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SELECTED RESEARCH ACTIVITIES OF THE INSTITUTE OF NUTRITION OF CENTRAL AMERICA AND PANAMA (INCAP)

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PROGRESS REPORT ON SELECTED RESEARCH ACTIVITIES OF THE INSTITUTE OF NUTRITION OF CENTRAL AMERICA AND PANAMA (INCAP) DURING 1965*

As in previous reports to the PAHO/ACMR, no attempt is being made to present a comprehensive review of INCAP research activities, but rather to summarize progress in specific projects. Three areas were selected: food and agricultural sciences, epidemiological studies of interaction between nutrition and infection, and clinical studies of severely malnourished children.

I. THE USE OF COTTONSEED PROTEIN CONCENTRATE AS THE MAIN PROTEIN SOURCE IN SWINE, POULTRY AND RUMINANT FEEDING

It is a well-known fact that, as in many underdeveloped areas, there is a protein deficiency problem affliciting the human population of the Central American countries. There are several well recognized factors responsible for this deficiency, among which one of the most important is the low availability of the better dietary sources of protein, such as milk, meat and eggs. This low availability, in turn, can be traced to the lack of appropriate protein concentrates needed to formulate economical,

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simple and highly nutritive rations for the animal population at the industrial or home level. In short, both the human and animal populations are limited, in their respective potential of development and production, by a lack of good quality protein concentrates.

There is, therefore, a great need to increase efficiency in animal raising, particularly where swine and poultry are concerned, due to increasing need and demand for their products. Because of the availability of cottonseed in the area, research on an increased, safer and more efficient use of cottonseed protein in animal feeding was initiated 3 or 4 years ago, and expanded more recently to include swine, broiler and egg production, poultry, and ruminants.

A. Studies with Swine

Previous work indicated that weanling pigs could be fed cottonseed meal or flour as main protein source, without adverse physiological effects, when rations were supplemented with ferrous sulfate and calcium hydroxide. In this situation, feed conversion values and the weight gain of the animals were slightly lower than those resulting when soybean meal was used, but the difference was not significant. Since these results became available, additional studies have indicated differences in response with respect to the

type of process employed for the production of the cottonseed protein concentrate. Materials prepared by pre-press solvent extraction are nutritionally superior, and safer than cottonseed meal prepared by screw presses. Although ferrous sulfate and calcium hydroxide additions are beneficial in both flours, their need appears to be greater in the screw-press meals. Furthermore, screw-press meals fed to swine without Ca and Fe cause a high mortality, while pre-press solvent extracted meals do not, even if the available lysine values of both are equally high. In a recent study, swine fed soybean flour as main source of protein gained 53.8 kg in 12 weeks, while those fed pre-press solvent extracted cottonseed flour with added Ca and Fe gained 52.6 kg over the same period of time. Feed efficiencies were similar in both groups.

In the studies referred to so far, the animals were fed over periods of 12 to 15 weeks, attaining weights of 100-125 lb. This is still low for marketing. Therefore, studies have been carried out in which fattening finishing rations were tested, using corn, sorghum and wheat middlings supplemented by 8% cottonseed or soybean protein. Study results indicated that the addition of protein supplements, whether cottonseed or soybean, to corn and sorghum, produced daily gains of 0.9 to 1.0 kg in swine. In a 10 to 12 week period the animals had reached a market weight of

200 lb. These studies indicate, therefore, that cottonseed protein can be used efficiently in swine feeding from weaning to marketing weight, yielding results which are comparable to those obtained with soybean meal. There are still some aspects to be studied in order to render the use of cottonseed meal in swine feeding efficient and practical, which will be undertaken in the near future.

B. Studies with Baby Chicks

Several experiments have been conducted to determine the efficiency of broiler production using cottonseed flour as the main protein source. These studies have indicated that 50% prepress solvent extracted cottonseed flour will produce birds weighing around 410 grams in a 5-week period. These rations are deficient in lysine, due to which, in other studies, cottonseed flour was supplemented by 8% dehydrated whole fish meal. In these experiments, final weight of the birds in 5 weeks was around 700 g, with excellent feed conversion values. Not all fish meals tested gave good results. This was, however, attributed to a high salt concentration in the fish. These rations are easily prepared and the materials are economical and readily available.

