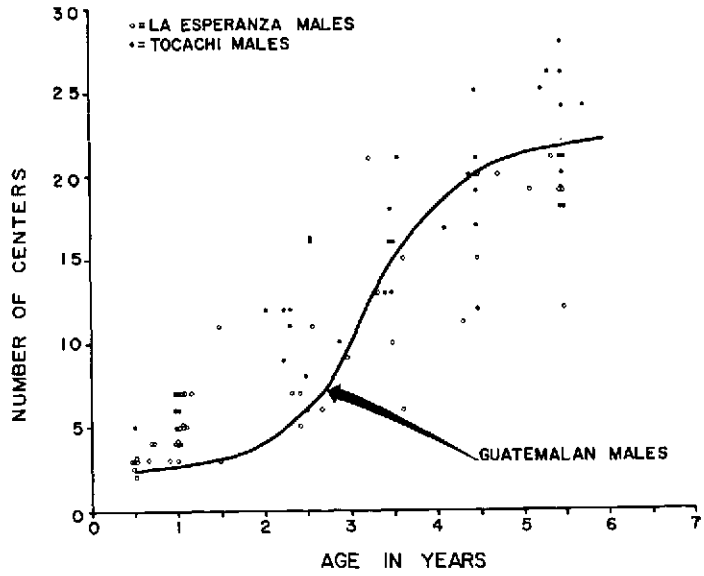

Figure 2. The distribution of Ecuadorian boys after therapy (1968) for the number of appearing hand ossification centers. The Tocachi boys fall significantly above the trend ($0.5 > p$) of Guatemalan males while the La Esperanza boys are randomly distributed.

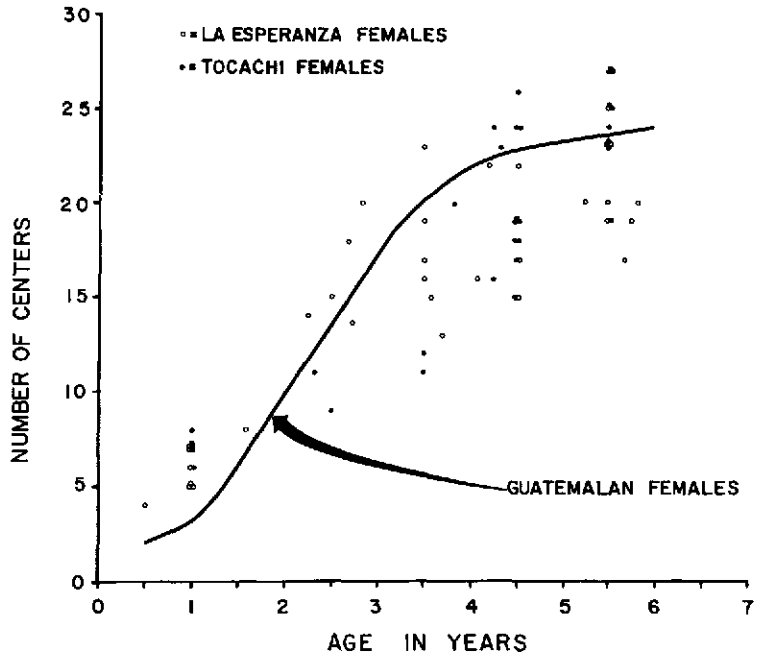

Figure 3. Post-treatment (1968) values for number of hand centers in girls. Both the Tocachi and La Esperanza girls distribute themselves in random fashion about the Guatemalan trend line.

14, 15). Essentially this involves three components, those being total width, cortical thickness, and medullary width. These reflect two of the three envelopes of bone as designated by Frost (3), periosteal and endosteal. Changes subperiosteally are mirrored in total diameter, endosteally in medullary width, and the thickness of cortex is the product of both.

A previous study in protein-calorie malnutrition and growth demonstrated that ossification status (number of centers) of the hand was unaltered while cortical bone (compact bone) of the metacarpal was severely reduced (4). Surprisingly, linear metacarpal growth was unaffected while the cortex was progressively thinning. In the study just cited the opportunity existed for autopsy evaluation of the skeleton. By suitably testing a specific bone (tibia), it was demonstrated that its length, when corrected for cortical thickness or area, correlated highly with total bone mineral (ash) ($r = 0.94$). While one should not presume that micrometric measurements on a single bone reflect the entire mineral content of the skeleton, it at very least seems to be a non-destructive method for estimating bone change in vivo.

Applying this to the Ecuadorian population, a clear picture emerges of change in the transverse diameter of the second metacarpal. Taking children no younger than 2 years of age in April 1968, the cortical width of the metacarpal at midshaft appears markedly increased over 1966 values. This is shown (Figure 4) longitudinally in that the growth velocity, as reflected in slopes of lines connecting cortex measurements in 1966 and again in 1968, among 28

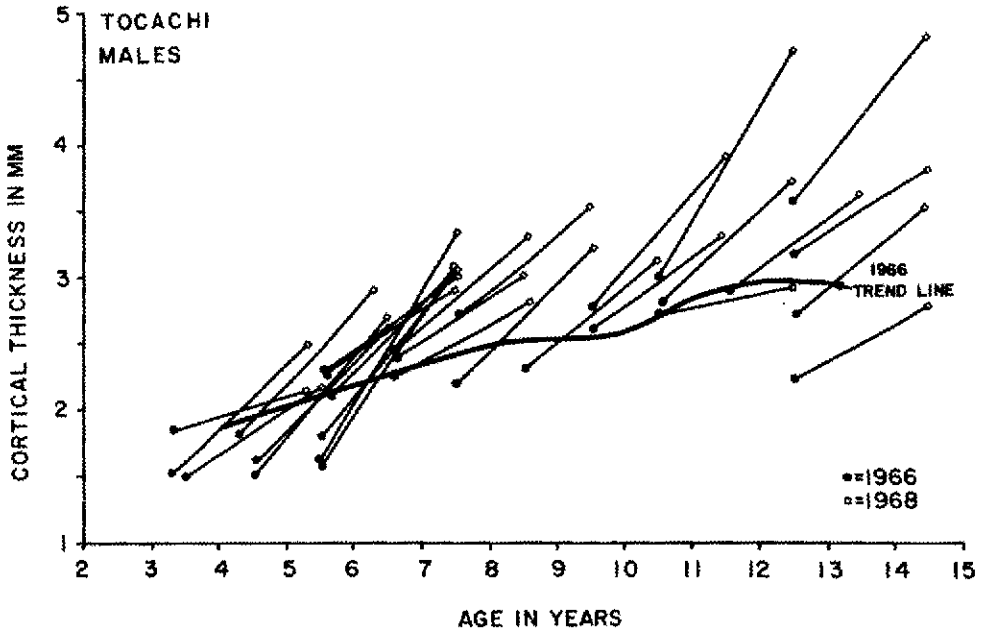

Figure 4. Growth velocity of cortical thickness among the treated group as reflected in slopes of lines connecting measurements in 1966 and 1968. Note that in all except two or three instances the rate of growth is greater following therapy as contrasted with the collective 1966 trend line.

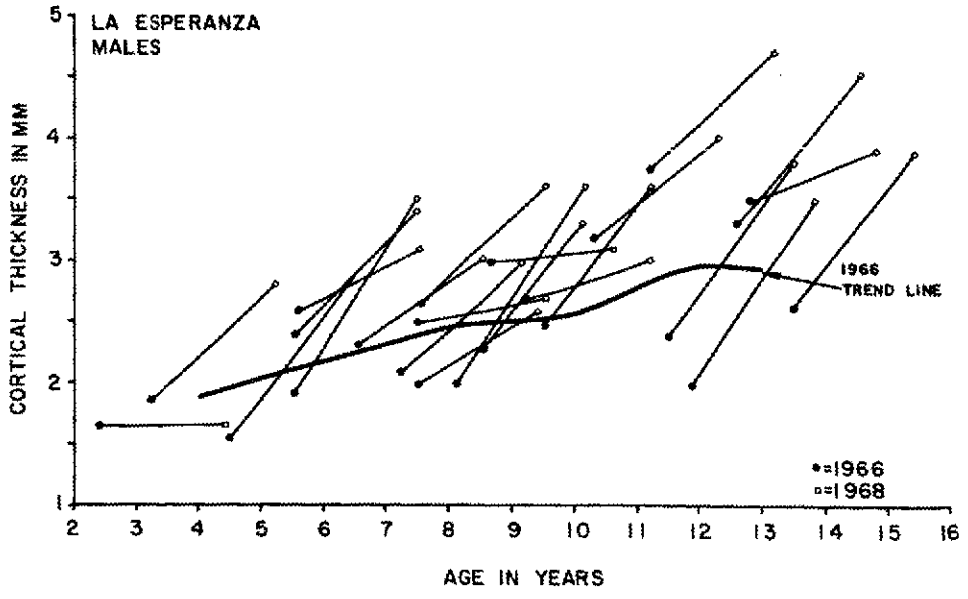

Figure 5. The accelerated gain in cortical thickness is apparent in this control group just as was noted among the treated patients in the preceding illustration. The one example demonstrating no growth over the two-year period has now been shown to be a result of error in identification. Therefore, no males fall below the 1966 trend line.

male Tocachi individuals exceeds any age comparable increment in the 1966 trend line except in two or three instances. Also there are only two individuals in 1968 who fall below the trend for amount of cortical thickness, while 16 did in 1966. The 1966 values for cortical thickness are randomly distributed above and below the line.

Not only are cortical thickness changes in Tocachi apparent, but those in La Esperanza are equally convincing (Figure 5). The velocity of growth on a group basis (1966) exceeds individuals in only four of the 23 cases. whereas 11 individuals were below the trend line in 1966 only one remains by 1968. The girls from both villages, not graphically presented, reflect precisely the same rapid and greater gain in cortical bone of the second metacarpal. To emphasize this the information was then handled cross sectionally; villages and sex separated trends in 1968 generated on the basis of mean thickness at given yearly intervals were matched with the appropriate 1966 values (Figures 6 and 7). The boys and girls from both villages in 1968 clearly demonstrated a striking gain in compact bone. This rapid addition of bone amounted to over 25 per cent in the older age group.

The gain in cortical thickness among males and females in both the treated and control villages is apparent. Although not represented here, the overall gain in cortex was similarly reflected in the total cross-sectional diameter of the bone, and suggested marked changes at the subperiosteal surface. The type of activity occurring at the endosteal surface or its role as a participant in cortical gain must wait until the medullary portion of the bone can be suitably evaluated and tested in the manner just described for the other transverse bony segments. Even without endosteal assessment there is little doubt that two years' time has seen considerable gain in cortical thickness or in compact bone (mineral mass).

BONE LENGTH

To this point there is little or no demonstrable change in ossification status, but there is a marked acceleration of compact bone gain and transverse growth among both villages. With these changes in mind, the metacarpal was appropriately measured (9) and tested for linear growth.

Data analysis was handled in precisely the same fashion as was bone diameter. The longitudinal sample matched against the pretreatment trend shows that the velocity of growth on an individual basis seldom if ever exceeds the slope of the collective curves (Figures 8 and 9). This holds for boys and girls in both Tocachi and La Esperanza. Not only is velocity unchanged, but there is no demonstrable shift among 1968 values from pretreatment random distribution (1966) about the trend toward any significant alteration two years later. Growth is in continuum, but not accelerated. The sex specific information from both villages handled cross sectionally substantiates the null findings (Figures 10 and 11). Simply put, there was no pronounced effect upon linear growth of the metacarpal over the two-year period in either village as was so clearly demonstrable in the transverse segment of the bone. One unusual feature is the fact that the greatest number of individual Tocachi males (Figure 8) fell below the 1966 trend line rather than being evenly distributed about it. This is the only instance among either the boys or girls from Tocachi or La Esperanza where there is a failure to randomize about the pretreatment trend, and it is likely a problem of sampling.

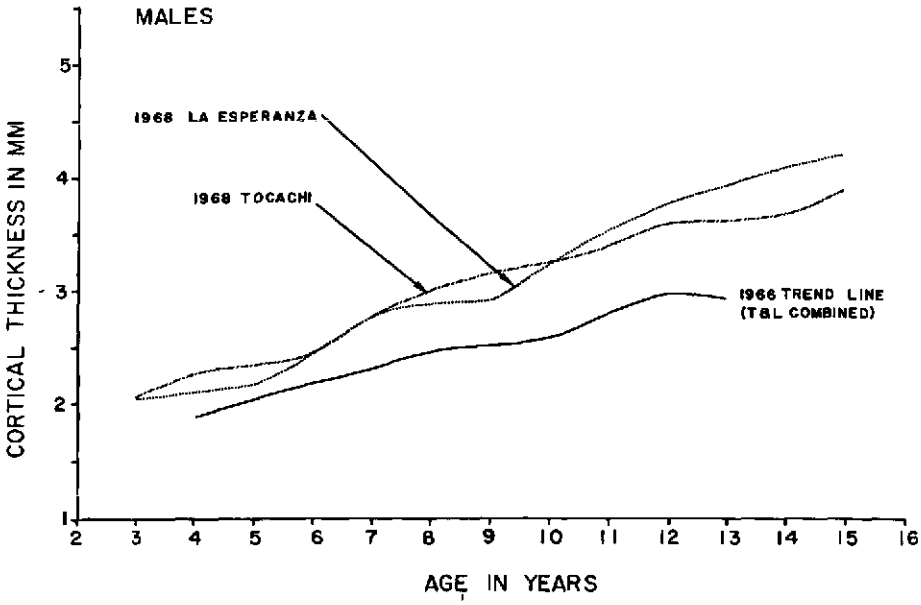

Figure 6. Trend lines generated on the basis of mean cortical thickness at given yearly intervals in each village treated separately in 1968 and matched against combined trend lines from 1966. This clearly demonstrates the gain in cortical thickness in both the control and treated villages.

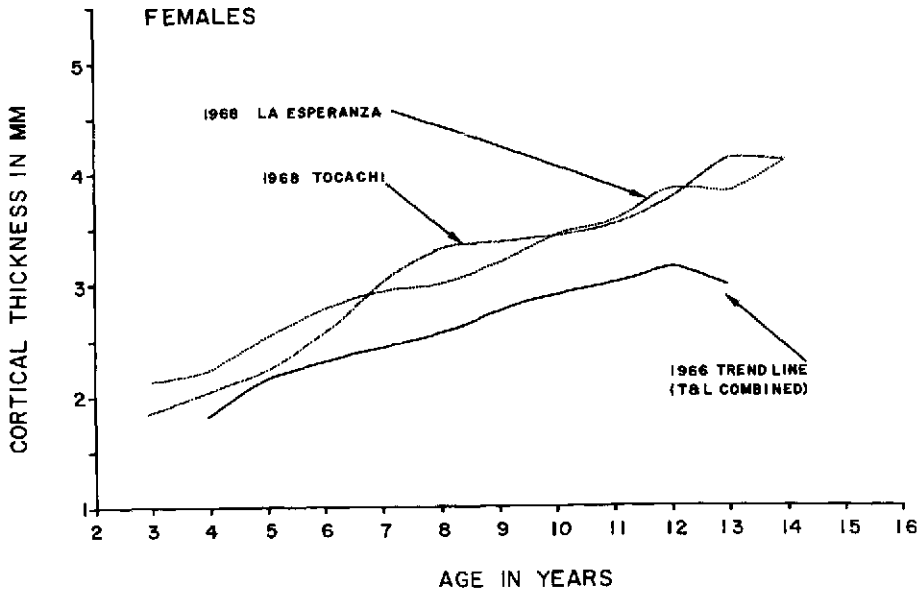

Figure 7. Female trend lines for cortical thickness again showing the gain in cortical thickness for both villages when matched against pretreatment values. The greater amount of cortical thickness amounts to nearly 25 % by age 12 in both village groups.

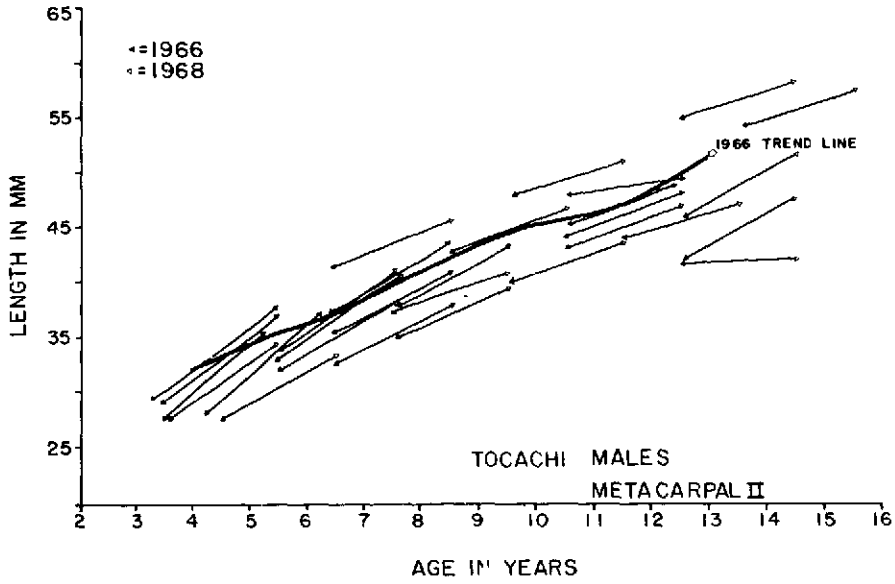

Figure 8. Bone length, handled in precisely the same manner as cortical thickness, on an individual basis fails to demonstrate that the acceleration noted in the transverse diameter also occurs axially. Velocity of linear growth over the two-year period remains unchanged when compared to 1966 values. Among these Tocachi boys, an inordinate number fall below the trend line in both 1966 and 1968. The failure of individual values to randomly distribute occurred in this instance only, and is likely a sampling problem.

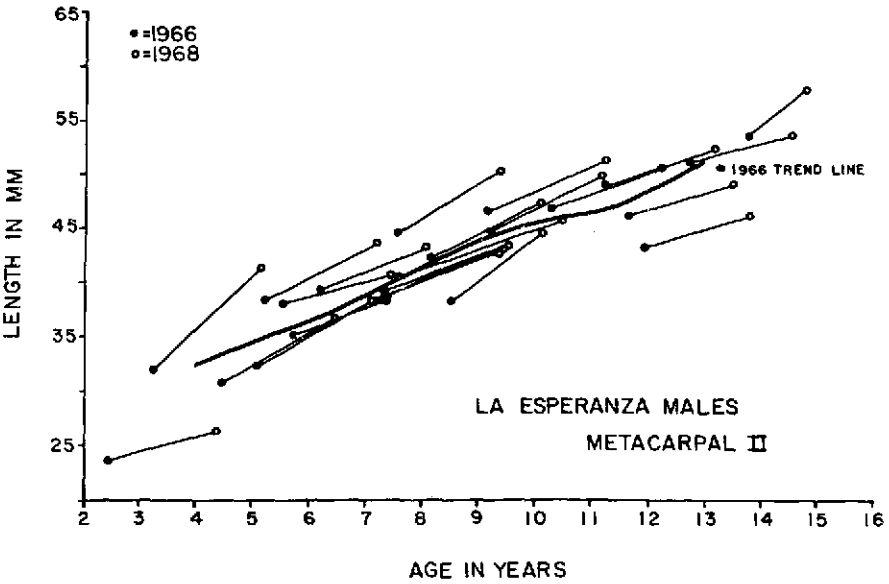

Figure 9. The La Esperanza males also show no increase in the rate of linear metacarpal growth during the two-year treatment period. These similar findings among La Esperanza and Tocachi boys followed for the girls from each village as well.