C. Studies with Hens

Cottonseed flour has also been tested as the main protein source in feeds for hens. It is well known that eggs from hens fed on rations containing more than 10% cottonseed meal become altered in physical appearance. The egg white turns pink and the yolk acquires a dark tint. In one of our studies, hens were fed the following rations: 1) control, no cottonseed protein, 2) cottonseed flour (pre-press solvent), 3) same as 2 but with 1% calcium hydroxide, 4) same as 2 with 0.1% ferrous sulfate, 5) same as 2 with both calcium and iron, 6) same as 2 but with calcium, iron and 2.5% cottonseed oil, 7) same as 2 with 2.5% cottonseed oil, 8) cottonseed flour plus lysine, 9) cottonseed meal, calcium and iron, and 10) cottonseed meal alone. The eggs collected were stored for two months, and then classified according to color changes observed by 5 persons. It was evident that eggs from hens fed rations 1 (control), 3, 4, 5 and 6 were equal and top grade. Eggs from hens fed rations 2, 7 and 8 were slightly colored; those from hens fed diet 10 were very dark and those fed ration 9 were dark, but not as markedly so, as those fed 10. These results were interpreted to indicate that pre-press solvent extracted meal is superior to press meals and that Ca and Fe help in decreasing

the damage caused eggs by feeding high levels of cottonseed flour to hens.

D. Studies with Ruminants

There are, in Central America, many agricultural by-products such as corn cobs, corn leaves and stalks, coffee pulp, lemon and citronella grass ,bagasse, which are used in animal feeding to a very limited extent only. These materials are characterized by a relatively high content of crude fiber and little protein. Since available amounts are quite large, it is desirable to use them in the feeding of ruminants capable of very efficient crude fiber utilization, provided adequate amounts of other nutrients are present in the ration. Studies have been undertaken using sheep to determine whether or not the cellulose in these by-products could be better utilized by feeding the animals these materials in conjunction with cottonseed meal as source of protein, and cotton-seed meal plus molasses as source of protein and readily available energy respectively. The results are being evaluated by measure-ments of increase in cellulose digestibility. Studies carried out so far suggest that corn stalk and leaves can be utilized more efficiently when they are supplemented with the minimum amount of protein requirement for sheep derived from about 80-120 g of cotton-seed meal and 5% sugar molasses.

II. ECOLOGICAL STUDIES ON THE INTERRELATIONSHIPS BETWEEN MICROORGANISMS AND THE HEALTH AND NUTRITIONAL STATUS OF THE HOST

A. Incidence of Diarrheal Diseases

The longitudinal study of the colonization of the intestine of babies and its relation to health and nutrition, on which a report has been presented to the PAHO/ACMR , has progressed satisfactorily according to plan. A preliminary analysis of the incidence of diarrheal diseases and their possible etiological factors was done for the period comprising the first 77 calendar weeks of observation. Thus, it was possible to make a study of a few 18-month-old children, several one-year-olds, and many under one year of age. (Table I).

Fifty-nine babies followed during the first 12 weeks of life and representing 730 person-weeks of experience, showed 25 episodes of diarrhea or 3.42 diarrheas per 100 person-weeks. This rate increased more than twice for 18-month-old children.

This type of analysis also permits an estimation of the incidence and relative importance of each agent in diarrheal disease.

TABLE I

DIARRHEAL DISEASE AND SHIGELLOSIS
SANTA MARIA CAUQUE (1964-1965)

Age (Weeks)	No. Infants	Person-Weeks At Risk	Diarrheal Disease		Shigellosis					
			No. of Episodes[1]	Episodes per 100 Person-Weeks	Clinical Episodes[2]		Carriers[3]		All	
					No. and Rate per 100 Person-Weeks		No. and Rate per 100 Person-Weeks		No. and Rate per 100 Person-Weeks	
					Number	Rate %	Number	Rate %	Number	Rate %
0-12	59	730	25	3.42	0	0	1	0.13	1	0.13
13-25	46	535	30	5.61	0	0	0	0	0	0
26-38	36	420	23	5.48	0	0	0	0	0	0
39-51	28	249	14	5.62	4	1.61	1	0.40	5	2.01
52-64	11	71	6	8.45	3	4.2	2	2.8	5	7.0
65-77	5	45	4	8.9	1	2.2	2	4.4	3	6.7

1 Episodes were considered independent if separated by 14 days or more.

2 A case was considered as such if it began during the period stated.

3 A carrier was considered as such if this condition arose within the period stated. A new carrier was considered as such if more than 14 days had elapsed after previous one.