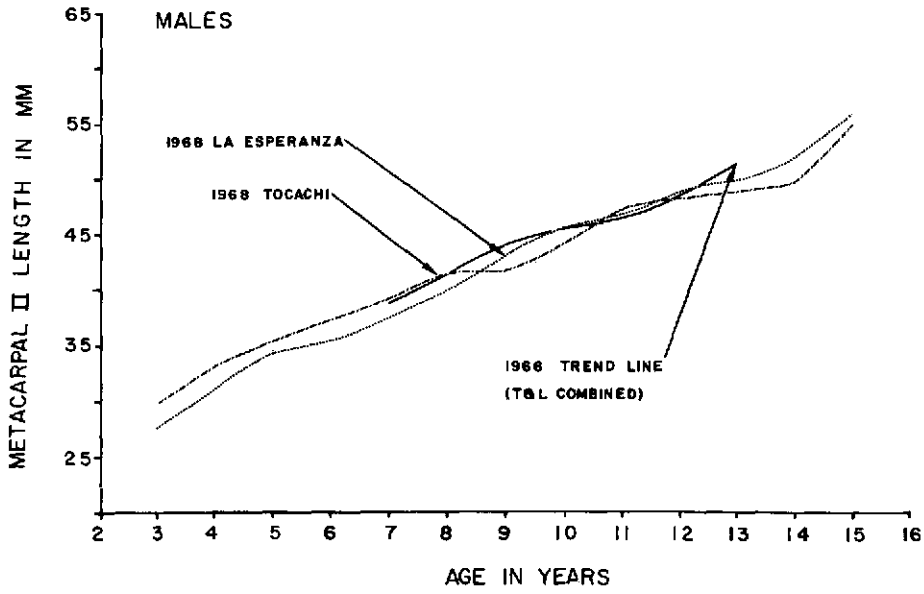

Figure 10. Metacarpal length information treated on a cross sectional basis further demonstrating failure toward marked change axially over the two-year period in both the treated and control groups.

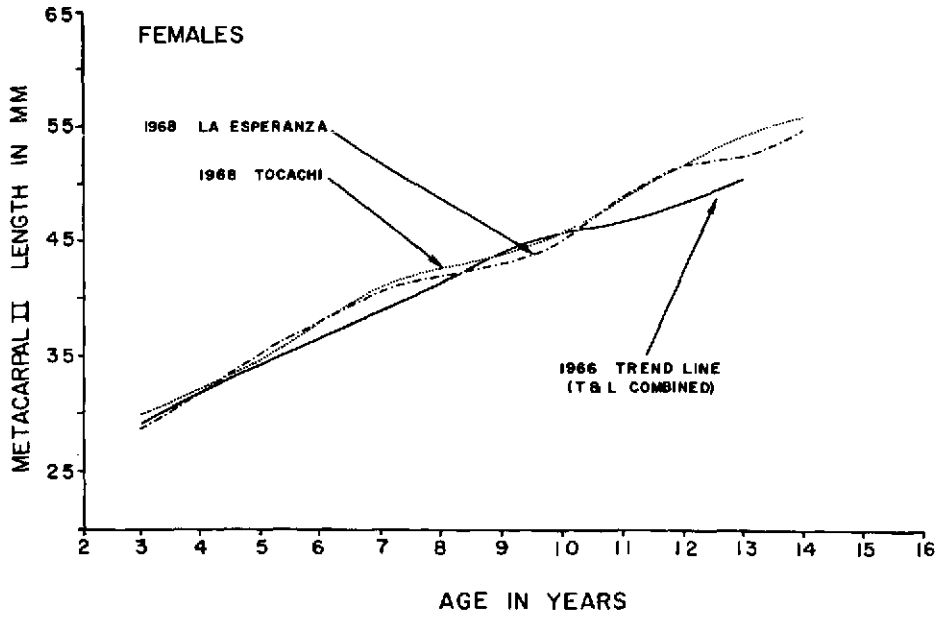

Figure 11. The females also show no acceleration in linear growth of the metacarpal in either village when compared to the combined 1966 pretreatment values.

CRETINS

The hand skeleton among cretins is variably affected, as one would expect from the fact that the gross morphological abnormalities are likewise variable (12). Some of the cretin children surveyed showed maturational delay, while others did not. This can be explicitly demonstrated far more easily than it can be tested for, as shown in Figure 12. The top portion shows 12-year-old Ecuadorians. The hand radiograph on the left of a cretin (A) matches favorably in maturational progress with a representative Ecuadorian noncretin (b) of similar age and sex. Conversely, the cretin on the right (C) is clearly maturationally delayed as attested by overall size and shape of the carpal bones as well as progression toward mineralization and fusion of the epiphyses. The hand films of the two cretins (A and C), besides pointing out the fact that maturation delay is only a sometimes occurrence, also reveal that linear hand growth is retarded in some, but not all. From these films and others, it becomes increasingly more clear that lag in skeletal maturity and retardation of linear growth of the hand-finger bones is a simultaneous situation in cretinism. This is not surprising since others have recognized skeletal maturation and height growth are together delayed in many diseases (11). The final film on this illustration (D) demonstrates extreme maturation delay and size inhibition, if in fact the stated age of 14 years is valid.

The next illustration is a group of adult males; the emerging skeletal pattern of the hand is quite varied (Figure 13). On the left (A) a 25-year-old cretin demonstrates no visible abnormalities of the hand and in fact is not distinguishable by any unusual aspect from the noncretin (B) in the center. But the cretin on the right (C) has a markedly deformed skeletal pattern. The metacarpals appear very short and bulbous on the ends. The phalanges, while shortened, appear less affected than the metacarpals, and the epiphysis of the ulna appears still to be open. All the others seem to be fused, but the lines of demarcation are still prominent. Actually, the cretin on the left is non-dwarfed while the one on the right is dwarfed (132 cm).

In review of the hand radiographs of the cretins, the following pattern seems to emerge. Some cretin children are delayed maturationally and some are not. Those who are, also appear to be slowed in linear growth of the hand. It is likely then that dwarfed cretins are the ones who are delayed maturationally. Among adults an extreme disproportion in size between the metacarpals and phalanges produces a bizarre hand skeletal pattern which appears to be mainly a result of the malformed metacarpals. Again, this abnormality appears restricted to dwarfed cretins. Interestingly, the hand skeletal deformity (metacarpal-phalangeal disproportion) was not observed in any children, but only in adults.

At present chemical studies are not available on these cretins, but the findings of Harrison and others (8) (cf. also Chapter 31) shed some light on the status of the endemic cretins of the Tocachi and La Esperanza areas. They reported that the basal levels of serum growth hormone were in the normal range and insulin hypoglycemia stimulated an exaggerated growth hormone secretion among dwarfed and non-dwarfed cretins, while serum thyroxin concentrations were in the low to normal range.

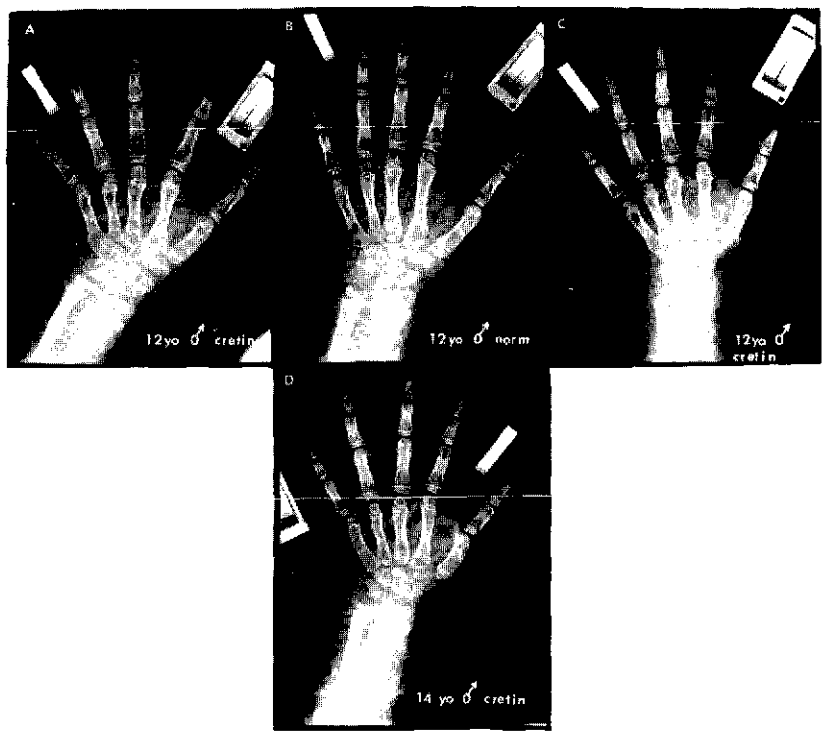

Figure 12. Hand x-rays of four Ecuadorian children of similar chronologic age; three (A, C, D) are cretinous while "B" is a goitrous but non-cretinous individual from the same area. Note that cretin "A" is not delayed maturationally or linearly when compared to the non-cretin while cretin "C" falls behind in both of these parameters. Hand film "D" is of a severely skeletally retarded cretinous individual. The metacarpal-phalangeal disproportion present among some adult cretins is not seen in any of the child examples.

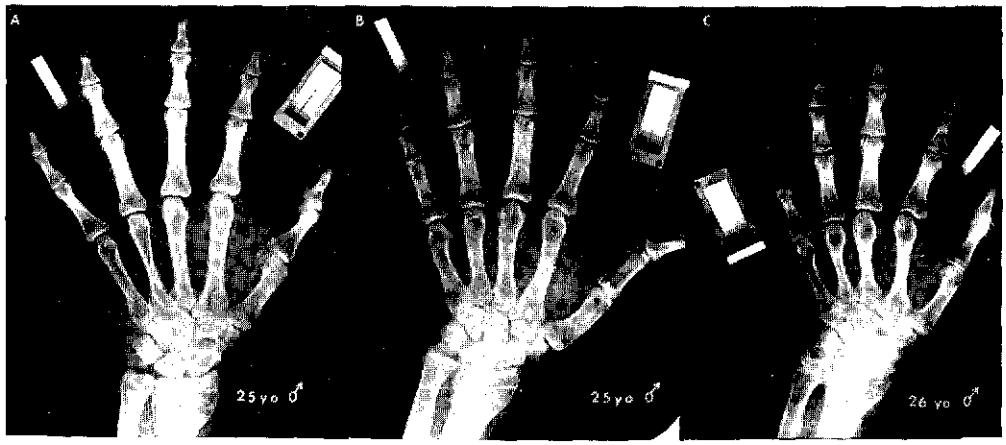

Figure 13. Two adult cretins (A and C) compared with a goitrous non-cretin of similar age (B). It is noteworthy that the hand x-ray on the right (C) displays severe shortening of the metacarpals while the cretin on the left (A) shows hand development comparable to the non-cretin (B). The cretin with the hand deformity is dwarfed while the cretin with normal hand configuration is non-dwarfed.

DISCUSSION

It appears from the findings presented here that the period from 1966 to 1968 has seen important changes in skeletal development among Ecuadorian children who participated in a study of iodine prophylaxis for endemic goiter and endemic cretinism. Not only did the treated village, Tocachi, gain materially, but the control village of La Esperanza has also reaped benefit, and in similar fashion. The positive aspects involve greater than otherwise expected gain in compact bone without appreciable advancement of the children toward skeletal maturity. Certainly, what has occurred for the good is not in any way as dramatic or convincing as "catch-up" seen in treated hypopituitary dwarfs or hypothyroid children, but the beneficial effects are nevertheless evident. An in-the-field nutrition program of this type could not realistically be expected to produce a generalized and clear-cut favorable response of the skeletal system. After all, only one deficient nutrient mineral was supplied. There was no attempt to alleviate any protein (especially meat), calorie, or vitamin deficiency if in fact these inadequacies actually exist.

The next point is that both the treated and control villages responded similarly over the two-year period. No statement of fact can be forthcoming, but there are a number of reasons to explain in part these findings. Both villages, for the first time, have ready access to a medical care facility and a physician, both villages are always responsive to the well-known vacillations of infectious disease of epidemic and nonepidemic proportions, both are agrarian and susceptible to similar climatic aberrations, which could affect the yield at harvest, and finally it is highly possible that socioeconomic progress has finally reached both areas. Lastly, there could be iodine cross-contamination, but this question appears to have been answered negatively (2).

The benefit to the skeletal system of the children of both villages is there, but iodine alone cannot be implicated as the sole agent, since there was a positive effect in both the control and treated populations.

SUMMARY

The Ecuadorian iodination program has been accompanied by demonstrable changes of the hand skeleton during the developmental years of the children in both the treated and untreated villages. This was manifest as a gain in compact bone of the metacarpal over the two-year treatment span begun in 1966. On the other hand, ossification status and linear growth of the metacarpals failed to exceed expectancy.

The apparent change in one respect and lack of change in the others was on a short-term basis. It is only reasonable then to stress that definitive results require an extension of the time period under which the children live supplemented with iodine. The picture of alteration in one parameter and unresponsiveness in two others could drastically change over an extended period of time. The changes could become either more or less profound.

ACKNOWLEDGMENTS

Appreciation is expressed to Dr. John Kevany of the Pan American Health Organization, and to Dr. John Stanbury and Dr. Andrés Querido, PAHO special advisors, for their contributions. The authors wish to thank Dr. Stanley M. Garn, Fels Research Institute, for assistance in data analysis. Appreciation is also expressed to Hellen O'Neil for manuscript preparation and illustrations and Barbara Birch for technical assistance.

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CHAPTER 29

NEUROLOGICAL ASPECTS OF ENDEMIC CRETINISM

Philip R. Dodge, M.D.,[1] Ignacio Ramírez, M.D.[2] and Rodrigo Fierro-Benítez, M.D.[3]

Although it is well known that the function of the nervous system is significantly impaired in endemic cretinism, reports in the literature of systematic evaluations of nervous system functions are few. In particular, formal psychological tests designed to assess mental function critically appear not to have been employed in the best available writings on this disorder. This brief report is concerned primarily with an analysis of the neurologic, including mental, aspects of endemic cretinism as encountered in the highlands of South America.

MATERIALS AND METHODS

Twenty-eight cretins residing in two small neighboring communities in the Ecuadorian Andes were examined. The populations of these two villages, of Quechua and Quechua-European stock, are currently the object of an intensive collaborative study, with amelioration of the symptoms and signs of iodine deficiency, including cretinism, by prophylactic iodine administration being the focal issue in the investigation. There were 11 males (ranging in age from 12 to 50 years) and 17 females (from 6 to 65 years) in the study population. The mean age in the males was 27.2, and in the females, 25.7 years. Better than half of the patients in both groups were between 20 and 50 years of age. Previous studies have shown that the iodine content of the soil and excretion of iodine in the urine of the populace are markedly low and that approximately 80 per cent of the adult population has goiter. Goiter was present in all but five of the patients in this study. Utilizing the international rating scale for goiter, four goiters were classified 0-B; 11 as Grade 1; six as Grade 2; and two as having Grade 3 tumors. All of the Grade 2 and 3 goiters were nodular. The patients were certified as cretins by a team of examiners, on the basis of the concurrence of several symptoms and signs as set forth by Stanbury and Querido (9) and by Choufoer, Van Rhijn, and Querido (5), and as defined by Querido elsewhere in this volume.

All patients were examined neurologically by the authors on at least one and, in many instances, on more than one occasion. Psychological tests

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included items from the Gesell (6), Leiter (7), and Ayres (2) tests. All of the tests employed demanded non-verbal responses and were specifically chosen because of the high prevalence of deafness among the study population.

Various parameters of thyroid function are available for some of the adult subjects included in this report. The values for protein-bound iodine (PBI) were normal and varied from 1.2 to 5.0 μg/100 ml in ten subjects. Serum thyroxine values ranged from 0.5 to 6.0 μg/100. These limited data are in accord with the generally accepted fact that the basic neurological abnormalities in cretinous subjects cannot be correlated with their thyroid status when this is assessed in adult life. The concentration of total protein in the serum was normal (> 7 gm/100 ml.) in every subject.

RESULTS AND COMMENTS

General appearance: Although the appearance of individual subjects varied considerably, all resembled other non-affected members of their communities. The features of some of the older cretins were judged to be excessively coarse, whereas impassive facies characterized the younger subjects; the tongue was prominent in a few subjects only. The hair, however, was remarkably dry and coarse in 22 subjects and judged to be normal in only two; four subjects were not examined for this feature. All cretins were short in stature, 16 adults ranging from 115 to 145 centimeters (3 ft. 10 in. to 4 ft. 10 in.) in height, but there was considerable overlap in height with unaffected members in the communities studied. It was impossible to obtain measurements in several of the cretins for a variety of reasons. The three children whose heights were measured were significantly short for age. Kyphoscoliosis was present in only three of the study population; thus, their short stature could not be ascribed to this deformity. Although head circumference was within normal limits in all but two of the adults in this series, it was approximately one standard deviation below the expected mean. It would appear, therefore, that a small head is not characteristic of cretinism, in spite of the marked impairment in linear growth. In this respect, these findings are at variance with those noted in protein-calorie malnutrition, in which disorder head circumference, although large for linear and ponderal size, is significantly small.

Neurological evaluation: The most dramatic abnormality relates to intelligence. All individuals in the series were markedly defective mentally. Formal tests were utilized in 24 cases. In the 16 subjects examined by the Leiter test, only four, or 25 per cent, scored at the third year level or above. More than half of the adults failed to complete test items normally accomplished by 2-year-old children. In confirmation of this finding was the failure of 35 per cent of the adult cretins to complete the form board item from the Gesell test; this is usually accomplished by the 2-year-old child. The best performance recorded on the Leiter test was at the 5-year level; two adults scored at better than the 4-year level, and one adult obtained a mental age of 3 years. Only one of nine subjects tested completed the Ayres form recognition test at the 2-year level.

Of particular interest is one patient who had not previously been recognized as being mentally defective. Her features were fine, although her hair was coarse, and she had a minor kyphoscoliosis and was deaf and dumb. The severity of her mental defectiveness came somewhat as a surprise as initially

she was not considered to be a cretin by her village associates or by the study team. This suggests that the deafmute population must be scrutinized carefully by formal tests to ascertain whether or not mental deficiency exists.

There is no reason to believe that the poor performance on these various tests by the study population reflected difficulty in communication beyond that which could be explained by their low intelligence. Unaffected individuals in the population studied comprehended the tests and completed them satisfactorily without verbal clues, indicating also that sociocultural factors were not the limiting ones.

Deafmutism: Twenty-seven subjects had impaired hearing and speech. Seventeen appeared to be totally deaf and mute, whereas ten persons had some, albeit slight, hearing. In three subjects there was minimal speech and hearing; a single individual had fairly good speech. In the field it was impossible to carry out highly sophisticated hearing tests and estimates as to the degree of hearing impairment were made by producing various commonly recognized sounds at different intensities. It is possible that the extreme mental retardation may have contributed to the language deficits in our cases, but clearly impaired hearing was primarily responsible. Maldevelopment of both middle and inner ear structures has been described in endemic cretinism. These changes have been reviewed recently by Bargman and Gardner (3) in an article describing the development of histopathologic lesions in the inner ear of chicks rendered hypothyroid during incubation of the fertilized egg.

Motor and reflex function: Some impairment in walking was present in 23 individuals. A stooped attitude with slightly flexed knees and shuffling gait was characteristic of 16 subjects. Two had significant adductor spasm with a tendency toward scissoring in walking; they also walked on tiptoes. Three subjects were unable to stand or walk, and in each of these there were marked flexion contractures at the knees. Difficulty in hopping was universal and none of the several subjects tested could walk tandem. Only four subjects had a reasonably normal gait.

Examination by passive stretch showed evidence of spasticity of the lower extremities in 19 cases; this was marked in two. There was no spasticity elicited in three, and questionable spasticity in two other subjects.

The stretch or tendon reflexes were likewise increased. Sustained ankle clonus was demonstrated in one subject, and unsustained clonus elicited at the ankles in six, and at the knees in one. In ten subjects tendon reflexes in the lower extremities were accentuated, but there was no sustained or unsustained clonus. In only two of the 20 cases examined were the tendon reflexes judged to be normal. It is interesting that in two instances reflexes were increased at the knees and markedly decreased or absent at the ankles, suggesting the possibility of an associated peripheral neuropathy of undetermined cause.