As an example, infection with Shigella is shown in Table I. No clinical episodes were observed in children under 9 months. A healthy carrier was observed, however, in a child under three months of age.

As age increased, clinical cases were more frequent, as well as convalescent carriers. This observation underlines the importance of studying very young infants in order to test the significance of agents in diarrheal disease. In the specific case of shigellosis, it seems that a considerable proportion of individuals, experiencing Shigella infections for the first time, become persistent carriers of the organism.

The Shigella carrier rate was 2 per 100 person-weeks of experience in the 39-51 week-age group, increasing to 6.7 per 100 person-weeks in the 65-77 age group. Of these 4.4 per 100 person-weeks were convalescent carriers.

B. Etiologic Agents and Diarrheal Disease

A longitudinal study provides one of the best ways to test the significance of microbial and parasitic agents in disease. The reason is that internal controls can easily be established and that these controls are probably the best from an epidemiological standpoint.

An analysis was made of the first 65 episodes of diarrhea experienced by a cohort of 45 children (1964-1965). For every child with diarrhea, one control was obtained without diarrhea, matched, case by case, by age, locality and season. In other words, the first fecal specimen collected from a child during the course of diarrhea was compared against the first sample collected during the same week from a control baby without diarrhea. The microbiological and parasitological results are presented in Table II.

It can be noted that agents that have been incriminated by others as important in diarrheal disease, were found more frequently in children with diarrheal disease than in the corresponding controls. It should be stressed that viruses were also more frequent in diarrheal disease, particularly viruses paralytogenic in sucking mice inoculated under 24 hours after birth. The differences were statistically significant. At the present time a total of 120 episodes of diarrhea and an equivalent number of controls are available for analysis. About 30% of all diarrheas, however, were without a demonstrable agent.

TABLE II

AGENTS IN 45 CHILDREN WITH AND WITHOUT DIARRHEA

SANTA MARIA CAUQUE (1964-1965)

Agent	Episodes of Diarrhea	Probability	Matched Controls
Total	65		65
Polio-like	3		7
Echo-like	3		3
Coxsackie-like	8		1
Other enteroviruses-like	9		4
Adenovirus	3		0
All viruses except polio-like	23	$P<0.01$	7
All viruses	25		14
Salmonella	2		1
Shigella	2		0
Entamoeba histolytica	4		0
Other parasites	10		4
Viruses (except polio-like)	-		-
Salmonella-Shigella	-		-
Entamoeba histolytica	31 (47.7%)	$P<0.001$	9 (13.8%)
Viruses (except polio-like) Salmonella-Shigella	44 (67.7%)	$P<0.001$	13 (20.0%)
Parasites	-		-

C. Studies on the Intestinal Biota

During the past few months an interest has been developed in our laboratories concerning the quantitative aspects of the intestinal biota and its relationships to health and disease. Shigella infection was selected as a model for the development of these quantitative studies. A methodology was succesfully established permitting quantitation of the following groups of bacteria: coliforms, slow lactose fermentors, Shigella, micrococci, Candida, enterococci, streptococci, lactobacilli, clostridia, and bacteroides. It was possible in this manner to quantitate the number of Shigella excreted which was shown to range from 10^2 to 10^8 bacilli per gram of wet feces. A correlation of high numbers of Shigella with the acute phase of diarrhea, or with a recrudescence of symptoms, was observed. It seemed apparent that there was an inverse relationship between the numbers of Shigella and those of Escherichia coli.

Since there are no standards established for the intestinal biota of normal children, it is not possible to compare the findings of this study with preestablished patterns.

In general, it was evident that the various components of the intestinal biota as measured in the feces, will fall into

three categories: (a) organisms always found in large numbers in a consistent manner (bacteroides, lactobacilli, streptococci); (b) organisms found in low numbers, tending to increase under abnormal circumstances (Candida, clostridia, micrococci and staphylococci); and (c) organisms varying greatly from day to day, sometimes attaining high counts (enterococci, coliforms, slow lactose fermentors) (Table III).

From these data it is logically apparent that the pabulum, which is highly dependent on the food ingested, could be a determinant of the growth of some groups of bacteria, particularly those appearing in erratic numbers. It was of interest to note that the biota of children living in an Indian village, under a protein-deficient diet made up principally of corn, contains large numbers of coliforms (10^8 per gram of feces). As opposed to this, counts were 10^3 - 10^6 in children from an institution for convalescents in Guatemala City, where a diet with a high content of good quality protein is provided.

Diarrhea and fever were observed following alterations in the relative proportions of the biota components.

TABLE III

QUANTITATIVE DETERMINATIONS OF MICROORGANISMS IN THE FECES OF 6 CHILDREN WITH *SHIGELLA* INFECTION

(number of microorganisms expressed in $\log_{10}$ per g of feces)

Child	Period observed (days)	No. of samples	Coliforms	SLF	Shigella	Enterococci	Microaerophilic lactobacilli	Anaerobic[1] lactobacilli	Microaerophilic streptococci	Anaerobic[1] streptococci	Bacteroides[1]	Clostridia[1]	Staphylococci	Candida
F.V.	35	35	5.5±1.2 (<3-8) 2 days<3	5.1±1.0 (<3 - 7) 1 day<3	5.1±1.6 (<2 -7) 4 days<2	7.3±0.7 (<5 -9) 1 day<5	8.1±0.8 (7 - 10)	9.2±1.0 (7 - 10)	8.4±0.9 (6 - 10)	9.2±0.8 (8 - 10)	8.2±0.7 (7 - 9)	20 days<7	3.7±0.6 (<3 - 5) 11 days<3	3.2±0.6 (<3 - 4) 16 days<3
S.C.	35	35	4.8±0.9 (<3-7) 5 days<3	5.4±0.9 (<3 - 7) 2 days<3	4.2±1.3 (<2 -6) 10 days<2	7.1±1.2 (<5 -10) 1 day<5	7.2±1.0 (6 - 9)	8.5±1.0 (7 - 11)	8.8±0.6 (8 - 10)	9.0±0.8 (7 - 10)	8.1±0.8 (7 - 9)	20 days<7	3.5±0.6 (<3 - 5) 15 days<3	3.2±0.4 (<3 - 4) 16 days<3
N.G.	35	35	7.8±1.2 (4-9)	6.1±0.9 (<4 - 7) 14 days<4	3.6±1.9 (<2 -7) 5 days<2	6.8±1.0 (5 - 9)	8.4±0.8 (6 - 9)	9.0±1.2 (7 - 11)	8.3±1.0 (6 - 10)	8.7±1.0 (7 - 10)	7.9±0.7 (7 - 9)	8.4±0.8 (7 - 10)	3.6±0.6 (<3 - 5) 11 days<3	3.1±0.3 (<3 - 4) 23 days<3
J.R.	28	28	5.5±1.2 (<3-9) 1 day<3	5.7±0.9 (4 - 7)	5.0 (<2 -5) 27 days<2	6.8±0.9* (<5 -8) 2 days<5	7.7±0.8 (7 - 10)	8.2±0.6 (7 - 9)	8.3±0.9 (7 - 9)	8.7±1.1 (7 - 11)	8.6±0.7 (8 - 10)	7 days<7	3.2±0.2 (<3 - 4) 9 days<3	3.0±0.0 (<3 - 3) 13 days<3
A.A.	55	16	8.5±0.8 (6 - 9)	6.9±0.9 (5 - 8)	2.8±1.2 (<2 -6) 11 days<2	8.5±0.9 (7 - 9)	7.3±0.8 (6 - 8)		8.5±0.8 (7 - 10)				3.7±0.8 (<3 - 5) 3 days<3	4.1±0.6 (<3 - 5) 1 day<3
E.G.	32	12	8.2±0.2 (7 - 9)	6.6±1.1 (<4 - 8)	3.7±2.1 (<2 -7) 3 days<2	7.6±1.4 (<5 -9)	(8.7±1.0 (<6 -10)		8.6±0.7 (7 - 10)				3.9±2.0 (<3 - 6) 4 days<3	3.9±1.0 (<3 - 7) 1 day<3

1 Observed only during last 20 days.

* Observed only during last 7 days.