It was difficult to elicit plantar responses because of the extreme callus formation of the soles, a condition attributable to the absence of shoes. Nevertheless, a Babinski or Oppenheim response was demonstrated in nine subjects, and a questionably extensor or no response to plantar stimulation was found in an additional nine cases. In only two subjects was the plantar response clearly flexor. Accentuation of reflex activity and of specific motor involvement

of upper limbs was suggestive in the majority of these subjects although, admittedly, a quantitative assessment of this was difficult.

It is evident that the vast majority of subjects had pyramidal tract dysfunction involving predominantly the lower extremities. The level of the responsible central nervous system lesion cannot be stated with accuracy because a spastic diplegia can result from lesions of spinal cord and of various levels of brain. The absence of clear-cut sensory changes although, admittedly, careful sensory examinations were impossible, and of bladder dysfunction would point away from the spinal cord and suggest a cerebral locus of disease.

A general clumsiness in movement beyond that attributable to the pyramidal tract disease was also noted in the majority of subjects. In four subjects cerebellar dysfunction might have contributed, as there was a slight intention tremor, but in 14 subjects there was no evidence of tremor. Thus, cerebellar involvement would not appear to be the explanation for the general disturbance in coordination. In all probability, diffuse affection of the central nervous system was responsible. In this regard it is appropriate to mention that even today the neuropathology of cretinism remains surprisingly obscure considering the long history of this disorder and the magnitude of the clinical problem. The state of our knowledge is well summarized by Adams and Rosman (1): "The inconsistency of these observations (referring to the histopathologic findings in cretinism) serves to emphasize the considerable doubt that exists as to whether the light microscope has successfully demonstrated any reliable brain lesions in hypothyroidism, even when cretinism has been accompanied by life-long idiocy."

Clearly, a critical study of the neuropathology and chemical anatomy of the brain of cretins utilizing present-day techniques could provide important clues to our understanding of this disorder. Certain abnormalities in structural organization of the nervous system are known to occur only at certain periods of prenatal development. If, for example, faulty migration of nerve cells, as evidenced by a disordered relationship among cortical neurones and heterotopias were found, one could state with confidence that the nervous system had been damaged during the early weeks of prenatal life. Such abnormalities in brains of retarded patients with muscular dystrophy have been described recently (8). On the other hand, faulty myelination or retarded growth and elaboration of neuronal processes would indicate that the adverse influence was exerting its effect upon the nervous system at a later period in gestation or during early postnatal life. The experimental studies of Eayres and his associates (4) have shown that hypothyroidism induced during the neonatal period in rats does, in fact, result in a paucity of axodendritic connections and more densely packed neurones. Although not proved, it seems most probable that the defects in cretinism arise as a consequence of fetal and early life hypothyroidism. Still the possibility must be admitted that some other associated, and as yet unknown, factor is operative.

SUMMARY

Significant neurological impairment was common to all subjects with endemic cretinism studied. Mental deficiency of a very severe degree was universal. A spastic diplegia of variable severity and probably of cerebral origin was found in the majority of cases. All subjects had significant

hearing impairment and most were mute. The concordance, without exception, of mental defect and deafmutism suggests that all patients with deafmutism in endemic goiter areas should be examined with formal intelligence tests. The techniques used in this study, although not free of cultural bias, appear to be useful, and their further application is planned.

ACKNOWLEDGMENTS

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CHAPTER 30

EFFECT ON INTELLIGENCE OF IODINE IN OIL ADMINISTERED TO YOUNG ANDEAN CHILDREN—A PRELIMINARY REPORT

Philip R. Dodge, M.D.,[1] Helen Palkes, M.A.,[2]
Rodrigo Fierro-Benítez, M.D.,[3] and Ignacio Ramírez, M.D.[4]

Evidence accumulated to date from studies in several regions of the world where iodine deficiency goiter and cretinism are endemic indicates that the administration of iodine in oil results in a dramatic reduction in the prevalence and size of goiter in the population. Early results also suggest that iodine prophylaxis in women of childbearing age may prevent the development of cretinism. An issue requiring resolution is the possible effect on intelligence of iodine administered to children at various ages after birth. The present study is concerned with this question, and although the results are preliminary and inconclusive they underscore the need for critical study of this problem.

SUBJECTS AND METHODS

The Goodenough Draw-A-Man test was administered to more than 100 first and second grade boys and girls living in the villages of Tocachi and La Esperanza, Ecuador, in an attempt to establish a baseline of intelligence for children in an endemic goiterous region of the Ecuadorian Andes. This test, introduced about 40 years ago by Goodenough (1) and modified in recent years by Harris (2), has been used successfully throughout the world in many populations of different sociocultural backgrounds. Recent studies have demonstrated its usefulness in children of Indian and Indian-European cultures in the Western Hemisphere (3). Because of the non-verbal character of the test and its ease of administration, it is readily applicable to large populations of schoolchildren experienced with the use of pencil and paper and not unfamiliar with their use in drawing. Our subjects, who varied in age from 6 to 10 years, had been enrolled in school for a few months to about four years. They represented a majority of school-age children within the ages studied and varied from good to poor students. No cretins were included and clinical evidence of hypothyroidism was lacking in these children.

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4/ Research Fellow, National Polytechnic Institute, Quito.

All of the children in Tocachi had been injected with iodized oil two years earlier, whereas iodine had not been administered to those in La Esperanza. The inhabitants of these two villages were similar in other ways, including ethnic origins, socioeconomic status, and prevalence of goiter. Details regarding these communities and the iodine prophylaxis program are described elsewhere in this volume by Fierro Benítez et al. (cf. Chapters 26 and 27)

RESULTS

The initial hypothesis was that the iodine treatment of the children of Tocachi two years earlier at ages 4-8 years would not modify brain function and thus, scores obtained on the Draw-A-Man test. However, since there was this obvious difference, a comparison of Draw-A-Man results was made between the two populations. Table 1 presents a comparison of the chronologic age, mental age, and intelligence quotients for females in the two villages. There was no difference in chronologic age, but the girls in Tocachi scored significantly higher than did those in La Esperanza with regard to mental age and intelligence.

Table 1. Comparison of mean intelligence estimated from Draw-A-Man test in females 6-10 years of age.

	Tocachi n = 26	La Esperanza n = 20	t	p Value
Chronological age - months	92.23	96.40	1.18	n.s.
Mental age	90.34	81.70	2.85	<0.01
Intelligence quotient	97.38	84.50	3.62	<0.001

In the male population (Table 2) the chronologic age was also identical but again, mental age was significantly higher in the Tocachi group. The mean intelligence level in Tocachi was also higher although the differences were not significant when a two-tailed test was employed. When, however, a one-tailed test was used, based on the assumption that iodine prophylaxis would improve the performance, then highly significant differences were obtained for intelligence as well.

Table 2. Comparison of mean intelligence estimated from Draw-A-Man test in males 6-10 years of age.

	Tocachi n = 25	La Esperanza n = 25	t	p Value
Chronological age - months	89.84	89.16	0.234	n.s.
Mental age	92.50	83.88	2.10	<0.05
Intelligence quotient	102.92	94.24	1.69	n.s.

No obvious differences in overt behavior or in well-being between the children in the two villages were noted.

COMMENT

The defects in this study are obvious. We have assumed that pretesting of children from the two villages would have yielded no differences because of the apparent homogeneity of the two populations. Furthermore, the size of the study groups was small and only a single test instrument was used. Nevertheless, the results are of considerable medical interest for they admit the possibility that the cerebral function of children in endemic goiter regions may be influenced beneficially from iodine administration even when treatment is given as late as 8 years of age. Further studies which include testing of subjects before iodine administration, the use of additional testing instruments, and a larger study population are planned. If future studies confirm these preliminary results, then the implications for future prophylaxis programs are significant.

ACKNOWLEDGMENTS

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CHAPTER 31

GROWTH HORMONE IN RELATION TO ENDEMIC CRETINISM AND DWARFISM

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Since the introduction of a sensitive radioimmunoassay capable of detecting Immunoreactive Human Growth Hormone (IR-HGH) in plasma, basal levels of IR-HGH and response to I.V. insulin or arginine (32) infusion have been investigated in several conditions associated with disturbances of growth. IR-HGH has been studied in ateliotic dwarfs (26, 36, 37) and African pigmies (37) as well as in patients with hypopituitarism (15, 38), myxedema (38), thyrotoxicosis (4), Turner's Syndrome (8, 23, 38), Down's Syndrome (38), cystic fibrosis (18), diabetes insipidis (38), "genetic pituitary dwarfism" (21), and constitutional delay in growth and development (38). We have studied the IR-HGH response to insulin in endemic cretins of rural Ecuador and present our experiences in terms of the larger unanswered problems of human pituitary growth hormone physiology and its role in metabolism and growth.

CRETINISM AND HGH

Although endemic cretinism has never been rigidly defined, short stature, mental deficiency, defects in speech and hearing, motor abnormalities, a characteristic appearance, and a geographic relation to endemic goiter are all descriptive of it (5, 7, 9, 11, 12, 41). These features may be present in varying combinations and severity. A high incidence of these defects associated with severe endemic goiter and documented iodine deficiency is found in isolated villages in the Andes of Ecuador (11, 12), as well as in other well studied areas of Uele (the Congo) (9) and New Guinea (5). Defects of endemic cretinism are probably related to thyroid hormone deficiency in intrauterine or early neonatal life (5, 9, 41).

Dwarfism, abnormalities of sella contour, and several interesting observations on interrelationships of growth hormone, dwarfism, and thyroid function

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suggest an etiologic role for pituitary malfunction in endemic cretinism. IR-HGH levels are elevated and response to insulin is exxaggerated in the first 48 hours after birth, and Human Growth Hormone (HGH) undoubtedly plays an important role in subsequent growth (6). Isolated HGH and other pituitary trophic hormone deficiencies have been demonstrated in various forms of growth retardation and dwarfism (17, 36, 38). On the other hand, normal IR-HGH levels and response to insulin have been demonstrated in African pigmies (37). Since elevated levels have been found in hereditary dwarfs who responded to exogenous HGH (21) the question of a genetically determined but biologically altered protein hormone or of target organ resistance (21, 33, 37) has arisen.

Changes in growth hormone secondary to hypothyroidism have been demonstrated. The pituitaries of hypothyroid rats show degranulation of acidophils, and the electrophoretic bands corresponding to growth hormone in pituitary extracts are lost (22). The IR-HGH response to insulin-induced hypoglycemia is reduced in magnitude (3, 19, 38) and possibly delayed (20) in primary hypothyroidism in man. Both effects become reversed after treatment with thyroid hormone. With these observations in mind we have measured serum IR-HGH, cortisol levels and the responses to insulin-induced hypoglycemia in a group of dwarfed cretins in Ecuador as a sensitive test of pituitary function (15, 20).

POPULATION

The subjects of the study reside in Tocachi and La Esperanza, Ecuador, two isolated, primitive, mountain villages (altitude 2,952 meters) of 750 and 1,198 Indian and Mestizo persons, respectively. They are three miles apart and 40 miles north of Quito in the Province of Pinchincha in the north central region of the country.

The villagers are impoverished and highly inbred. Most of the adults are illiterate. They live and die in their villages, rarely venturing beyond their region. The diet staples are corn and potatoes grown on small plots of land around mud huts. Meat is eaten once or twice monthly. Most of the villagers have short stature; only a rare one reaches 165 cm (65 in).

The low iodine content of local salt (0.24 µg per gram) and water (0.85 to 1.00 µg per liter) and low urinary excretion of I^{127} (17.35 and 22.27 µg per 24-hour period in Tocachi and La Esperanza, respectively) are correlated with a high incidence of endemic goiter and endemic cretinism. In Tocachi, the incidence of endemic goiter is 54.5 per cent and in La Esperanza, 51 per cent; the incidence of mentally deficient, deafmute cretins is 7.4 and 5.5 per cent respectively. Severely dwarfed, mentally deficient, deafmute cretins account for 0.8 per cent of the population in Tocachi and in La Esperanza, for 0.5 per cent. Seventeen months prior to this study all inhabitants of Tocachi received iodized oil intramuscularly as part of another study designed to ascertain its prophylactic effect on endemic goiter and cretinism.

SUBJECTS

Basal blood samples for the determination of IR-HGH were obtained from 20 villagers. The study group was comprised of 14 cretins (six of whom were severely dwarfed), five indigenous "normals," and one non-cretin dwarf. At

least one blood sample was obtained from 16 of the 20 subjects after administration of I.V. insulin. The superstitions of a primitive people never before accustomed to medical attention proved to be considerable obstacles to a test requiring multiple blood samples.

Deafmutism was assumed when the subject could not hear (as determined by history), gave no response to sound, and did not talk. Mentally deficient subjects could not attend school. They did only manual household or field work, had difficulty placing shaped blocks in appropriate slots, and performed poorly or not at all on draw-a-man tests. The spectrum of deficiency was wide.

The stigmata of endemic cretinism may be present in the individual patient in varying combinations and severity. It has been claimed that short stature is a variable rather than a necessary feature of the disorder (5, 7). All the cretins of this report were severely affected deafmutes and mentally defective. They had the characteristic appearance of cretinism as well as short stature. Six severely dwarfed, typical cretins (130 cm or less) were chosen as extreme and unequivocal examples. Anthropometric data on the subjects appear in Table 1.

METHODS

The patients were transported from their huts to a small dispensary early in the morning before eating or exercising. They were allowed to lie quietly for one to two hours. After a basal venous blood sample was drawn, crystalline insulin (0.15 units per kg body-weight) was injected I.V. through the same needle. Additional timed samples were drawn from the anticubital vein as circumstances permitted. Signs of hypoglycemia appeared in 14 of the 20 subjects; two reactions (Subjects 2 and 13) were severe enough to require termination by I.V. administration of glucose.

Serum from venous blood samples was pipetted into lyophilization flasks, sealed, and immediately frozen. The samples were lyophilized in Quito, and the volumes reconstituted in Boston. Control experiments showed that growth hormone, thyroxine, and cortisol are stable to lyophilization. Glucose determinations by the Folin Wu micro-method, initiated in the field and read in Quito, proved unsatisfactory and are therefore not reported.

The concentration of IR-HGH was measured on duplicate serum samples at several dilutions (at least two), using a charcoal-dextran radioimmunoassay (42). Human Growth Hormone (NIH HS 968C) served for radioiodination and as a standard. Eight of the samples were also analyzed for IR-HGH content by Dr. Peter Sonksen at the Joslin Clinic in Boston, using a double-antibody assay method (1). Absolute values agreed within 20 per cent and are three-and-a-half to four fold lower than values previously reported from our laboratory in Boston (42).

Serum thyroxine levels were determined on aliquots of combined pre- and post-I.V. insulin samples from the same individual by the competitive protein binding method (27) (normal range, 4 to 11 μg per 100 ml). Serum cortisol levels were determined by New England Nuclear Biomedical Assay Laboratories, Boston, utilizing a double isotope technique (normal range, 5 to 25 μg per 100 ml).

Table 1. Anthropometric data on study patients.

	Subject	Age	Sex	Height (cm)	Weight (kg)	Goiter*	Treat-ment+	Remarks
Group I# (≤ 130 cm)	1	40	F	113	31.8	II	P	Typical dwarfed cretin
	2	22	M	95	30.4	0	P	Typical dwarfed cretin with severe contractures
	3	24	M	130	37.3	0	P&E	Cretin improved with Proloid therapy
	4	~17	M	103	20.4	0	E	Inadequate stimulation; values excluded from mean
	5	25	F	121	35.4	0	E	Test stopped at 30 minutes; values excluded from mean
	6	~40	F	123	41.5	III	P	Typical dwarfed cretin with severe contractures
Mean		23.0		114.2	32.8			Values from Subjects 4 and 5 excluded
± S.E.		3.9		5.4	2.98			
Group II** (> 130 cm)	7	32	F	147	52.3	I	E	Had "normal" child
	8	~20	F	~141	41.8		E	
	9	~60	F	~150	45.8	0	E	Had "normal" children; goiter before Ethiodol
	10	38	F	~142	41.8	II	E	Had "normal" child
	11	26	M	138.5	39.5	0	E	
	12	~40	M	144.5	48.7	III	E	Brother of Subject 15
	13	29	M	142.5	40.5	0	E	Test stopped due to hypoglycemia
	14	17	F	143	45.5	0	P	Has 2 cretinous, deafmute sisters; basal sample only
Mean		32.8		143.6	44.5			
± S.E.		4.8		1.3	1.57			
Group III Indigenous "normals"	15	~50	M	139.5	45.0	0	E	Lives in isolation with cretinous brother (Subject 12)

Table 1. Anthropometric data on study patients (continued).

	Subject	Age	Sex	Height (cm)	Weight (kg)	Goiter*	Treat-ment+	Remarks
	16	33	M	~138	47.3		E	Short, due to hunch back; Pott's disease of spine
	17	20	M	~160	63.6		0	Febrile illness; ? insulin dosage miscalculation
	18	28	M	~154	56.8		E	
	19	15	M	152	45.0	0	0	Mother stopped test
Mean				148.7	51.5			Values from Subject 17 excluded
± S.E.				4.2	3.7			
Non-cretinous dwarf	20	18	M	135	39.1	0	0	Intelligent dwarf without testes in scrotum; brother of Subject 17

* 0 = Thyroid not enlarged; I = Enlarged on palpation; II = Visibly enlarged; III = Grossly enlarged (visible from a distance).

\+ P = Proloid (approximately 260 mg. per week); E = Intramuscular Ethiodol (iodine in oil) 17 months previously; 0 = No medication.

\# Severely dwarfed, deafmute, and mentally deficient cretins.

** Deafmute and mentally deficient cretins.

RESULTS

The findings from the 20 subjects appear in Table 2 and Figure 1. The cretins are subdivided into two groups: Group I consists of the severely dwarfed cretins 130 cm or less in height (mean, 114.2 cm) and Group II of those taller than 130 cm (mean, 143.1 cm). The subjects in Group III had no external appearance of cretinism and were not deafmute or mentally deficient. One, a non-cretinous, intelligent dwarf, was not included in these three groups.

The 20 patients had normal basal IR-HGH levels. The level was undetectable (< 0.25 mμg per ml) in four. IR-HGH was detected, either before or after insulin, in all 20 subjects. The highest basal value, 6.0 mμg per ml, was found in Subject 5, who was a severely dwarfed, cretinous female. Of the 15 subjects from whom blood samples for IR-HGH were obtained 52 to 93 minutes after administration of I.V. insulin, 13 showed at least a five fold increase from basal levels. Subject 5, who had the highest basal level (6.0 mμg per ml), had a two fold increase in IR-HGH in a single sample drawn 30 minutes after the administration of insulin, before her test had to be terminated. Subject 4 showed neither an IR-HGH nor a cortisol response. The reason is not known, but failure to obtain adequate stimulation seems likely. Subjects 17 and 20 were brothers (tested simultaneously) in whom there was a possibility of insulin dosage miscalculation. The normal brother (Subject 17) had a modest IR-HGH response. The non-cretinous, intelligent, dwarfed brother showed a minimal cortisol response (increment 5.0 μg per 100 ml) and a markedly impoverished IR-HGH response (increment 2.2 mμg per ml). The values of IR-HGH from these subjects (Subjects 4, 5, 17, and 20) were therefore excluded in the computation of the means for each group.

The impossibility of obtaining regularly timed blood samples made accurate evaluation of the time of the growth hormone response impossible. Usually, only one sample could be obtained after insulin administration. It is impossible to know whether the levels were rising or falling at that point. Figure 1 indicates that isolated, post-insulin values were greatly increased over baseline values. One response (that of Subject 7) increased between 63 and 93 minutes; another (from Subject 1) fell between 68 and 92 minutes.