The number of children examined up to the present time is small. Further observations will be made to broaden understanding of the significance of intestinal biota, its interrelationships in connection with diet, and its effects on the general health and nutrition of the host.

D. Effect of Diarrhea and other Illnesses on Growth

Up the the age of 6-7 months, the growth of cohort children was generally good. During this period, children are exclusively breast-fed, and then supplementation begins with the introduction of fluids, thin gruels, thick gruels, and solids. By the time they are one year old, children receive supplements to maternal milk representing all foods consumed by the adult. All supplementary foods provided not more than 20% of the recommended allowances for proteins, calories and other nutrients, and although it has not been possible to quantitate the mother's milk ingested by these babies, it is very doubtful that it can supply the rest of nutrients needed.

At the age of 6-7 months, the weight curve departs from the lower Iowa standard limit. In order to see if there is a correlation between disease and malnutrition, analyses were made of the various parameters measured in this study. The analysis of weight and disease is presented as an example.

Weight curves were fitted by means of the least squares method, using the equation $y = a \pm bx$, where y = weight in kilograms; "a" and "b" are constants of weight at birth and growth rate (slope) respectively.

Since departure from normal standards occurs at the approximate age of 6 months, fitting was done for the period between 6 months and 1 year after birth. The "b" constants found for the first 24 children who had completed one year of life are presented in Table IV.

RATE OF WEIGHT CHANGE "b" IN 24 INFANTS DURING THEIR SECOND SEMESTER OF LIFE

SANTA MARIA CAUQUE (1964 - 1965)

Values of "b"			
1st Quartile	2nd Quartile	3rd Quartile	4th Quartile
.227	.154	.133	.030
.222	.152	.130	.027
.220	.150	.128	.014
.218	.149	.094	.009
.179	.146	.087	-.002
.158	.138	.069	-.082

It can be observed that the "b" values go from 0.227 for the child with the best growth rate, to -0.082 for the child with the poorest weight gain. For every child a comparison was made between "b" and total days of illness, which yielded a correlation of 0.39, which is significant at the 5% level.

In Table V, data are presented on the total number of days for each illness in two groups of 6 children each: one showing the largest "b" and the other the smallest "b".

It is evident that there was a significant association between disease incidence and growth for practically all observed clinical entities and combinations of same. It should also be mentioned that even greater correlations were found between growth and some indices of dietary intake. Whether children developed more clinical disease because they showed lower weight increments (due to malnutrition), or whether they had lower increments because they had experienced more days of illness, cannot be said. Further analyses and observations may help in clarifying this function.

TABLE V

DAYS OF ILLNESS IN 6 CHILDREN WITH THE LARGEST "b" (1st Quartile) AND 6 CHILDREN WITH THE SMALLEST "b" (4th Quartile) CALCULATED FOR THE SECOND SEMESTER OF LIFE[1]

Disease	In children with greater increments	In children with lower increments	Values of Chi square	Probability
Total	417	693	91.8	<0.001
Diarrheal disease	100	170	19.3	<0.001
Upper respiratory disease	106	115	0.3	N.S.[2]
Bronchitis and bronchopneumonia	7	24	9.2	<0.001
Conjunctivitis	49	94	14.6	<0.001
Thrush	5	16	5.7	<0.02
Stomatitis	15	23	1.6	N.S.
Measles	19	49	13.4	<0.001
Associated illnesses[3]	107	202	31.4	<0.001

[1] Morbidity includes all days of illness during the whole year.

[2] N.S. = not significant at the 5% level.

[3] Associated illnesses:

Conjunctivitis + Upper respiratory disease
Diarrhea + Upper respiratory disease
Diarrhea + Conjunctivitis
Diarrhea + Stomatitis
Diarrhea + Upper respiratory disease + Conjunctivitis
Cellulitis + Upper respiratory disease
Thrush + Conjunctivitis

III. STUDIES ON THE NATURE AND PREVALENCE OF ANEMIA IN SEVERE PROTEIN-CALORIE MALNUTRITION

A. Background

There have been many studies published which describe a moderate anemia that is in most instances normochromic-normocytic, as a constant feature of severe protein-calorie malnutrition. When there is a marked degree of anemia, other causes besides protein-calorie deficiency have been incriminated. These are primarily deficiencies of folic acid, vitamin B_{12}, iron, vitamin E and probably zinc and copper. The evaluation of the degree of anemia has been made only in terms of concentrations of hemoglobin or red cells per 100 ml of blood. The usual anemia has been characterized by a content of 8 to 12 grams of hemoglobin and 25 to 35% packed red cells per 100 ml of blood. Also stated is the fact that the majority of cases respond to a complete diet with recovery of hemoglobin concentration, simultaneously with the correction of the protein and calorie deficiency.

A complicating factor in the study of the anemia of protein-calorie malnutrition, as well as of other manifestations of the syndrome is the difficulty in quantifying the degree of protein deficiency.

The Biomedical Division of INCAP has found in the Creatinine-Height Index a valuable tool for the quantification of this deficiency. The Index reflects the degree of muscle mass for the height of the patient in relation to the normal muscle mass expected for his height. It has been found that children appearing to be fully recovered -according to the usual criteria of normal serum protein concentration, normal levels of serum enzymes, normal weight for height, and absence of clinical signs of any deficiencies- may still have a low muscle mass for their height. This indication of protein nutriture usually recovers more slowly than the other parameters utilized to judge protein repletion.

Studies in other areas of physiological function in protein-calorie malnutrition have disclosed that there is a significant correlation between the recovery of these functions and the protein repletion as measured by the Creatinine-Height Index.

Another important consideration in our studies is that, based on the previous findings, we have wondered if the anemia reported in protein-calorie malnutrition on the basis of hemoglobin concentrations is a true anemia, considering the definition of anemia as an amount of total circulating hemoglobin inadequate to fulfill the oxygen transport demands of the total active

tissue mass. If the severely malnourished individual has lost a large amount of metabolically active tissue, his total circulating hemoglobin could be reduced and yet still be sufficient to supply the needed amounts of oxygen to the tissues. Since the vascular space is not as reduced as the active tissue mass, this adaptive phenomenon could be accomplished by a mechanism of blood dilution. This would become manifest by means of a decreased hemoglobin concentration, such as has been reported. If this were the case, this lowered hemoglobin concentration should not be considered as a true anemia, in physiological terms.

B. Specific Aims of Present Studies

1) To determine whether or not there is a true anemia in protein-calorie malnutrition, evaluated in terms of oxygen transporting capacity in relation to oxygen demands.

2) To attempt to establish the specific roles of protein deficiency and the interactions between protein deficit and other erythropoietic substances in the production of the blood picture observed in severe protein-calorie malnutrition.

C. Materials and Methods

Thirteen children with protein-calorie malnutrition have been admitted into INCAP's Metabolic Ward following careful

screening designed to make certain that they had the least possible complications. These children were observed very closely, with particular attention given to the presence of infections which could interfere with erythropoiesis. During the first 24 to 48 hours they have received treatment directed toward the normalization of water and electrolyte imbalances. Crystalline penicillin is administered intramuscularly since it has proved very valuable in the prevention of bronchopneumonias in severely malnourished children. During this period, all the original hematological studies are carried out. Then the children are placed at random in two plans of study. Plan No. 1 consists of the administration of vitamin-free casein supplemented with methionine, at a level of 0.7 grams per kilo for a period of 10 to 14 days, after which folic acid and vitamin B_{12}, in doses of 50 micrograms and 0.01 micrograms per kilo respectively, are administered every day intramuscularly for the duration of the study. Protein intake is maintained at 0.7 grams per kilo during an additional period of 10 to 12 days, at the end of which it is increased to 3 grams per kilo with methionine-supplemented casein. This diet is continued until the end of study period. After 10 to 12 days of this intake, oral vitamin E is started at 50 milligrams/day; and 10 to 12 days later, ferrous sulfate, equivalent to 60 milligrams of elementary iron, is supplied orally every day.