The majority of IR-HGH values after insulin administration were high in all three groups when compared to values obtained in our laboratory on 22 subjects from the United States, who did not show evidence of endocrine disorders 60 minutes after administration of insulin intravenously (mean, 9.3 ± 6.7 mμg IR-HGH per ml; range, 0.6 to 31.0). The highest IR-HGH value after insulin stimulation (78.0 mμg per ml) was obtained from the single patient (Subject 11) who also had the lowest serum thyroxine level (3.3 μg per 100 ml).

Initial cortisol levels were all within normal limits (5 to 25 μg per 100 ml) and increased significantly after insulin administration in 7 of the 8 subjects on whom this determination was made. Only Patient 4 failed to show a rise in serum cortisol concentration.

Thyroxine concentration was in the lower range of normal in 19 of the 20 subjects (normal range, 4 to 11 μg per 100 ml calculated as thyroxine). Only one patient had an abnormally low value of 3.3 μg per 100 ml.

Table 2. Serum thyroxine, cortisol, and growth hormone in study patients.

	Subject	Serum thyroxine (μg/100 ml)	Serum cortisol* μg/100 ml before	after	Serum IR-HGH mμg/ml before	after
Group I+ (≤ 130 cm.)	1	5.7	11.0	18.2	2.5	58.0
	2	6.6	8.2	15.6	0.7	31.0
	3	5.0	--	--	0.4	46.0
	4	7.1	10.2	9.4	0.3	1.0
	5	7.2	--	--	6.0	13.5
	6	5.8	--	--	0.7	--
Mean		6.2	9.8	16.9	1.8	45.0
± S.E.		0.3	0.8	1.3	0.9	7.8
Group II# (> 130 cm)	7	4.1	7.2	16.4	0.25	52.0
	8	7.1	--	--	0.25	9.2
	9	5.2	--	--	3.6	18.6
	10	4.5	--	--	0.3	38.0
	11	3.3	--	--	0.25	78.0
	12	7.0	--	--	0.3	26.0
	13	5.8	--	--	3.6	--
	14	4.5	--	--	0.8	--
Mean		5.2			1.1	37.0
± S.E.		0.5			0.3	10.2
Group III Indigenous "normals"	15	7.9	11.2	16.2	0.3	24.6
	16	8.4	9.1	18.3	0.7	66.0
	17	6.2	--	--	0.8	16.2
	18	6.4	4.2	16.1	0.25	23.8
	19	4.8	--	--	0.8	--
Mean		6.7	8.1	16.9	0.5	38.1
± S.E.		0.6	1.9	0.7	0.2	13.9
Non-cretinous dwarf	20	4.6	11.2	16.2	1.3	3.5

* "Before" refers to basal values; "after" refers to peak values post-I.V. insulin. Timing of post-insulin samples was not uniform. Maximum values only are given for Subjects 1, 2, and 7, who had more than one post-insulin sample.

\+ Severely dwarfed, deafmute, and mentally deficient cretins.

\# Deafmute and mentally deficient cretins.

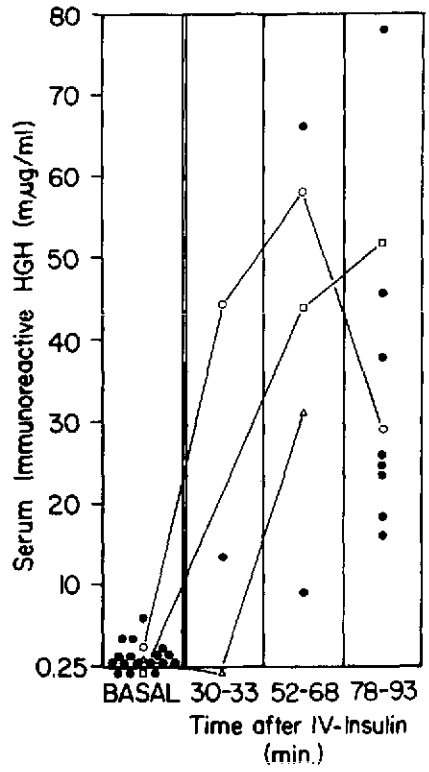

Figure 1. IR-HGH response after 0.15 units per kg insulin administered intravenously. Black dots represent values in subjects on whom only one sample was obtained after insulin. Several post-insulin samples were obtained on three subjects: Subject 1 = o; Subject 2 = Δ ; Subject 17 = □ . Values for Subjects 4 and 20 are omitted.

Some cretins may have low thyroid hormone concentrations in the blood. One such patient from La Esperanza (not a subject for insulin IR-HGH assay) was a severely retarded cretin with a huge multinodular goiter (Figure 2). A severe kyphoscoliosis and spastic diplegia contributed to her short stature (111 cm). Plasma thyroxine was 0.4 μg per 100 ml. Her 16-month-old infant was severely retarded and had a plasma thyroxine level of 0.7 μg per 100 ml. The father of this infant was not known. The maternal grandfather of the infant was 143 cm high and had a large nodular goiter and a plasma thyroxine level of 2.7 μg per 100 ml; the maternal grandmother was 134 cm high, had a large nodular goiter also and a plasma thyroxine level of 0.8 μg per 100 ml. Fasting ambulatory serum samples contained detectable IR-HGH (0.68 to 4.0 mμg per ml) in all four members of this family.

No significant differences were found in mean thyroxine, cortisol, and IR-HGH or in the response of cortisol or IR-HGH to insulin-induced hypoglycemia among the two groups of cretins and normals.

Figure 2. Severe cretinism in La Esperanza. This patient, both parents, and a child were hypothyroid by plasma protein-bound iodine concentration.

DISCUSSION

This study reveals that endemic cretins and others in the severely endemic, goitrous villages of Tocachi and La Esperanza have normal basal levels of serum IR-HGH and respond to insulin-induced hypoglycemia with ample secretion of IR-HGH. This indicates an intact hypothalamic--anterior pituitary--growth hormone pathway. Thus, there is no evidence to implicate primary pituitary malfunction in the etiology of the short stature and associated defects of the endemic cretins of Tocachi and La Esperanza. It should be kept in mind that the patients were not considered hypothyroid by chemical analysis when they were tested (mean serum thyroxine level: 6.0 μg per 100 ml). Four typical dwarfed cretins had received proloid, and most of the others (all those from Tocachi) had received iodized oil 17 months before our study. It is, of course, impossible to rule out an abnormal growth hormone response to thyroid deficiency in early life.

Whether chronic thyroid deficiency in Tocachi and La Esperanza can alone account for the general shortness of stature as well as the severely dwarfed cretins is not known; the long-range study which is in progress there may provide an answer to this question.

In terms of elucidating the physiology of HGH and its role in growth and development this study on IR-HGH response to insulin in endemic cretins has several limitations. As in all immunologic assays, immunoreactivity is not necessarily synonymous with biological activity. Normal or high levels of IR-HGH and responses to insulin in endemic cretins, African pigmies (37), and hereditary dwarfs (21, 33) may not reflect biologically active HGH. Moreover, isolated levels of IR-HGH are not adequate for evaluation of pituitary HGH function because IR-HGH levels flucuate widely with such factors as time of day, activity, nutritional status, relation to recent meal (13, 15, 29, 31). A more meaningful evaluation would be a measurement of total daily secretion of IR-HGH. This information might be obtained from HGH turnover studies or from measurement of urinary IR-HGH excretion (40).

Since basal IR-HGH levels are often undetectable, tests which stimulate secretion of HGH are often helpful. IR-HGH response to insulin-induced hypoglycemia can be different from IR-HGH response to amino acid (arginine) infusion; some patients suspected of HGH deficiency because of failure to respond to one may respond to the other (34).

There is reason to believe that HGH has different functions in childhood and adulthood (6). Adequate IR-HGH levels and responsiveness during adulthood do not disprove early childhood abnormalities. Disturbances in growth are intimately related to hereditary, nutritional, and endocrine factors. The influence of a single endocrine such as HGH cannot be evaluated outside the context of these inadequately understood interrelationships. It is possible that genetic factors play a role in the short stature of the cretins and villagers in the highly inbred populations of Tocachi and La Esperanza. It has been said that iodine deficiency and hypothyroidism rarely give rise to endemic cretinism until several generations have inbred. While general nutritional factors may play a major role in disturbances of growth in Tocachi and La Esperanza, elevated basal levels of IR-HGH reported in protein malnutrition states (30) were not detected in our subjects.

Table 3. Outline of responses to insulin and arginine in disturbances of growth and metabolism.

Condition and reference	Response to I.V. insulin		Response to I.V. arginine	
	Blood sugar	IR-HGH	IR-HGH	IRI
Normal subj. (10,13, 15,25,26,32,37)	N Nadir at ~ 30 min; return by ~90 min	N Peak > 5-fold; rise at ~60 min	N Peak > 5-fold; rise at ~60 min	N Peak ~ 80 mu/ml. at ~30 min
Hypopituitary dwarfs (16,28,38,39)	N or prolonged hypoglycemia	0	0	-
Ateliotic dwarfs (26,36,37)	Prolonged hypoglycemia	0	0	↓
Ateliotic dwarfs (treated with HGH)(33)	-	-	-	-
African pigmies (37)	Prolonged hypoglycemia	N or ↑	N	↓
Hereditary and some proportionate dwarfs (33)	Prolonged hypoglycemia	↑	↑	↓*
Hereditary and some proportionate dwarfs (treated with HGH)(33)	-	-	-	↓*
Ecuador cretins	-	N or ↑	-	-
Myxedema (3,19,35,38)	Prolonged hypoglycemia	↓	↓	N or ↑
Diabetes mellitus (10, 13,14,24)	-	-	↓	↓
Diabetes mellitus (treated with HGH)(10)	-	-	-	N

N - Normal response.
0 - No response.
↑ - Higher than normal in magnitude.
↓ - Lower than normal in magnitude.
* - IRI response in one proportionate dwarf.

The changes in Immuno-Reactive Insulin (IRI) and IR-HGH which follow infusion of amino acids have yielded interesting information relative to the problems of HGH physiology (10, 14, 24-26, 32-35). The responses of serum glucose and IR-HGH to I.V. insulin and the responses of IR-HGH and IRI to arginine stimulation in various disturbances of growth and metabolism appear in outline form in Table 3.

The IRI and IR-HGH responses to arginine infusion are especially interesting. It has recently been demonstrated that patients with diabetes have decreased IRI responses (2, 13, 14, 24) and that these return to normal after treatment with HGH (10). The magnitude of the IRI response to arginine stimulation is diminished in ateliotic dwarfs (26) but substantially improved after treatment with exogenous HGH (33). Like ateliotic dwarfs, African pigmies have a prolonged hypoglycemic response to exogenous insulin and a decreased IRI response to arginine or glucose, but normal IR-HGH (37). The same prolonged hypoglycemia and decreased IRI have recently been demonstrated in a proportionate dwarf with elevated, non-suppressable levels of IR-HGH; pretreatment with HGH did not increase IRI response to glucose load (33). In both the African pigmies and this proportionate dwarf the question of an abnormal HGH molecule or tissue unresponsiveness has been raised. It is possible that the IRI response to arginine is dependent on the presence of biologically active HGH. However, one of the authors (S.R.) studied six severely myxedematous adults who had normal or exaggerated IRI responses to arginine (35).

The factors influencing the changes of IR-HGH and IRI that follow arginine infusion are intriguing. Clarification of the complex functions of these hormones in the regulation of metabolism may define their roles in the physiology and pathology of growth and development.

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SECTION VIII

ENDEMIC GOITER IN MEXICO

CHAPTER 32

ENDEMIC GOITER IN MEXICO AND ITS CHANGING PATTERN IN A RURAL COMMUNITY[1]

Jorge A. Maisterrena, Enrique Tovar, and Adolfo Chávez

It has been said that endemic goiter appeared in America after the Spaniards came to this continent (8, 18), but in our opinion it was here long before. It seems more probable that certain secondary etiologic factors have influenced its severity. Living conditions changed radically after the Spanish conquest. Many of the natives found themselves in abject misery and poverty, or in slavery, or moved into remote areas, or went to work in the mines. Even now, some of the most affected zones of endemic goiter are those of mining towns.

Goiter in Mexico was known before the fifteenth century. The Aztecs called it "Quechpezahuailiztli" and the Mayas "Pjadsisi" (19). Endemic goiter in Mexico was not adequately studied until the end of the nineteenth century, when Orvañanos (17) presented the first map with the geographic distribution of goiter in the country. This was obtained by inquiring from the medical authorities the prevalence of goiter in different rural zones. Castillo Najera (2) continued this type of work, and in 1930 Darío Fernández and his group (7) obtained wider information about the distribution of goiter. His student, Martínez Catalán (16) surveyed the State of Puebla in 1931, and Jiménez (10) published his survey of the State of Oaxaca in 1932. These studies showed the magnitude of the endemicity in the highlands.

In 1937 Herbert Stacpoole (20) became chief of the "National Campaign against Endemic Goiter." As a result of personal surveys in eight states he has presented a more accurate geographic distribution. In recent years surveys have been made in the Federal District and the State of Morelos by Chavarría (3) and in the State of Puebla by the Military Medical School in 1963 (6). The geographic distribution in the mountainous region, and the level of iodine content in the water in Mexico are shown in Figure 1.

In recent years the National Nutrition Institute has been interested in the relationship between nutritional status and endemic goiter. Thorough surveys of 22 communities throughout the country were made in 1961. It is evident that proper assessment of iodine nutrition requires longitudinal study of nutrition of the people in relation to thyroid function.

Mexico City is surrounded by mountains some of which are always snow-capped. Previous studies have shown that endemic goiter is present on the

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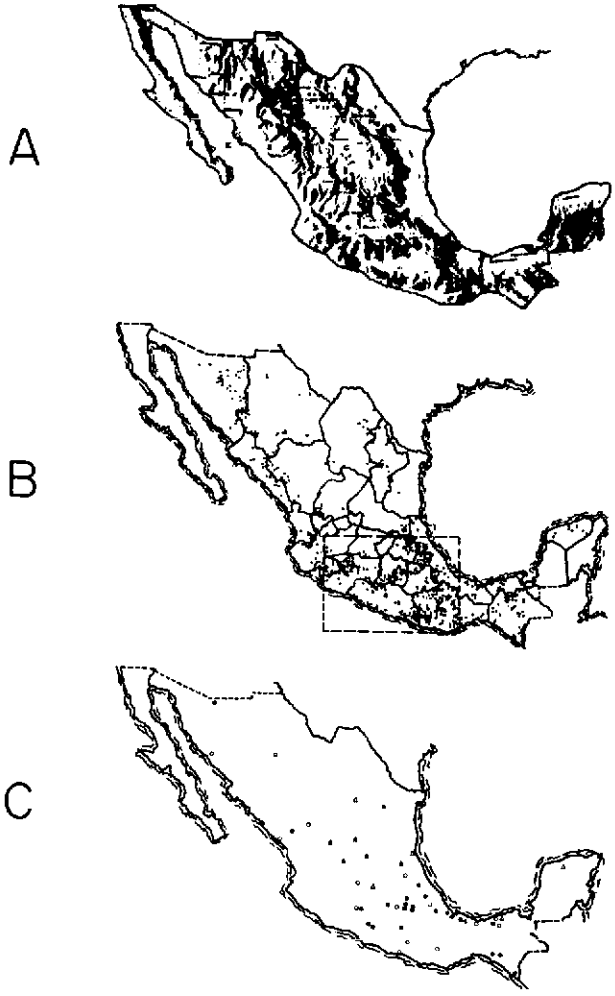

Figure 1. Mountainous regions (a), endemic goiter areas (b), and low iodine content in water (c) show a similar geographic distribution in Mexico.

slopes of these mountains and in the nearby valleys. Tepetlixpa, a small town in the State of Mexico, was selected for intensive and longitudinal study of the school population. The community had a high incidence of endemic goiter. The population is 5,000 inhabitants. It is located in the foothills of the volcano Popocatepetl at 2,300 meters above sea level and is 71 km from Mexico City. It is easily reached by paved road, a convenience for handling equipment and specimens which permitted in most of the cases laboratory procedures to be done at the Thyroid Clinic in the National Nutrition Institute. A metabolic unit consisting of two bedrooms, kitchenette, and a laboratory was set up in the town. Iodine nutrition in the schoolchildren and factors that may influence iodine intake have been studied in this unit and in the village since 1962.

Early in the study, stable iodine balance showed that intakes near the normal recommended allowances gave strongly positive iodine balance. Fecal iodine excretion was within normal limits and larger than the urinary excretion. The addition of iodide supplements to the diet resulted in a slow even fall in radioiodine avidity to a new steady state after several weeks (12). Iodine intake was found to be more deficient in children than in adults and to be correlated with their diet. Food was the major source of iodine and, because of the alimentary habits of the rural population, consisted primarily of corn and beans. Iodine intake varied widely according to the origin of the food consumed. Long seasonal swings in iodine balance have been observed (11-15). Observations through the years have shown a gradual increase in iodine intake levels and a tendency to normalization of thyroid function, and also a gradual decrease of goiter prevalence. Nutritional status has changed little, and the changes are not significant. The cause of the increasing iodine intake levels is presumably the diversification of foodstuffs (14, 15).

Although the analysis of duplicates of dietary intake may be considered as the most accurate method for epidemiological studies, the estimation from basal urinary iodine excretion may have some advantages since it appears to be independent of variations in the diet and the degree of thyroid function and seems to be a good way to investigate the usual iodine intake levels prior to a study (14, 22, 23).

The work done in the school population of Tepetlixpa since 1962 has given us the opportunity to observe the evolution of iodine nutrition as reflected in various parameters of thyroid function, to observe the slow transformation of the endemic, to investigate the different factors that may influence the iodine intake levels, and to estimate the amounts of iodine necessary to maintain normal iodine metabolism over a long period of time. The changing patterns of endemic goiter in this area may be the consequence of socioeconomic changes and may reflect the situation of other goitrous zones in the country.

GOITER PREVALENCE

Thyroid enlargement was found in 92 per cent of the 866 children of both sexes with ages ranging from 6 to 14 years in the 1962 survey. The size was variable. Most of the glands were small, 21 per cent were nodular, and no sex difference was found. Evaluation of goiter was done according to a modification of the criteria advised by the Third FAO-WHO Conference in Caracas, Venezuela, in 1953. Since the interest of this work was to observe the changes in small goiters, the classification was modified to include goiters of two to three times the normal thyroid volume. This group was called Grade I, and the original FAO-WHO groups were called Grades II, III, and IV (12). The goiter surveys were performed by the same two persons (J.A.M. and E.T.) in order to avoid different criteria.

The second survey done in 1965 gave a prevalence of 68 per cent. The third in 1967 gave a prevalence of 50 per cent, and finally in 1968 an incidence of 38 per cent was found. The results of the surveys appear in Table 1. The presence of thyroid nodules also diminished successively from 21 per cent to 13 per cent, to 5 per cent, and finally to 2 per cent in 1968. Goiter prevalence against age, compared during the four surveys, appears in Figure 2. It is clearly shown that the prevalence has principally diminished in the early age

Table 1. Goiter prevalence in the school population of Tepetlixpa in different years.

Age	No. of patients	Goiter No.	Goiter %	Grade I %	Grade II %	Grade III-IV %
		1962				
6-8	297	274	93	51	38	11
9-11	355	334	94	41	40	19
12-14	214	199	93	34	33	33
Total	866	807	93	44	37	19
		1965				
6-8	330	190	58	71	25	4
9-11	422	303	72	60	31	9
12-14	182	142	78	57	22	11
Total	934	635	68	65	27	8
		1967				
6-8	347	117	34	100	-	-
9-11	377	210	56	92	7	6
12-14	268	167	62	81	16	3
Total	992	494	50	91	9	1
		1968				
6-8	273	76	28	95	5	-
9-11	316	125	40	93	7	-
12-14	213	106	50	91	9	-
Total	802	307	38	92	7	-

groups of the schoolchildren. When the changes in the size of the goiter were studied in the same child it was found that 48 per cent of the 255 children who were examined in the first two surveys showed shrinking of the thyroid, whereas 16 per cent showed slight enlargement of the goiter and 36 per cent showed no change (15). In the last three years 74 children, out of a representative sample of 100, were examined in the two surveys. Sixty-nine per cent showed shrinking of the thyroid, against 8 per cent who had slight enlargement, and 23 per cent who showed no change.