Plan Two differs from Plan One in that, after the initial studies are completed, the child is placed at 3 grams of methionine-supplemented casein per day, adding folic acid and vitamin B_{12}, vitamin E and iron at intervals of 10 to 12 days in the same doses as given in Plan One. With this therapeutic scheme we have tried to discover whether or not there is a response to high protein levels alone, to vitamin B_{12} and folic acid in the presence of a low and high protein intake, and later on whether or not there are specific responses to vitamin E and iron.

From the beginning of treatment, all the children are supplemented with vitamin C, vitamin A and B complex with the exception of folic acid and vitamin B_{12}.

These two therapeutic diets have been applied to all children admitted into the metabolic ward but, due to several complications which arose during treatment of the children under study, one has only been able to follow three of each group longitudinally. Only one child has died of severe staphylococcal pneumonia. In the near future, we hope to complete a series of 5 from each group and then alter the sequence of experimental treatments by providing iron or vitamin E as the first supplement introduced to the children receiving the two different levels of protein.

During the period of study a series of examinations are performed as follows:

Daily reticulocyte counts throughout the whole period of study; and the following each time that there are changes in the treatment or when considered necessary: complete blood counts with red cell size determination; bone-marrow aspiration for the study of cell morphology, spicule iron and sideroblast counts; blood volume and total circulating red cell mass and hemoglobin determined by means of ^{51}Cr labelling of red cell and Evans Blue dilution; red cell life span obtained by means of ^{51}Cr tagged red cells; serum levels of iron, total iron binding capacity, vitamin B_{12} and folates, vitamins E, A and C and total proteins and their electrophoretic fractions; red cell reduced glutathione and glucose-6-phosphate dehydrogenase levels as well as osmotic, saponin and peroxide fragilities; formiminoglutamic acid and imidazol acrylic acid urine excretions after a histidine load.

Urine erythropoietin is quantitated repeatedly in 24-hour urine specimens which are sent to Doctor Clement Finch in Seattle, Washington. Nitrogen balance and urinary creatinine excretions are also determined during the period of study.

D. Results Obtained So Far

All the children under study have markedly low total serum proteins on admission, as reported by many workers.

The Creatinine-Height Index indicated a marked deficit in muscle mass, ranging from 30 to 60% of normality for the individual child's height. The red cell mass and total circulating hemoglobin are also markedly low, measuring an average of 35% expected for their height. This marked reduction is independent from the values obtained on packed red cell volume and hemoglobin concentrations which were within the normal range in 5 of the 13 patients studied on admission.

The Creatinine-Height Index attains normal values approximately two and a half months after initiation of the high protein intake. The red cell mass and total circulating hemoglobin remain low until the Creatinine-Height Index approaches 70% of normality, at which time they rise, approaching the normal levels for their height. The total blood volume, in contrast, begins to rise as soon as the therapeutic diet is instituted, therefore producing a drop in hemoglobin concentration and hematocrit during the initial period of recovery.

The red cell life span has been found moderately shortened in five of the six cases followed longitudinally the decrease ranging from 24 to 11.4 days.

Specific responses to the various treatments, as followed by reticulocyte counts, have disclosed that five out of the six children under study respond to casein administration. The other child, already ingesting 3 grams of protein per kilo, responded to folate and B_{12}.

Response to protein has been accompanied by a drop in serum iron and an increase in total iron binding capacity, both initially low. Initially, the reduction in iron binding protein was greater than in the serum iron and, therefore, the percentage of saturation was high.

It is important to mention that although we have not found iron in bone marrow spicules in any of these children, the sideroblast counts have been normal to high upon admission, indicating that iron has not been the limiting factor in erythropoiesis. Following the reticulocytosis obtained with protein or with protein plus folic acid and B_{12}, the sideroblast counts dropped to very low levels, indicating that iron may then become limiting in blood production.