THYROID FUNCTION

Protein-bound iodine determinations and measurements of tendon reflex time were within normal limits from the first survey and were of no help for comparative studies in different years. Thyroid function as measured by the 24-hour ^{131}I uptake proved most useful. Results of radioiodine thyroid uptake in 37 children on whom measurements were made in two or more years appear in Figure 3. There has been a fall from 91 per cent in 1962 to the upper limits of normal in the last three years. Similar results were found when different groups of children were measured in the same years (Table 2).

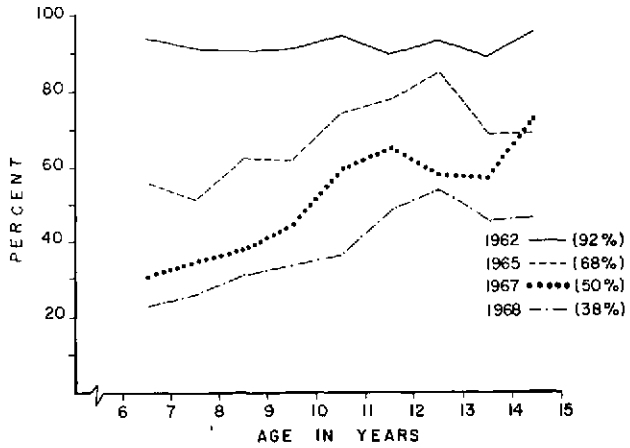

Figure 2. Changing patterns of goiter prevalence. Prevalence has diminished considerably and changes are more significant at early age in the schoolchildren of Tepetlixpa.

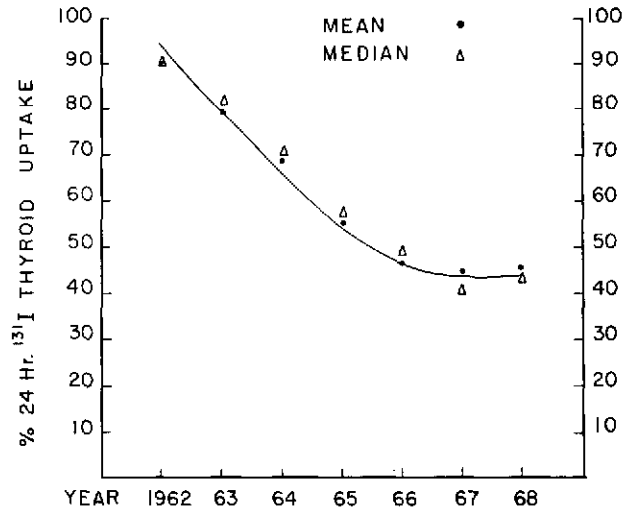

Figure 3. Twenty-four hour ^{131}I thyroid uptake shows a decrease, which has reached the upper limits of the normal range during the last two years.

From these results it appears that the uptakes in these children have reached a steady level, but when uptakes were done in different seasons during a whole year important seasonal variations were found in many instances. Figure 4 shows these changes in 24 subjects in whom the uptake was repeated two or more times during a year. The changes were thought to be due to different levels of iodine intake, particularly in those cases where normalization of iodine balance had not been completely achieved. This figure suggests two

Table 2. Twenty-four hour thyroid ^{131}I uptake in different years.

Year	No. cases	% Uptake			
		Mean	Median	Mode	Range
1962	102	85	89	91-95	50-98
1963	50	77	81	81-85	26-96
1964	12	71	70	66-70	45-90
1965	46	48	49	56-60	26-75
1966	70	47	40	41-45	25-75
1967	125	45	41	36-40	21-88
1968	101	46	44	41-45	19-86

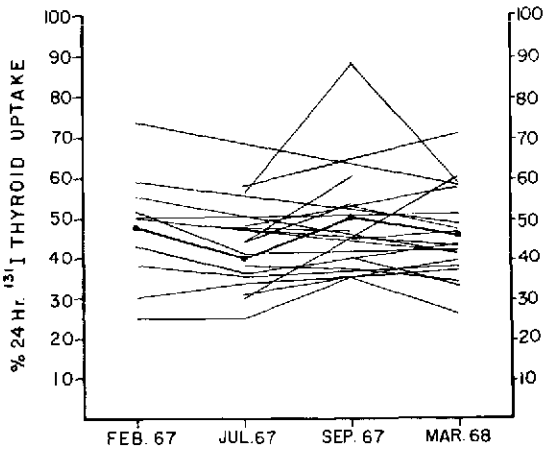

Figure 4. Seasonal changes in the 24-hour ^{131}I thyroid uptake in 24 children.

seasons of higher iodine intake. One was identified in the first study in 1962 (12), when the town population had to obtain food from the open market because the local crops were gathered generally during October and November. The second one was in July during the rainy season, when children eat fruits from other regions, such as watermelon, canteloupe, pineapple, mango, tangerine, banana, and others which were not eaten at the time of the 1962 survey.

In March 1968 a new group of children came to school because a railroad is being built through the town. Approximately 200 to 300 railroad workers will be in town for a year or two and their children will be attending school. Thyroid ^{131}I uptakes done in 20 of these children showed a mean of 33 per cent, a median of 35 per cent, a mode of 35 to 40 per cent, and a range of 16 to 48 per cent.

IODINE INTAKE

During the six years of the study iodine intake levels have been determined in groups of children by several methods. In 1962 and 1963 daily dietary supervision was done by a dietitian on a three-visit a day schedule. Aliquots of foodstuffs were taken for iodine determinations (12). From 1964 on, direct measurements of iodine intake were made on duplicates of total food ingestion. For this each child was given a four-liter wide-mouth polyethylene jar and was requested to place in the jar an exact duplicate of everything eaten during the day, including beverages (24). They were supervised by the dietitian at meal time. The diet with these two methods was ad libitum and in accordance with their family habits.

From 1965 on, estimates of the average iodine intake were calculated from the urinary iodine excretion rate during fasting, plus the fraction of the hormonal iodine secretion excreted in the feces (4). From basal urinary iodine excretion and the radioiodine uptake, the thyroid hormonal secretion rate was calculated according to Stanbury (12) as follows:

$H = \frac{EU}{1-U}$, where H is secretion rate, E is urinary iodine excretion, and U is ^{131}I thyroid uptake.

From the beginning of these studies iodine balance at the field metabolic unit has been used to measure iodine intake levels. These levels can be calculated approximately from the relationship between the excretion and the intake of iodine, as suggested by Dworkin et al. (5). In a similar way, iodine balance can be calculated by using the relationship between iodine balance and iodine intake at different levels of ingestion. Thus by drawing a regression line which crosses the line of iodine balance one shows the balance point representing the usual iodine intake level (14).

Figure 5 shows the progressive increase in iodine intake level through the six years as determined by the average results of the different methods employed. The increase follows a line that is almost a mirror image of that of the radioiodine thyroid uptake.

URINARY IODINE EXCRETION

Urinary iodine excretion has been determined in various groups of children through the six years and in different seasons of the year. Usually it was measured on 24-hour collections at home in a large number of children. Some were measured in small numbers of children during balance studies but through longer periods of time. Others were obtained in larger numbers of children from the urinary iodine excretion rate during fasting corrected to 24 hours.

Similarly to the iodine intake curve, the urinary iodine excretion has shown a tendency to increase gradually, although the changes are not so marked in the last two years, as shown in Figure 5. In this figure may also be observed the two seasons of high iodine intake as mentioned before, corresponding to June and July during the rainy season, and September, October, and November, when the populace eats produce from other regions.

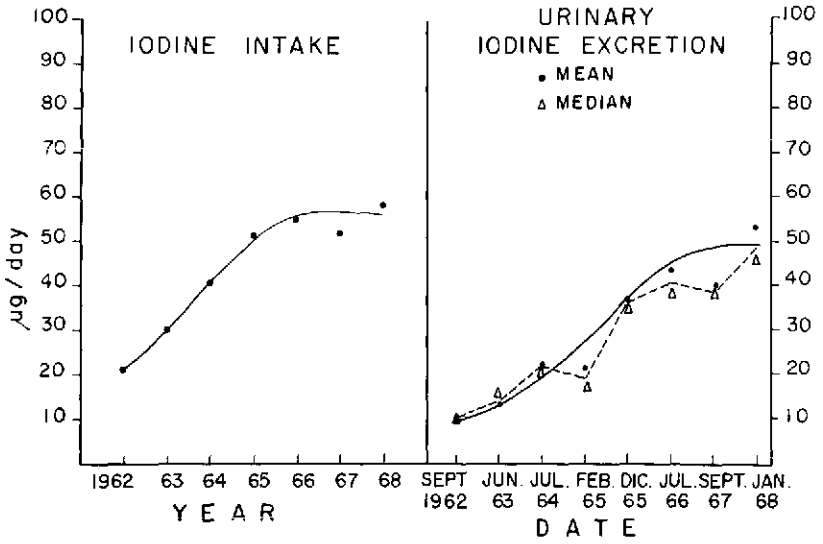

Figure 5. Change in dietary daily iodine intake and 24-hour urinary iodine excretion. The points correspond to the average values obtained for different methods. Seasonal changes in the urinary iodine excretion are shown by the broken line.

IODINE BALANCE STUDIES

Iodine balance studies in the field metabolic unit, as well as dietary iodine intake and excretion in children living at home, have also been measured in different years. The iodine balance studies proved their usefulness in detecting misinterpretations of the results obtained in 1962, when four euthyroid goitrous children were found to be in marked positive balance with an intake of approximately 140 µg of iodine per day according to the intake levels calculated from aliquots of food. These studies demonstrated that the real average, or usual iodine intake was not 140 µg, but between 20 and 30 micrograms, as was proved later (12). Further studies corroborated this tendency to positive balance even on intakes lower than 100 µg of iodine per day (11). In 1964 ten girls receiving an average of 65 µg of iodine daily were in positive iodine balance, although not as marked as in previous years (26).

Since equilibrium between intake and excretion would be obtained only when the average or the usual iodine intake is given, these children were fed higher amounts of iodine than the average intake received at home before each study. Further studies in later years showed that the children were in balance with iodine intakes varying from 60 to 70 µg, as shown in Figure 6. These levels of iodine intake are slightly higher than those obtained when larger groups of children were studied at their home, but it must be noted that these balances were always done in a small and selected group of girls who did not represent the general school population.

When iodine intake is increased or decreased, there is a lag period during which the subject is in positive or negative balance before equilibrium is achieved at a new level of intake. The response of iodine excretion during a period of different levels of iodine ingestion was investigated in six girls in 1965. The iodine intake was varied by addition of 100 and 200 µg of iodine

to the constant diet of three-day periods. Iodine intake and urinary iodine excretion showed good correlation, but fecal iodine excretion did not correlate with iodine intake. It was almost constant in these children, and corresponded to the rest of the iodine balance studies done for other purposes. With each increase in iodine intake there was a corresponding increase in iodine retention. The experimental points fitted a linear response in the first four girls but not in the last two (Figure 7). The change between determinations of 24-hour ^{131}I thyroid uptake was considered to be a measure of the constancy of iodine kinetics. When expressed as per cent of the initial uptake, the first four showed no significant change, but on the last two girls iodine supplementation caused a decrease of uptake, and in one of them the uptake was already at a normal low level.

In two of the girls iodine balance was measured during the low iodine season and repeated in the high iodine season, as shown in Figure 7 (girls nos. 1 and 2). It can be seen that after two months of higher iodine intake, iodine balance was close to equilibrium.

NUTRITIONAL SURVEYS

Early in the study, in order to know the alimentary values of the town, eight representative families were studied. Daily dietary survey during a week showed low values of Vitamin A and riboflavin, as is known to be true for much of rural Mexico. The survey included assessment of anthropometric data, skin fold thickness, and signs of nutritional deficiency.

In 1964 malnutrition and iodine deficiency in relation to growth and maturation were studied in 150 children. Over 50 per cent of them had some deficiency signs but none was severely malnourished. Ideal weight for sex and age was deficient in about 90 per cent, and height was proportional to weight. One of the principal findings was retardation in bone maturation. Other laboratory data were within normal limits, except for low plasma albumin levels in 20 per cent of the cases. Malnutrition and goiter were not correlated, and delayed growth and bone maturation were correlated with malnutrition but not with goiter.

There was a change through the years toward diminishing the proportion of malnutrition, but the changes were not very significant.

GOITROGENIC FACTORS

During 1963 the effect of native foods on the ^{131}I uptake gradient was studied. At that time the thyroid uptake was abnormally high. When ^{131}I was given to fasting children the curve showed a rapid rise, approaching its maximum level during the first four hours. When food was given two hours after the tracer dose, no difference from the fasting controls was found(12).

Since Vitamin A deficiency has been considered an etiologic factor in endemic goiter (1, 9), and since in our nutritional survey Vitamin A and riboflavin deficiencies were found, a double blind study was performed by administering both vitamins and placebos, but no change in the radioiodine thyroid uptake was found after 12 weeks.

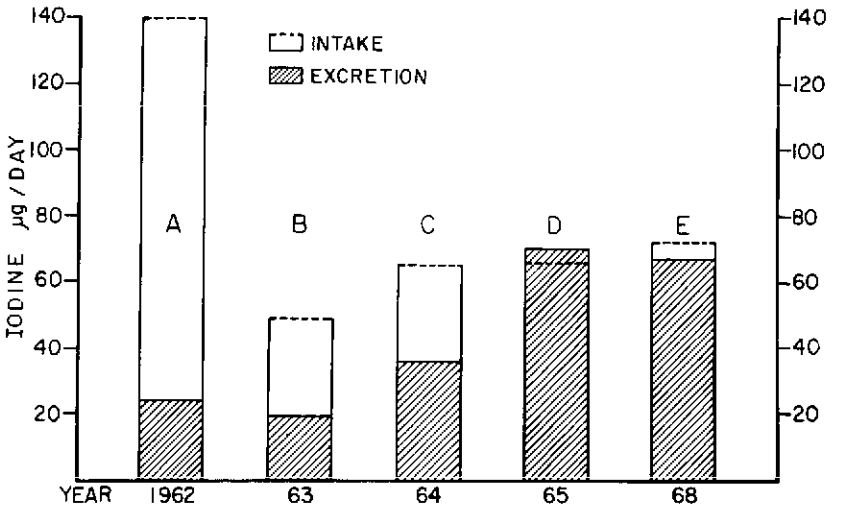

Figure 6. Iodine balance studies in the field metabolic unit in different years. The positive iodine balance during the first three years was no longer found in recent years on similar iodine intakes.

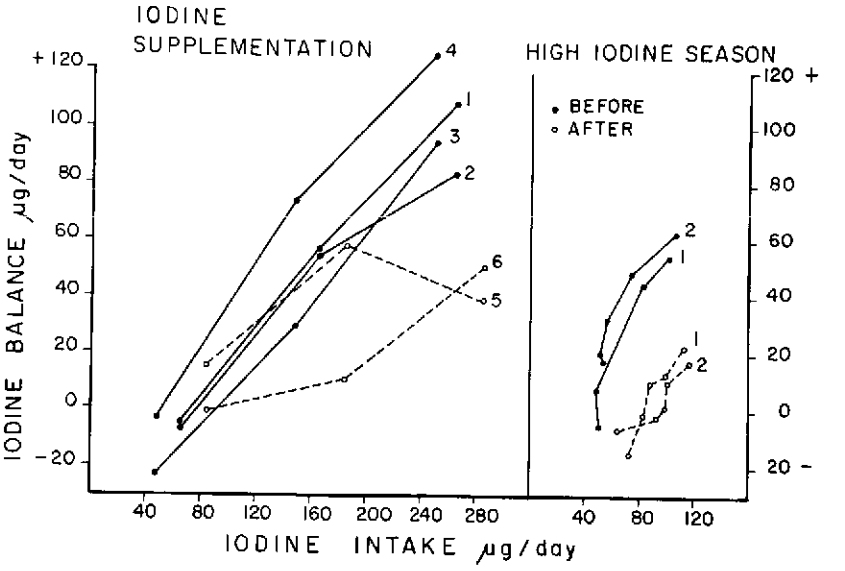

Figure 7. A different iodine balance response was obtained in two of six girls on iodine supplementation and in two girls before and after eight weeks of the high iodine season. The different iodine balance response is probably in relation to the degree of iodine depletion.

The effects of small amounts of methimazole on the synthesis of thyroid hormone was studied by measuring the $PB^{131}I$ levels at 72 hours. The local diet of the children was modified while the iodine intake and turnover was maintained constant. The preliminary results in 1967 were negative but some technical problems have to be solved before rejecting the existence of a goitrogenic factor in the community

DISCUSSION

The repeated surveys have demonstrated that goiter in Tepetlixpa had tended to disappear. If nodularity is related with the severity of the endemia, the almost total disappearance of thyroid nodules also supports this hypothesis. Although disappearance of goiter occurred in the same child examined in different years, it is interesting to note that the prevalence of goiter has diminished principally in the younger group of schoolchildren. This suggests that new generations probably will be free of goiter.

The thyroid uptake of radioiodine has declined in accordance with the changes in the goiter prevalence. The changes can be reasonably attributed to a natural increase of daily iodine intake. Figure 8 shows a good correlation between these parameters. The fact that during the past three years the uptake has been in the upper limits of the normal range while the prevalence of goiter has continued to decrease is suggestive of the existence of a lag period between attainment of normal iodine handling by the gland and disappearance of goiter. This suggestion fits the well-known fact that endemic goiter does not necessarily coexist with iodine deficiency: adequate nutritional levels may be reached long before the endemic disappears. On the other hand the thyroid uptake in these children, in spite of average results within the normal limits as a group, should not be considered as normal since many of them are subjected to variations in relation to iodine intake as determined by the seasonal changes. Moreover, Figure 9 shows that although the frequency distribution of the radioiodine thyroid uptake in 1968 was similar to the control done in 1962 in schoolchildren from Mexico City, the small group of children of railroad workers who have recently arrived in the town have lower values.

The increase in iodine intake is probably the result of many factors. Among the most important should be considered: better standards of living in general, better communications including transportation facilities and increased traffic through the road, the occasional use of iodized salt, and mainly the variety of food in the diet (14). This last factor is primarily responsible for the seasonal changes in iodine intake levels throughout the year.

Iodine balance studies corroborated the increasing levels of iodine intake and their seasonal changes. The high positive iodine balance has tended toward equilibrium in the last three years on 60 to 70 μg of iodine per day. Iodine supplementation (Figure 7) caused strongly positive iodine balance and gave a significant correlation between iodine intake and the response of iodine balance in four subjects, and slight positive iodine balance in girls nos. 5 and 6. These findings help to explain the lack of correlation between iodine intake and excretion found previously (26) in a group of girls at different iodine intake levels. Balance studies demonstrated that a small but persistent increase in iodine intake was more physiological and had a better effect that higher doses for short periods. Subject 1 and 2 had a high positive

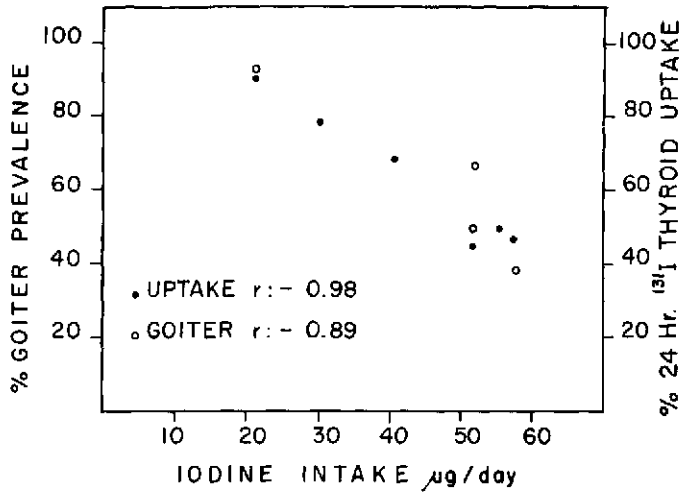

Figure 8. Correlation between iodine intake and 24-hour radioiodine thyroid uptake and between iodine intake and goiter prevalence in the school population during six years. Changes of iodine intake through the years correlate well with the corresponding changes of 24-hour ^{131}I thyroid uptake and goiter prevalence.

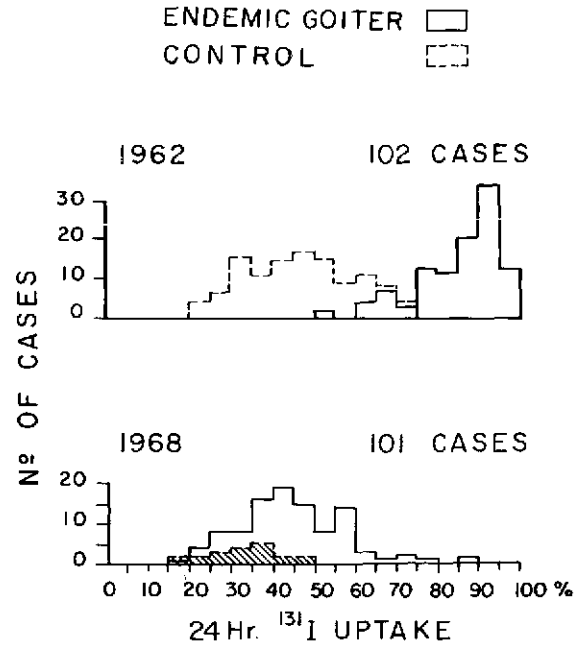

Figure 9. Frequency distribution of 24-hour radioiodine thyroid uptake in the schoolchildren of Tepetlixpa and a control group from Mexico City. Children recently arrived at the town in 1968 are shown by the shaded area.

balance with a linear response during iodine supplementation, and after two months of the high iodine season were in equilibrium on intakes varying from 70 to 120 μg per day.

Better living standards may account for the slight improvement in the general nutritional status. The marked improvement in goiter suggests that the most important factor in the disappearance of goiter has been the increasing iodine intake due to diversification of foods.

Our studies agree with the hypothesis that iodine deficiency is the main if not the sole cause of endemic goiter in these regions. Nevertheless, the literature contains many reports suggesting the existence of other factors. On a very low iodine intake level in New Guinea, thyroid function was nearly identical in subjects with and without goiter (4), and endemic goiter has been found also in people living on relatively high iodine intakes (25, 27). The search for goitrogenic factors up to now in Tepetlixpa is probably of no value since their action, if any, is a slight one and it may not be apparent on normal or high iodine intakes. At the present time the school population is in borderline normal iodine intake. It might prove profitable to investigate further the extent that the seasonal changes in iodine intake levels influence goiter.

SUMMARY

Six-year studies in the school population of a rural Mexican community with endemic goiter have shown a fall in goiter prevalence and a gradual increase in iodine intake as indicated by direct determinations of iodine intake, iodine excretion and the tendency to normalization of iodine handling by the thyroid gland.

Iodine intake was found to vary greatly according to the origin of the food consumed. Long seasonal swings in iodine balance have been observed. During higher iodine intake than usual in 1962 the patients subsisted in strongly positive iodine balance. Addition of iodide supplements to the diet resulted in a slow even fall in radioiodine avidity to a new steady state in terms of weeks.

Recent studies have shown that iodine balance reached equilibrium at higher iodine intakes in only a few days and that radioiodine uptake also fell rapidly. These results clearly show a varying response to iodine supplementation according to the degree of iodine depletion and explain the iodine balance data obtained in goitrous and nongoitrous people.

Data obtained regarding the goiter endemic in the highlands and along a paved road across the country were consistent with iodine deficiency as the main if not the sole cause of the disease. In spite of a gross correlation between goiter prevalence and iodine intake, regions were found with a similar average iodine intake and different goiter prevalence. This may be explained by a changing situation as shown in the longitudinal studies.

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CHAPTER 33

IODINE NUTRITION LEVELS OF SCHOOLCHILDREN IN RURAL MEXICO[1]

Enrique Tovar, Jorge A. Maisterrena, and Adolfo Chávez

Endemic goiter has been a problem of public health in Mexico, but a recent appraisal reveals that previous estimates were limited to small areas in the country (6). During the last meeting of the PAHO Scientific Group on Research in Endemic Goiter (Cuernavaca, Mexico, 1965) it was generally agreed that "the school population (6-14 years) of both sexes provides a captive sector of the community which at the same time represents a sufficient susceptible group in terms of disease prevalence." Because of this it was decided to carry out initial studies on the schoolchildren of selected Mexican rural areas prior to examining the whole population. Twelve towns from 1,000 to 2,000 inhabitants were chosen along the Pan American highway that crosses the country from north to south, and a control group from a private school in Mexico City was used.

Because of the difficulties of direct measurements of dietary iodine intake in rural areas and also because of the virtually impossible complete collection of 24-hour urine samples, casual urine specimens were utilized to assess iodine nutrition levels. Our previous studies in Tepetlixpa (4, 7) had shown that estimation of dietary iodine intake from the basal urinary iodine excretion was in close agreement with direct measurements of iodine in duplicates of dietary intake and with calculation of the balance point from iodine balance data. Further support of this method was given by the determination of urinary iodine excretion during prolonged fasting or when a constant and fixed diet was given at equal intervals. These studies showed that the basal urinary excretion does not change significantly during the day and keeps a constant ratio to urinary creatinine excretion (8). It was also shown that basal iodine excretion corresponds to the median value of the dietary iodine frequency distribution obtained from duplicates of the food intake during 14 days in six schoolchildren in Tepetlixpa.

Finally we have considered the possibility that the basal urinary iodine excretion values may be a useful index of iodine nutrition levels, free of the daily changes of dietary intake and compatible with the recent past history of dietary iodine intake. If so, investigation would be far easier since urinary iodine could be determined in a casual urine sample collected after an overnight fast.

The study encompassed a survey of 50 to 70 male children from 9 to 13 years of age chosen at random in each town in order to have a selected but comparable sample from community to community. The children were examined as

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1/ From the National Nutrition Institute, Mexico City, Mexico.

to nutritional status and urine samples were obtained during fasting between two carefully timed voidings. Most of the children also had a 24-hour ^{131}I thyroid uptake measurement. Iodine and creatinine determinations were done in Mexico City by the Benotti and Benotti (1) and the alkaline picrate methods, respectively.

Goiter was found in more than 10 per cent of the children in five towns but was mainly of the small, non-visible type. Figure 1 shows the goiter incidence in relation to the frequency distribution patterns of the urinary iodine excretion in nine representative towns. Iodine deficiency as a cause of goiter is suggested by the larger number of children with urinary iodine excretion below 20 μg per day as goiter incidence increases. A clear-cut difference was found only between the extreme situation of one town with a 24 per cent incidence of visible goiter and urinary iodine excretion from 3 to 42 μg per day, and the control children from Mexico City with no goiter and urinary iodine excretion from 40 to 300 μg per day. The intermediate group showed a large overlap of urinary iodine excretion values in the presence of a variable percentage of goiter prevalence.

The usual inverse excretion in these children appears in Figure 2. According to this relationship, thyroid uptake would be above 50 per cent, arbitrarily considered as the upper limit of the normal range, when urinary iodine excretion levels are below 30 μg per day. This correlation is indirect evidence for iodine deficiency as the cause of goiter in the children under study.

Since no differences were found among goitrous and nongoitrous areas, except when extreme situations were considered, goitrous and nongoitrous children living in the same towns were also compared. Figures 3 and 4 show that no significant differences were found either in the urinary iodine excretion levels or in the 24-hour radioiodine thyroid uptake values. There is a tendency toward low iodine excretion levels and toward high thyroid uptake in the goitrous children. This might be explained by an exaggerated influence of the figures from one of the towns in the series.

Finally, Figure 5 shows the frequency distribution of the urinary iodine excretion of the whole population under study. Average values were 30 μg per day for the median and 20-30 μg per day for the mode. If one allows a constant amount of 10 μg per day for fecal iodine excretion, the usual dietary iodine intake of these children may be estimated as 30 to 40 μg per day. The average goiter incidence in these children was 17 per cent, but diminished to 7 per cent when only visible goiters were considered.

Iodine deficiency may be the only cause of endemic goiter in Mexico, as suggested by this and previous studies (2, 3), but the influence of other factors must be considered unless the changing endemic pattern as shown in Tepetlixpa (5) explains the difference in goiter incidence in children on a similar iodine intake.

The rural school population in Mexico apparently subsists on an iodine intake which is near the lower border of the normal range. Nevertheless, it is most difficult to establish valid limits of normality in iodine requirements since there are wide individual variations.

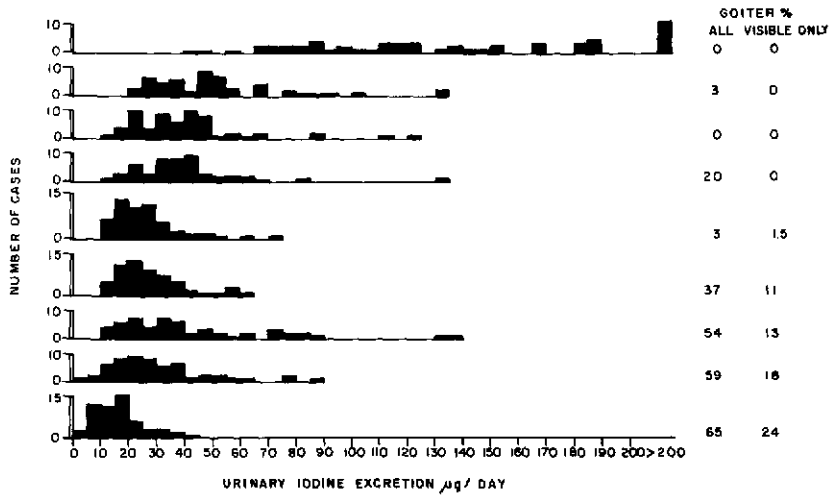

Figure 1. Prevalence of goiter and frequency distribution of basal urinary iodine excretion of children from nine representative towns.

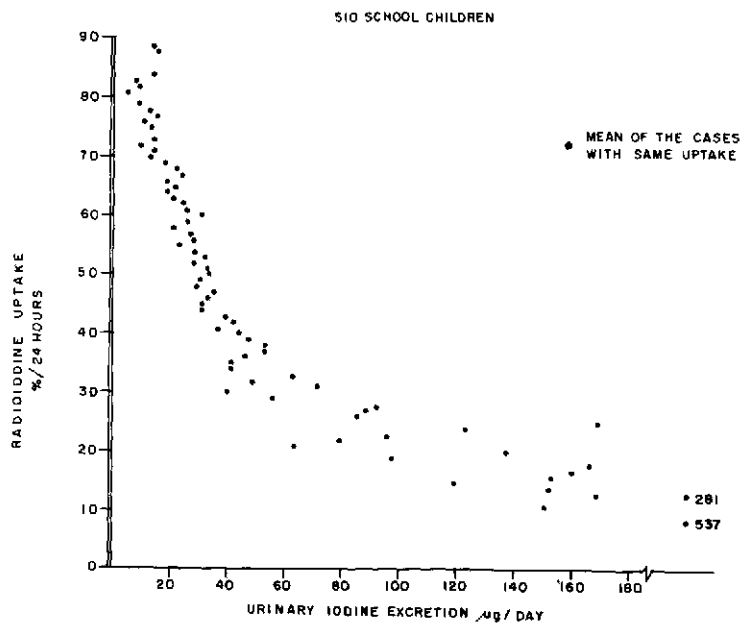

Figure 2. The usual inverse correlation between 24-hour ^{131}I thyroid uptake and urinary iodine excretion.

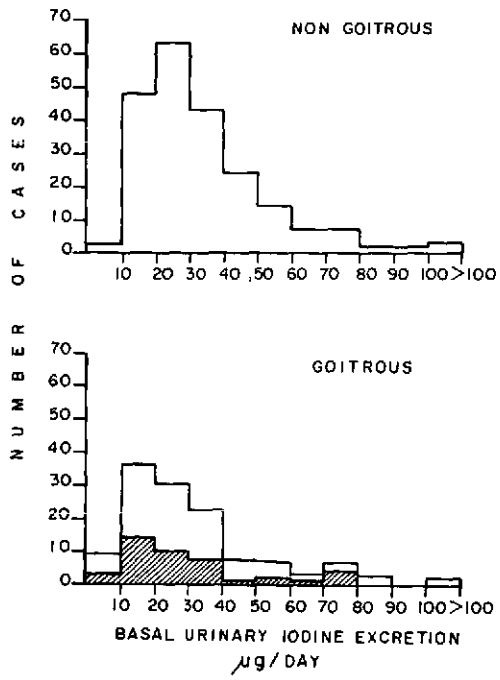

Figure 3. No significant difference was found in the urinary iodine excretion between goitrous and nongoitrous children living in the same area. Visible goiter is shown by the shaded area.

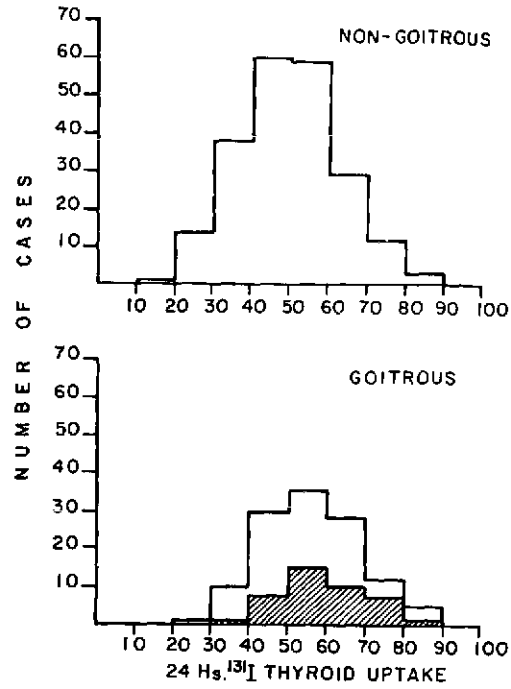

Figure 4. No significant difference was found in the 24-hour ^{131}I thyroid uptake between goitrous and nongoitrous children living in the same area. Visible goiter is shown by the shaded area.

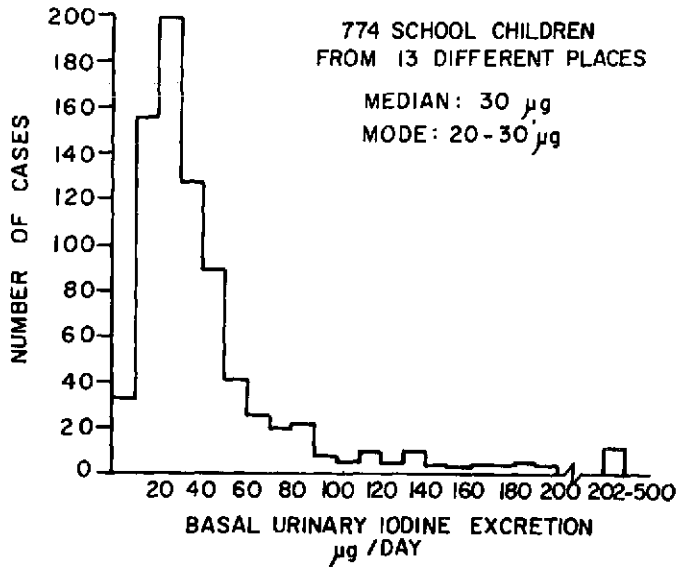

Figure 5. Frequency distribution of the basal urinary iodine excretion in the whole population of children under study.

SUMMARY

Previous studies in the endemic goiter area of Tepetlixpa, Mexico, have shown that estimations of daily iodine intake from the basal iodine excretion were in close agreement with the direct measurement of iodine in duplicates of food intake or calculation of the iodine balance point from iodine balance data. Further support of this method was given by the determination of urinary iodine excretion during prolonged fasting and when a constant diet was given at equal intervals. These studies showed that the basal urinary iodine excretion does not significantly change and that there is no diurnal rhythm in the excretion of iodine but only from the dietary intake.

Evaluation of iodine nutrition levels of schoolchildren from rural villages throughout the country showed a good negative correlation with the 24-hour ^{131}I thyroid uptake. According to this relation, the thyroid uptake would be above 50 per cent when the iodine intake is below 30 µg per day. Iodine intake levels also showed a large overlap between goitrous and nongoitrous areas and between goitrous and nongoitrous children in the same area.

Since endemic goiter does not necessarily coexist with iodine deficiency other factors must also contribute to the different goiter prevalence at similar iodine intake levels.

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SECTION IX

ENDEMIC GOITER IN PERU

CHAPTER 34

ENDEMIC GOITER IN RURAL PERU: EFFECT OF IODIZED OIL ON PREVALENCE AND SIZE OF GOITER AND ON THYROID IODINE METABOLISM IN KNOWN ENDEMIC GOITROUS POPULATIONS

E. A. Pretell, F. Moncloa, R. Salinas, R. Guerra-García, A. Kawano, L. Gutiérrez, J. Pretell, and M. Wan[1]

The existence of endemic goiter during the Incan rule of 1150-1533 was described by Lastres (19). The historical evidence has been vehemently attacked by Greenwald (13), but the disease was clearly prevalent during colonial times (1533-1821), when a Papal Bull of Pope Paul III (1534-1549) ordered missionaries to consider goitrous and cretinous people as beings with a soul and worthy of conversion to Christianity. More recently, Lorena (20), Monge (25), Burga-Hurtado (4, 5), Salazar-Noriega (31), and Marroquín (22) have made goiter surveys in the sierra and the jungle. Salinas (32) and the National Nutrition Institute began a survey in 1963 of the prevalence of goiter in school populations involving more than 220,000 subjects throughout Peru. Although still at the evaluation stage, the survey has shown a higher goiter prevalence in the sierra and jungle zones and less than 10 per cent prevalence on the coast.

Realizing the importance of the goiter problem, the Peruvian government has installed factories for salt iodization in areas more affected by the endemia; mechanical difficulties have limited operations to only three installations at present. These by no means fulfill the needs of all the goitrous zones, as designated by the National Nutrition Institute. In view of the difficulties confronting the salt iodization programs, and as a result of the recommendations of the Second Meeting of the Pan American Health Organization Scientific Group on Research in Endemic Goiter (Cuernavaca, Mexico, 1965), we have undertaken a pilot study on the use of iodized oil as a prophylactic measure against endemic goiter, as well as to test the effectiveness of iodine administration in preventing the occurrence of endemic defective people. The program is based on satisfactory results previously obtained in New Guinea (16, 23).

The first part of this study was supported by the Pan American Health Organization (HP-A-Peru-4201). The follow-up studies are being supported by the National Health and Welfare Fund and the Special Public Health Sevice, Lima, Peru, Contract No. 51.

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MATERIALS AND METHODS

In order to study the prophylactic effect of a single intramuscular injection of iodized oil, three central sierra villages, Tapo, Huasahuasi, and Ataquero in the Province of Tarma, were selected. A growth rate of 3 per cent assured a high percentage of children. Tarma, a nearby town equipped with laboratory and hospital facilities, served as headquarters for the field work. More detailed laboratory work was carried out in Lima, about five hours away by automobile. The location and general characteristics of the endemic areas are shown in Figure 1 and Table 1.

Table 1. General data on the endemic areas studied.