On admission, the serum folic acid levels have been under 5 nanograms in all but one child, who had 10 nanograms per ml of serum. Four children had folic acid levels below 3 nanograms per ml of serum. Following response to protein the folic acid levels descended and then rose with the administration of folic acid with one exception: the child who responded to folate and B_{12} administration and whose serum folate levels rose very slowly. All the serum B_{12} levels have been found normal or high, showing moderate depression whenever there was a response to protein.

The urinary excretion of formiminoglutamic acid has never been high in any period of study. The opposite is true of imidazol acrylic acid, the excretion of which has been abnormally high in all children examined on admission, returning to normal levels with the administration of a high protein diet. The fact that formiminoglutamic acid excretion did not appear abnormal, even after administration of the high protein diet and a return to normal of imidazol acrylic acid excretion, suggests that folic acid deficiency in the children under study is not severe.

The bone marrow morphology has been characterized by the presence of mild maturation arrest with the production of megaloblastoid changes. Only one case showed megaloblastic changes

on admission. However, this child's serum folic acid was 5 nanograms per ml and his serum vitamin B_{12} was normal.

The serum vitamin E levels have been found to be under 3 micrograms per 100 ml in 5 out of 13 cases on admission. These children had an abnormally high peroxide fragility. The serum vitamin E, however, rose to normal levels before vitamin E was administered, suggesting mobilization of stores of this vitamin and impaired transporting capacity in the severely malnourished child, as occurs with vitamin A. None of the children under study responded to the administration of vitamin E.

Both red cell glutathione and glucose-6-phosphate dehydrogenase have been found below normal in the great majority of children studied. These two substances increase rapidly upon institution of the high protein diet. The red cell size distribution indicates a broad base with occasional double peaking in the macrocytic size. Upon recovery the peak assumes normal characteristics.

It has been found that osmotic fragility decreased in all the children on admission, returning slowly towards normal. The saponin hemolysis indicates a flat sigmoid curve which returns to normal upon treatment, indicating that there are cells with increased as well as others with decreased resistance to this surfactant.

E. Discussion

The following suggestions emerge from the results obtained in this study:

1. Total circulating hemoglobin and the red cell mass are markedly reduced in children with protein-calorie malnutrition, independent of the concentrations of hemoglobin or red cells. They slowly return to normal upon protein repletion, blood volume increase preceeding increase of red cell mass. They both show a correlation with the Creatinine-Height Index, suggesting that they are related to the active tissue mass in the children.

If the above is confirmed by further studies which we have undertaken, including basal oxygen consumption and arteriovenous oxygen saturation studies, it will appear that the so-called anemia of protein-calorie malnutrition is not an anemia in physiologic terms and more likely reflects a decreased oxygen transport demand.

2. In 5 out of 6 children longitudinally studied, the limiting factor in erythropoiesis seems to be protein. The fact that there is initially a high percentage of sideroblasts in the bone marrow indicates that iron is not a limiting factor and that this mineral is delivered to the red cell precursors. It appears, therefore,

that another step in the synthesis of hemoglobin which may very well be the synthesis of globin, is limiting in protein-calorie malnutrition. The iron reserve in these children is undoubtedly low as reflected by the biochemical and tissue signs of iron deficiency which take place whenever there is an increase in red cell production in the absence of iron administration.

3. Vitamin B_{12} is not a limiting factor. In one of our cases, folic acid appears to be limiting in erythropoiesis. The fact that this child had normal serum folate levels and did not show an increase in formiminoglutamic acid urinary excretion after a load of histidine, together with the finding in another child who had megaloblastic changes with normal serum folate levels, suggest that there may be an abnormal metabolism of folate in protein deficiency. Red cell folate levels will be determined in order to attempt to discover whether or not tissue levels are low even in the presence of normal serum levels.

4. The findings of decreased life span, low red cell glutathione and glucose-6-phosphate dehydrogenase levels, a flat sigmoid saponin hemolysis and a broad distribution of red cell sizes, indicate an intrinsic red cell defect in severe protein-calorie malnutrition. This may be due to the many causes which are being explored by means of various special techniques.