Village	Altitude (m)	Urban Population	No. of Persons Examined	Visible goiter prevalence %		
				Females	Males	Both Sexes
HUASAHUASI	3,531	1,934	1,672	58.1	50.5	54.5
TAPO	3,311	1,830	1,287	49.0	38.4	44.3
ATAQUERO	3,100	400	166	57.4	56.9	57.2

Prior to injection of the iodized oil a basal study of goiter endemicity was carried out using the criteria given by Pérez et al. (29) as modified in the First Meeting of the PAHO Scientific Group on Research in Endemic Goiter (Caracas, Venezuela, 1963). These may be summarized as follows:

0_a - No goiter at all

0_b - Small goiter, detectable only by palpation

I - Goiter visible with extension of the neck only

II - Goiter visible at normal position of the neck

III - Goiter visible at a distance

IV - Huge visible goiter

In the present study, visible goiter (Vg) refers to goiters from I to IV degree while palpable goiter (Pg) refers to all degrees of goiter including 0_b.

In a house-to-house survey, 3,125 persons from a total population of 4,164 were examined by teams of two observers each. When a discrepancy arose, the lower value was recorded. Concurrently a nutritional survey was made by the National Nutrition Institute; the results will be published elsewhere. Thyroid function tests for control values were performed as follows.

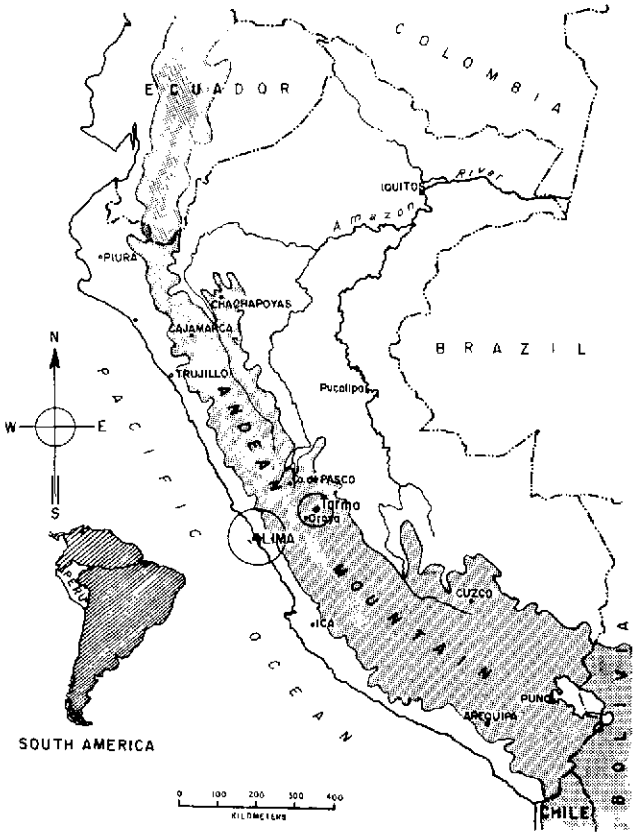

Figure 1. The location of the endemic goiter areas of Peru. The project was conducted in the vicinity of Tarma shown in the circle.

Determinations of urinary iodine were done on casual samples by a modified chloric acid procedure (Zak) as described by Benotti et al. (2). The 24-hour urinary excretion of iodine (EUI) was calculated from the iodine creatinine ratios of the same samples, according to Jolin and Escobar del Rey (17), whose work is based on that of Vought and London (35). The adequacy of the formula used was confirmed by collecting 24-hour urine samples. The actual creatinine and iodine values were in fairly good agreement with those estimated by the formula. The number of determinations is given in the tables.

Protein-bound iodine (PBI) and total serum iodine (SI) were measured according to Benotti et al. (2). Total thyroxine ($I\text{-}T_4$) levels were determined in serum by the competitive protein-binding analysis of Murphy et al. (26) according to a modified method of Nakajima et al. (27).

The thyroid uptake at 24 hours was estimated after an oral tracer dose of 5 to 10 μc of a carrier-free ^{131}I. These studies were carried out in the villages of Tapo, Huasahuasi and Ataquero under field conditions using a transportable power supply generator, a 1.5 inch crystal shielded with a lead collimator, and a phantom for the standards.

A group of 1,771 subjects were injected with iodized oil or a placebo. Ethiodol^{R}, a poppy-seed oil containing 475 mg of iodine per ml, was obtained from Laboratoires André Guerbet (France) as marketed by E. Fougera & Co. (U.S.). The placebo, also poppy-seed oil but without iodine, was obtained from Guerbet. Nine hundred and twenty-three subjects in the three villages were injected with iodized oil, and 848 with the placebo (Table 2). Injections were done in people of both sexes up to 45 years of age. Men over 18 years were excluded. Among the schoolchildren, groupings for the placebo and the iodized oil were predetermined at random within a given age. Care was taken to match the size of goiter. In other groups, random injection of placebo and iodine was accomplished by consecutive alternation of iodine and placebo injection. The doses used were the following: less than 1 year old, 0.5 ml; from 1 to 5 years old, 1.0 ml; over 6 years old, 2.0 ml. In adult women with nodular goiter the dose was reduced to 0.2 ml. All the injections were performed in the first week of October 1966.

At the time of injections two other surveys were performed, one by Dr. Harry Israel for bone maturation and the second by Dr. Philip Dodge for neurologic development. The results of these studies will be reported elsewhere.

In the general follow-up of the injected population evaluations have been made by comparison between placebo (Gr-P) and iodized oil (Gr-I) injected groups, except when otherwise indicated. In the Gr-I a distinction is made between adults injected with 2.0 ml of Ethiodol and those injected with 0.2 ml. These two subgroups are treated separately in the results.

Table 2. Distribution of population at the time of injection according to age and sex.

SEX	AGE	PLACEBO		IODIZED OIL	
		Number of cases	%	Number of cases	%
♀	0 – 5	89	11	109	12
	6 – 12	173	20	177	19
	13 – 18 *	83	10	87	9
	19 – 45	131	15	155	17
♀	0 – 45	476	56	528	57
♂	0 – 5	108	13	116	13
	6 – 12	196	23	198	21
	13 – 18	68	8	81	9
♂	0 – 18	372	45	395	43
♀ ♂	All groups	848	100	923	100
* Child bearing ♀		165	19	186	20

The adequacy of the samples re-examined at follow-up surveys is described in Table 3. Distribution by sex and age was quite similar to that in the total population injected. Re-evaluation of the prevalence of goiter was made at six, 12, and 18 months after injection. At the time of re-evaluation the examiners were ignorant of both the prior estimated gland size and the type of injection. The size of goiter and presence of nodularity were recorded. Clinical assessment was made by members of the same team throughout the program. Careful attention was paid to the possibility of any side effects, especially thyrotoxicosis. The response of the iodine-deficient thyroid gland to the injected iodized oil was assessed at different periods of time throughout the follow-up program by means of ^{131}I-thyroid uptake and thyroid hormone determinations in serum. The samples for these evaluations were taken from the same individuals surveyed before the injections, some of whom were injected with placebo and others with iodized oil.

The kinetics of iodine metabolism was investigated in a small sample from Gr-I to determine the thyroid radioiodine clearance, as well as plasma inorganic iodine (PII) and absolute iodine uptake (AIU). These studies were made according to standard procedures (36) after an intravenous injection of carrier-free Na ^{131}I in a sterile solution of 0.14 M NaCL. In cases of low thyroid uptake at a given time, the extrathyroidal neck radioactivity was calculated (36).

The duration of the effectiveness of the injected dose of Ethiodol was evaluated in a sample from both Gr-I subgroups, that injected with 2.0 ml and that with 0.2 ml, by measuring UEI at the 6th, 9th, 13th, 15th, and 19th month

Table 3. Distribution of population (by age and sex) at different stages of the study.

	Age & Sex	PERCENTAGE DISTRIBUTION OF POPULATION			
		PRIOR TO INJECTION	6 th month	12 th month	18 th month
PLACEBO	0 - 5	11	8	8	6
	♀ 6 - 12	20	23	21	22
	13 - 18	10	9	9	9
	♀ > 19	15	16	18	17
	♂ 0 - 5	13	10	10	11
	♂ 6 - 12	23	17	24	24
	13 - 18	8	16	9	10
		848	551 (65%)	587 (69%)	543 (65%)
IODIZED OIL	0 5	12	9	9	8
	♀ 6 - 12	19	23	21	22
	13 - 18	9	7	9	7
	♀ > 19	17	16	18	19
	♂ 0 - 5	13	11	10	9
	♂ 6 - 12	21	24	21	21
	13 - 18	9	10	12	13
		923	565 (61%)	592 (64%)	557 (61%)

after the injections. At the 19th month urine samples were taken in children under 5 years of age who received different doses of Ethiodol.

Particular attention was paid to the females in the childbearing age (16 to 45 years). As many pregnancies as possible occurring in the injected population from both Gr-P and Gr-I groups were followed throughout gestation. Newborns were recorded for the purpose of future evaluation. In addition, serum levels of thyroid hormone were investigated in pregnant women, as well as iodine content in human milk from lactating mothers.

RESULTS

Baseline Epidemiologic and Thyroid Function Studies

Summaries of the results of the preliminary survey and studies performed before injection appear in Tables 1, 4, and 5. As can be seen, the three villages had a similar prevalence of goiter as well as UEI and thyroid uptake. Accordingly, all three are considered as a whole in all other tabulations. The changing rate of goiter with respect to age and sex is shown in Figure 2. Fifty per cent of the population was affected with goiter during the first five years of life. This was primarily of the 0_b degree; thereafter, more than 90 per cent were goitrous. Size of the goiter and nodularity increased with age (Figure 3). The prevalence of "endemic cretinism" and other endemic defectives varied from 1.0 per cent to 3.6 per cent in the three villages. The baseline results of I-T_4, PBI, and total serum iodine are recorded in Table 6.

These data characterize an endemic goiter area with a high goiter prevalence, high thyroid uptake, low UEI, and moderately low levels of serum thyroxine.

Effect of Iodized Oil on Prevalence and Size of Goiter

Follow-up studies demonstrated that goiter prevalence in Gr-I decreased steadily for the 18 months after the injection program (Figure 4). A striking fall from 58 per cent to 16 per cent in visible goiter as well as from 86 to 52 per cent in palpable goiter occurred in Gr-I, while Gr-P changed only slightly from 52 to 45 per cent in the former and 81 to 78 per cent in the latter. In both groups the decrease was more marked in males than in females. In addition, the decline appeared to be more pronounced in the 13-18 and 0-5 year-old groups than in the 6-12 year-old subjects (Figure 5).

The progressive increase in per cent of subjects without goiter (0_a) in Gr-I is shown in Figure 6. The per cent of 0_b increased from pre-injection control values to the 18th month, while the per cent of goiters of larger size decreased proportionally. There were some small decreases in Gr-P.

Occurrence of Side Effects

In spite of careful observation for side effects, no instance of "Jod-Basedow" (18) or hypothyroidism (28) was recorded. In the case of one adult female with a large nodular goiter who through error received a full dose of Ethiodol (2.0 ml), a suspicion of thyrotoxicosis arose by the sixth month when she was observed to have an increased pulse rate, slight tremor, and warm skin,

Table 4. Prevalence of visible goiter (VG) and palpable goiter (PG) in the injected population.

VILLAGE	SEX	VG %	PG %	N° CASES
TAPO	♀	50	86	354
	♂	55	81	303
HUASAHUASI	♀	58	85	565
	♂	54	82	417
ATAQUERO	♀	62	82	85
	♂	55	70	47
ALL VILLAGES	♀	56	85	1004
	♂	55	81	767
	♀+♂	55	83	1771

*Table 5. Comparative urinary excretion of iodine (UEI) and ^{131}I thyroid uptake in the endemic areas and Lima**

	TAPO	HUASAHUASI	ATAQUERO	LIMA (26)
Urinary Iodine (µg/24h)	17.1 ± 1.5** (82)	18.1 ± 1.8 (77)	12.8 ± 1.8 (10)	115.1 ± 13.0 (10)
^{131}I-Uptake (% at 24h)	76.3 ± 1.03 (108)	72.8 ± 0.76 (98)	74.5 ± 3.42 (10)	34.4 ± 2.50 (10)

* *Capital of Peru, non-endemic.*

** *Mean ± SE.*

( ) *Number of cases.*

Table 6. Thyroid function in subjects from the Tarma endemic areas.

	Nº	Mean ± SE	Range
Urinary Iodine (µg/24 h)	159	17.2 ± 1.1	1.7 – 64.2
^{131}I – Uptake (% at 24 h)	206	74.7 ± 0.6	40.3 – 99.0
Serum Iodine (µg/100 ml)	30	5.5 ± 0.3	2.3 – 7.3
Serum PBI (µg/100 ml)	32	4.9 ± 0.2	2.2 – 6.9
Serum I-T_4 (µg/100 ml)	26	4.1 ± 0.3	1.7 – 6.4

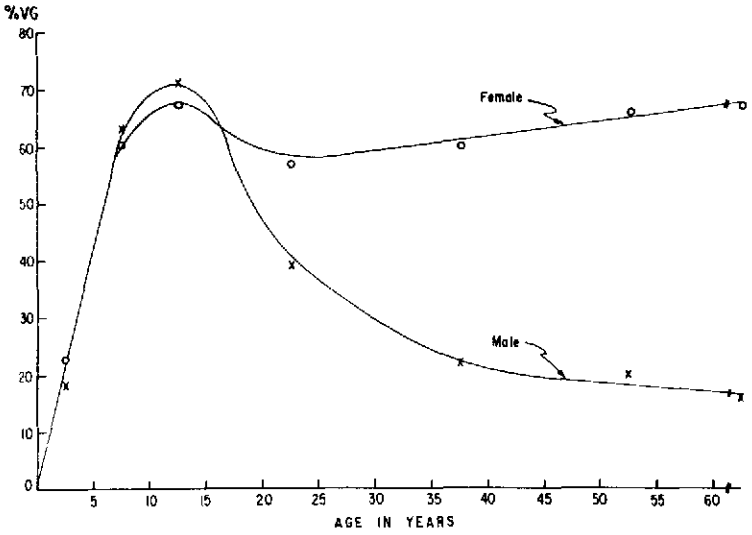

Figure 2. Distribution of visible goiter among male and female subjects with respect to age.

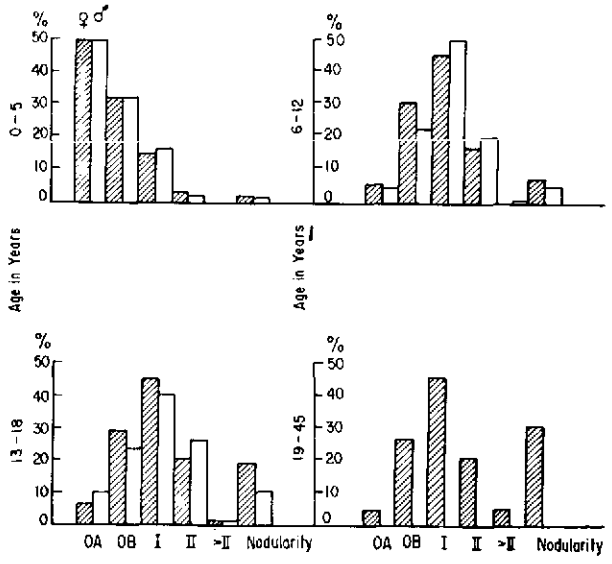

Figure 3. The relationship of size of a goiter and nodularity to age and sex in four different age groups.

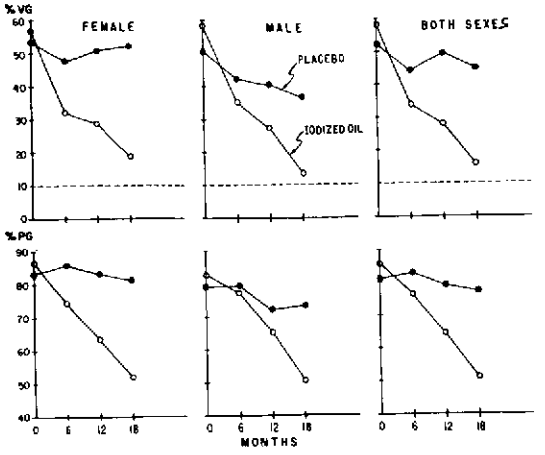

Figure 4. The changing level of goiter after administration of iodized oil compared to the trend after administration of a placebo. Upper figures: visible goiter; lower figures: palpable goiter.

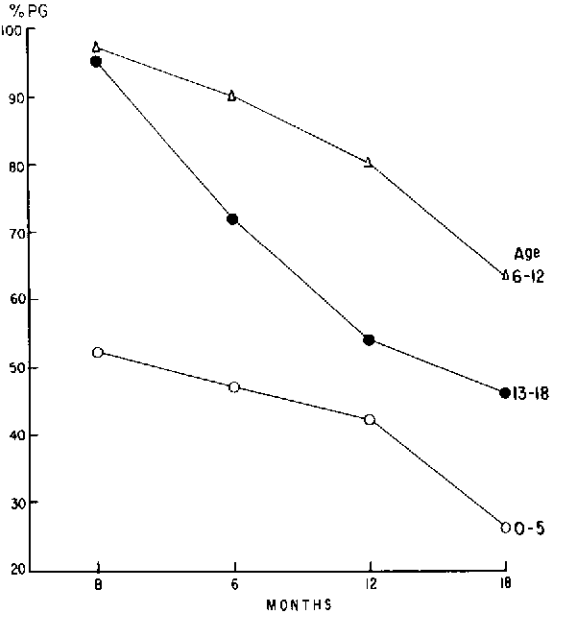

Figure 5. The fall in goiter prevalence in three different age groups after institution of the iodized oil program.

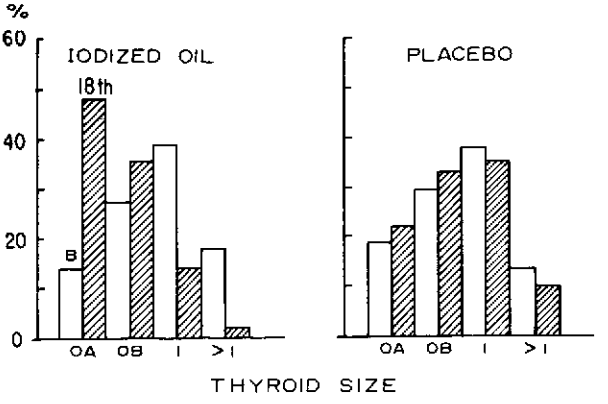

Figure 6. The progressive increase in the frequency of subjects found without goiter after administration of iodized oil as compared to the group receiving placebo.

but no laboratory documentation was possible at that time, and later, the patient appeared clinically normal. Neither iodine thyroiditis (8), nor "iodism" (37), nor local reactions were observed. Most of the newborn children from Gr-I mothers were seen within a few days or weeks after delivery, and none was found with goiter.

Changes of Thyroid Function in Response to Administered Iodized Oil

Changes in thyroid function tests at different stages of the study in Gr-I - 2.0 ml dose are shown in Figure 7. The 24-hour thyroid uptake fell from a control level of 75.1±0.9 to 15.8±1.4 per cent at nine months, but increased slightly to 19.5±0.3 per cent and 21.9±1.1 per cent at 13 and 19 months respectively. This change corresponded inversely to a rise above normal levels of both total serum iodide (SI) and PBI. These reached maximum levels by the ninth month and then fell to normal by the 19th month. Serum I-T_4 remained within normal limits. Thyroid radioiodine clearance in the patients studied was still depressed by the 19th month; PII, on the other hand, decreased from the 13th to the 19th month, but remained high. This resulted in an AIU within the normal range (Figure 8).

At nine months the group of women injected with only 0.2 ml of Ethiodol had 24-hour ^{131}I-thyroid uptake, SI, and PBI values which were not significantly different from those of subjects injected with 2.0 ml. However, by the 19th month, SI and PBI values for the 0.2 ml group showed no significant difference from those of Gr-P, while ^{131}I uptake and UEI values were still significantly higher in the former. Gr-P values before and after 19 months were similar (Table 7).

Urinary Excretion of Iodine

The values for UEI in relation to the dose of Ethiodol at two different age levels appear in Table 8. The 6-12-year-old group, which received about double the dose of iodine per kg of body weight (41.5 mg), demonstrated a higher UEI at six months than the 13-year and over group, which had an average dose of 23.0 mg. However, values in both groups continued to fall to equivalent levels throughout the later periods. The UEI for the latter group fell exponentially after six months. The slopes, and therefore the t 1/2, were similar for both 2 ml and 0.2 ml doses in this group (Figure 9).

The UEI after 19 months in children under 5 years of age demonstrated a direct correlation with the dose of Ethiodol injected (Figure 10).

Observations Made in Pregnant Subjects and in Newborn Children

A total of 92 deliveries were recorded among the women of child-bearing age. Of these, 58 were in Gr-I, the newborn children showing no apparent clinical difference from those in Gr-P over the observation period. The I-T_4 levels in the pregnant Gr-P had a mean value of 5.2±0.4 µg per 100 ml during the last five months of pregnancy, and thus failed to rise, as normally occurs. A mean value of 7.9±0.7 µg per 100 ml was found in the Gr-I subjects. The content of iodine in milk from nine women injected with 2.0 ml of iodized oil was 9.7± 1.2 µg per 100 ml. In four women injected with 0.2 ml, it was 1.9±0.7 µg per 100 ml, and in four injected with placebo, the range was from 0.0 to 0.5 µg per 100 ml. These observations were performed at the time of the 19th month control.

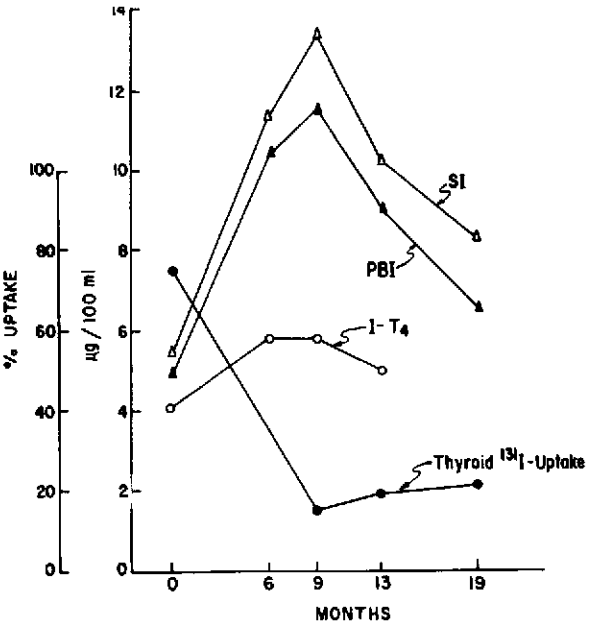

Figure 7. The change in radioactive iodine uptake, total serum iodide, protein-bound iodine, and plasma thyroxine in successive months after administration of iodized oil.

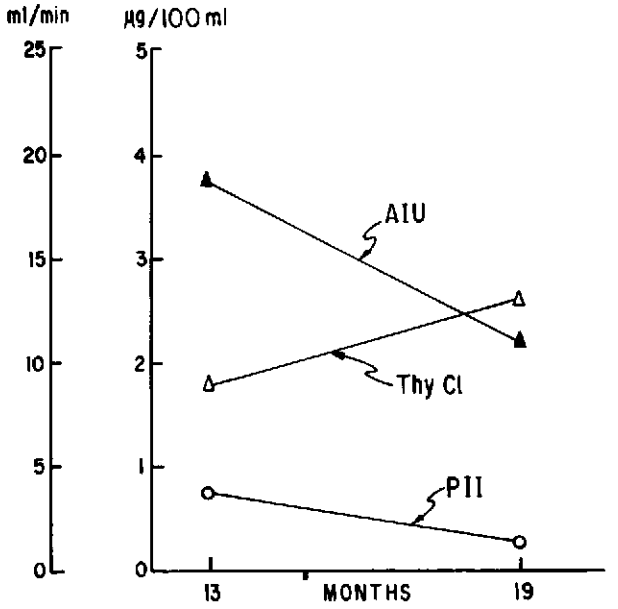

Figure 8. Change in the absolute iodine uptake, thyroid clearance, and plasma inorganic iodide after administration of iodized oil.

Table 7. Thyroid function tests 19 months after injection in the iodized oil groups and in placebo group.

	Before Injection	IODIZED OIL		PLACEBO
		Dose 2 ml	Dose 0.2 ml ≠	
^{131}I- UPTAKE % at 24 h	75.1 ± 0.9* (77)	21.9 ± 1.1 (19)	57 ± 3.1 (7)	77.2±1.5(41)
SERUM IODINE µg/100 ml	5.5 ± 0.3 (30)	8.3±0.7 (9)	5.6 ±0.7 (6)	5.1±0.3 (6)
SERUM PBI µg/100 ml	4.9 ± 0.2 (32)	6.6±0.7 (9)	5.6 ±0.7 (6)	4.5±0.3(6)
URINARY IODINE µg/24 h	16.0 ± 1.4 (58)	169.0±11.0 (70)	52.6 ±5.9(16)	14.6±1.6 (46)

≠ *Comparison of iodized oil 0.2 ml to placebo by the student's t test as an expression of the P value: Uptake P= < 0.001; SI, P= > 0.4; PBI, P= > 0.1; UEI, P= < 0.001.*

* *Mean ± SE.*

( ) *Number.*

Table 8. Urinary excretion of iodine (µg/24 hr.) in two different age groups injected with different doses of iodine (Ethiodol).

Age yr	Dose Ethiodol ml	Dose Iodine mg/Kg b.wt.	MONTHS AFTER INJECTION 6	9	13	15	19
6 – 12	2.0	41.5±1.1*	1079 ± 101	497 ± 49	346 ± 29	274± 24	169± 15
13 - >	2.0	23.0 ± 0.7	859 ±64	621 ±47	345 ± 29	253± 25	168± 16
	0.2	2.1 ± 0.1	190 ±27	--	81 ± 14	65 ± 7	53±6

* *Mean ± SE.*

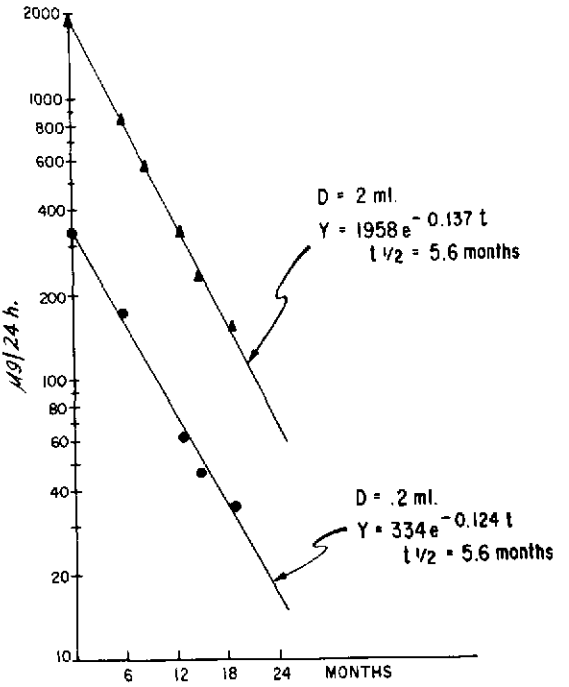

Figure 9. The fall in daily excretion of iodide in the urine after the administration of iodized oil.

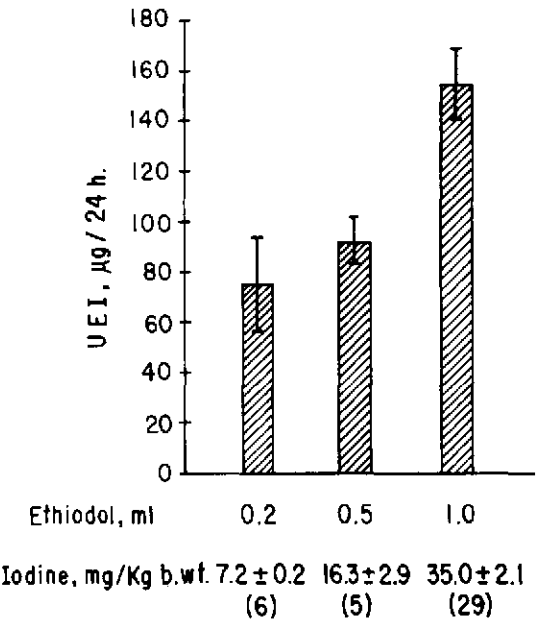

Figure 10. The relationship between dose of iodized oil and the urinary excretion of iodide 19 months after administration of iodized oil in children under five years of age.

DISCUSSION

The three villages selected for this study, Tapo, Huasahuasi, and Ataquero, are similar not only in socioeconomic level, ecology, and altitude, but also in relative iodine deficiency as demonstrated by high 24-hour ^{131}I-thyroid uptakes and low UEI. These findings are customarily found where there is widespread prevalence of goiter. For statistical purposes these villages have been treated as one. A progressive parallel increase in the prevalence of goiter was observed in boys and girls from birth to puberty, but after puberty goiter tended to persist in females but to regress spontaneously in males, a phenomenon previously noted by others (14, 21). Although a high thyroid iodine clearance is found in these subjects (10), the low PII may cause the AIU to be subnormal. This is reflected in the mean I-T_4 values not only in the lower normal range (3.7-7.9 µg per 100 ml in our laboratory), but also lower than the PBI values. Many individual values were below normal, but clinical hypothyroidism was not seen. An increased turnover rate of T_4 with a reduction

of the extrathyroidal organic iodine pool in goitrous patients, as demonstrated in the studies of Beckers et al. (1), may be one explanation for this observation. Alternatively, a more efficient biosynthesis of T_3 (15) may be another explanation.

In the injected population 63.1 per cent of those subjects without visible goiter were found to have enlarged glands by palpation. The overall visible goiter rate of 55 per cent corresponds to 83 per cent of palpable goiter. By both criteria of evaluation, the three villages were quite similar. In this population, which excludes males over 18 years, the per cent of large goiters and nodularity in both sexes progressively increased with age, but tended to be higher in women after puberty (Figure 3). The injected population was fairly well divided between Gr-P and Gr-I with regard not only to goiter prevalence, but also to age, sex, and percentage of women of child-bearing age (Table 2). These well-matched control groups permit the possibility of highly significant comparison of results. At the three follow-up surveys a similar distribution of the population has also been examined (Table 3).

The effect of the iodized oil injections on the prevalence and size of goiter is illustrated in the longitudinal follow-up carried out every six months (Figures 4, 5, and 6). A decline has been more evident in visible goiter than in palpable goiter at all intervals (72 per cent and 40 per cent respectively by the 18th month) as a result of progressive shrinkage of goiters of large size. Many of these have not completely disappeared, and are represented by palpable goiters of O_b degree as demonstrated in the rise in per cent of O_b degree (Figure 6). Whether these will completely disappear in time remains to be seen. A more marked fall of visible goiter in males than in females seems to be the result of the effect of iodized oil in addition to the spontaneous regression with aging in males observed in our initial survey and by others (14, 21) and in our control group over the 19 months as well.

The effectiveness of the iodized oil treatment as a preventive method in goiter is demonstrated by the fact that only 2.7 per cent of new cases were recorded in Gr-I versus 6.8 per cent in the Gr-P during the last two controls. These observations are in accord with expected results and confirm the previous observations in New Guinea (6, 16, 23), as well as preliminary results in an iodized oil preventive program in Ecuador (11).

Local reactions, such as those observed in New Guinea (16), or thyrotoxicosis, as reported by Stanbury (33) and as was suspected in some adult women with large nodular goiter in Ecuador (34) were not detected in our subjects.

The results of thyroid function tests at successive periods of time in the Gr-I fitted well with expectations for an iodine-deficient population. Shrinkage of goiter was accompanied by a depression in the high initial ^{131}I uptake to below normal values by the ninth month (when first tested) at a time when PII was above normal. This increased slightly at the 19 month survey, while the PII fell to normal values. The thyroid clearance rate of iodine was similarly low, and AIU became normal. In spite of depressed thyroid uptake and high levels of PII, the first aspect of the Wolff-Chaikoff effect (38) was not present at six months. Adaptation to high levels of iodine (3) must have been in operation by this time because there was no interference in thyroid hormone synthesis. T_4 values rose from the low basal level to the normal range.

In cases where only 0.2 ml of Ethiodol was administered ^{131}I thyroid uptake and values were still higher at 19 months than in those administered 2.0 ml. Values in the placebo group before and after 19 months were similar (Table 7). The latter finding indicates that the severe iodide deficiency remained the same in this endemic area. In the former group UEI values were high at the sixth month (Table 8) and it must be assumed that both UEI and PII values were probably even higher in the earlier months. Therefore, it may be assumed that this dose would not prevent the risk of thyrotoxicosis in those who are susceptible (9).

We do not think that it is necessary to await the reappearance of goiter to consider that the effect of the injection of iodized oil has ceased. The maintenance of UEI at a minimal value of 50 μg per 24 hours above basal excretion levels should be considered. An empirical formula for predicting the effect of a given dose on this minimal maintenance of UEI (Figure 11) is derived from the slopes of UEI over 19 months in both the 2.0 ml and 0.2 ml injected groups. These lines differ only in intercepts (Figure 9). By 15 months the UEI in the 0.2 ml group has reached this minimum, while the 2.0 ml group may be expected to drop to about this level by the 27th month. This has its counterpart at the 19th month control when the 24-hour thyroid uptake was 57.0±3.1 per cent in the 0.2 ml group and 21.9±1.1 in the 2.0 ml group. It may be predicted from this that subjects in New Guinea injected with 4.0 ml Neo-Hydriol (similar to Ethiodol) would show a fall to the minimal level by the 33rd month after injection. Actual results seem to fit with this prediction as judged from UEI value at 36 months (6).

If the exponential is used to calculate the total iodine excretion in the present study, then only 42 and 76 per cent of the injected dose was accounted for. Therefore, it is assumed that data on UEI reflect only the last component of a multiple exponential and thus only a part of the iodized oil contained in Ethiodol is responsible for the long-acting effect. These results also suggest that larger doses result in higher urinary losses during the first months after injection. Further support for this impression is provided by observations made in the 6-12-year group (Table 8) and in children under five (Figure 10). In the former, the dose of iodine in mg per kg body weight was about twice that in the over-13 group, but this resulted in no higher UEI values at 19 months after injections. However, in the latter urinary excretion of iodine at 19 months is found to be a semilogarithmic function of the injected dose. It is possible that iodized oil (or iodine) once absorbed from the muscle is in part stored not only in the thyroid gland, but also in other tissues such as the reticuloendothelial system, adipose tissue, or others. A slow release from these tissues may occur; thus its effect on the thyroid might continue longer than expected from UEI levels. Results published by Buttfield et al. (6) from studies in New Guinea four and a half years after an injection program seem to bear out this last possibility.

No differences in birth rate have been observed between Gr-P and Gr-I, nor has any case of goiter been recorded in the Gr-I. This latter observation favors the safety of the injection in women of child-bearing age. Although the fetal thyroid takes up proportionally more iodine than the mother's gland in the last trimester of pregnancy at a time that placental transport of iodine is also increased (30), the amount of iodine crossing the placental barrier in these subjects was not enough to produce goiter as has been reported in other

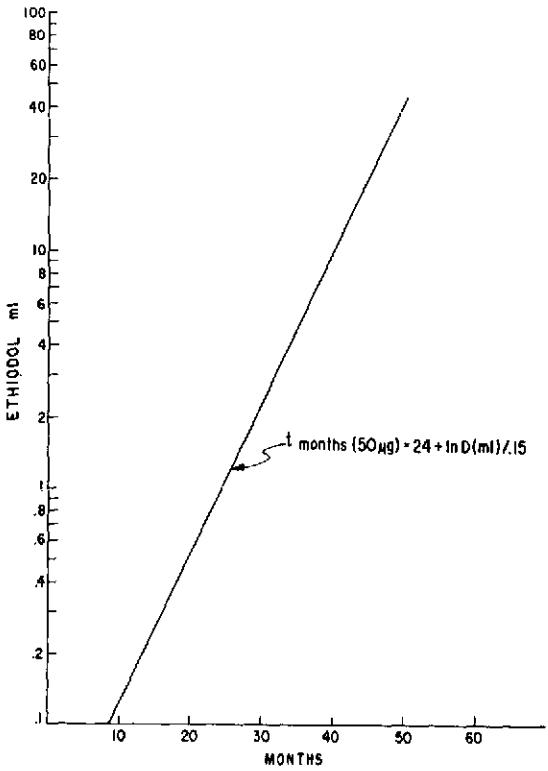

Figure 11. A theoretical formulation of the relationship of a given dose of Ethiodol versus time which is necessary to maintain a minimal urinary excretion of iodine. The plot is semi-logarithmic. For explanation see text.

circumstances (12). While the newborn children from both Gr-P and Gr-I, nine months after the injections, show no differences in clinical appearance, it is still too early and the number too small to realize the effect of iodized oil in prevention of mental deficiency or neurological and motor defects. It is possible that these conditions may become more obvious at a large age. A full evaluation of newborn children at 2 years of age, as well as a long follow-up, will provide more reliable information in this regard. The measurements of iodine in milk of mothers from Gr-I indicate that the newborn children have been receiving a good iodine intake, while the supply of iodine by this means to this group in Gr-P is probably suboptimal.

A finding of possible significance is that in pregnant women the physiologic rise of PBI during normal pregnancy failed to occur in Gr-P. Many of these values were in the hypothyroid range, and corresponding I-T_4 values were even lower. Although an impaired thyroxine-binding protein system may have been the cause of this phenomenon, it seems to be ruled out by contrasting rising values of I-T_4 in Gr-I as in normal pregnant women. Choufoer et al. (7)

made the same observation in New Guinea natives and found that TBG was normal. A few cases have even decreased their I-T_4 values as delivery approached. The fetal thyroid undoubtedly shares the low iodine supply from the diet with the mother's gland. This sharing would result in a lower amount of iodine available for the mother's gland, which in turn would be unable to supply the required hormone levels (7). In any case, it may be expected that the hormone contribution of the mother to the fetus must be small, if any, because free T_4 in such a subject might be decreased during pregnancy. Whether the fetus is capable of synthesizing its own T_4 is unknown. The absence of goitrous newborns in endemic areas suggests that the fetal hypothalamic-pituitary control is not disturbed, and therefore that the thyroid hormone levels are normal, but this necessarily needs further investigation.

SUMMARY

Three villages in the sierra of Peru with a high prevalence of endemic goiter and endemic cretinism were selected to study the prophylactic effect of a single intramuscular injection of iodized oil (Gr-I) or a placebo (Gr-P). The dose varied from 0.2 ml to 2.0 ml (475 mg iodine per ml) according to age and nodularity. Evaluations have been performed periodically through an 18-month follow-up.

The Gr-I showed a decrease in the prevalence of goiter from 58 per cent to 16 per cent (visible) or from 86 per cent to 52 per cent (palpable). Only 2.7 per cent of new cases of goiter were observed in Gr-I versus 6.8 per cent in Gr-P. The injection of iodized oil resulted also in a decrease in the initially high ^{131}I thyroid uptake and increase in the PII, with AIU in the normal range. Despite a high PBI, serum I-T_4 remained normal. UEI rose from low basal values to high levels by the sixth month. Thereafter a decrease toward preinjection levels was observed following an exponential function. The slopes were similar with both 0.2 ml or 2.0 dose. The results have been used to propose an empirical formula to predict the time when UEI would be 50 μg over basal values.

Iodine determinations in the milk of lactating mothers showed that newborns in Gr-I continued to receive a good supply of iodine. The rise of PBI (or total T_4) occurring during normal pregnancy was not observed in Gr-P, but Gr-I showed an increase.

Although no clinical differences have been observed between newborns in both groups, it is still too early to know the effectiveness of iodine administration in preventing the occurrence of endemic defectives. Neither side effects nor goitrous newborns have been recorded among Gr-I subjects.

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