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THIRTEENTH MEETING OF THE
ADVISORY COMMITTEE ON MEDICAL RESEARCH

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DENTAL CARIES IN TWO COLOMBIAN COMMUNITIES

DENTAL CARIES IN TWO COLOMBIAN COMMUNITIES*

During the course of a visit to Colombia as a PAHO Short Term Consultant in 1971 my attention was directed to the existence of an unusually low incidence of dental caries among the inhabitants of the village of Heliconia, a semi-isolated community situated in the mountains to the east of the city of Medellín in the province of Antioquia.

In most modern urban communities dental caries is virtually a universal disease from childhood onwards, and it is extremely unusual to find caries-free individuals in such populations. The ubiquity of dental caries has long been attributed to the consumption of highly refined foodstuffs, and, in particular, to diets rich in fermentable carbohydrate. Several studies have linked caries incidence to the consumption of sucrose, usually in the form of confectionery, sweetened beverages, and between-meal snacks of sugary food. The weight of evidence incriminating these common components of modern diets has tended to obscure the role of oral micro-organisms in the pathogenesis of tooth decay. There is, however, solid evidence to show that sugar alone is incapable of destroying tooth substance. The deleterious effect is produced by mouth organisms fermenting dietary sugar to acids which, when retained on sequestered areas of the tooth surface and shielded from dilution or dispersion, are capable of initiating the breakdown of tooth enamel. For teeth to be attacked therefore, two components are necessary - a fermentable substrate in the diet and organisms capable of fermentation in the mouth. Such is the diversity and multiplicity of organisms comprising the oral flora that the presence and activity of organisms capable of fermenting dietary carbohydrate is virtually taken for granted, and conventional attempts at caries prevention are traditionally directed towards the imposition of dietary restrictions. The fact that such measures have not proved successful to any significant extent is attributable to the ubiquity of fermentable carbohydrate in modern diets, to the undoubted palatability of sucrose, and to the ingrained

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habits of between-meal snacks and the consumption of "convenience foods" - such as candy-bars, cookies, and artificially flavoured beverages.

That all these facets of modern urban living favour the caries process has been borne out by experiment and by observation. Sugar rationing during wartime has been accompanied by reduced dental caries, and primitive communities in all parts of the world have been found to suffer a deterioration in dental health after the introduction of refined and fermentable foodstuffs from the developed world. Whether sugar-fermenting organisms are also introduced to such communities, or whether they are already endemic, is not a matter that is easily determined and, in any event, the blatant correlation between so-called civilized diets and the onset of caries tends to command the attention of observers to the exclusion of other considerations.

It is not altogether surprising therefore, that a remote and relatively isolated community should be found to exhibit a low incidence of dental caries. Many such communities are to be found in the less developed areas of South America, Africa, Asia, and Australasia. What is unique abount Heliconia however, is that the apparent resistance to caries occurs in a population whose diet is particularly rich in readily fermentable carbohydrates. A staple component of this diet is panela, prepared by heating crushed sugar cane until it crystallizes into a solid brick of sucrose with a caramelized surface. This sugar is consumed not only at meal-times but also in drinks and between meals. Moreover, the people of Heliconia share these dietary customs with other villagers in the province of Antioquia, but Heliconia alone appears to enjoy relative freedom from dental caries. In otherwise comparable communities the incidence of the disease is every bit as high as would be expected when the daily intakes of sucrose are so numerous and of such quantity. Scientists from the University of Antioquia who were the first to identify the discrepancy between Heliconia and other villages in respect of dental caries, found that children in Heliconia averaged only one third as many

damaged teeth as their counterparts in other villages.

Possible Causes

In seeking a reason for this remarkable difference a careful analysis of dietary intake was carried out. No differences between Heliconia and four other towns could be found in respect of food consumption and food habits. The weight of carbohydrate, lipids, and proteins consumed was the same in Heliconia as in the high-caries communities. No difference could be found in regard to the ingestion of water. In one of the high-caries towns considerably more milk is consumed than in Heliconia, where milk is in short supply.

Of all the natural phenomena investigated in relation to dental caries none is more important than the fluoride content of water. The discovery that caries incidence is low in areas where water is naturally fluoridated formed the basis for the most valuable public health measure yet devised for reducing the incidence of this disease. For this reason the first possibility thought likely to account for the dental health of Heliconia was an optimal concentration of fluoride in the local water supply. Investigation proved otherwise. Analyses of the water showed a content of less than 0.2 parts per million which is less than one fifth of what is generally regarded as the optimum level. To exclude the possibility of a high F intake from other sources, F excretion in the urine of school-children was measured and low values established beyond doubt that the low caries incidence in Heliconia did not reflect the benefits associated with F intake.

Studies carried out in different parts of the world have shown that trace elements other than fluorine can affect caries incidence. An analysis of 21 different trace elements on numerous samples did reveal differences between Heliconia and the comparable town of Don Matias. Specifically, the water in Heliconia showed higher values of magnesium, calcium,

molybdenum and vanadium, while the content of manganese, iron, and copper was higher in Don Matias. These differences are not thought likely to account for the difference in caries incidence between the two populations.

Racial factors have been proposed to account for various differences in disease experience. While genetic factors can be postulated to play a part in susceptibility to dental disease, the evidence available is less than far-reaching in its significance. Studies were carried out however to determine whether genetic differences were apparent in respect of blood groups and physical anthropometry. The resultant findings indicated no genetic difference between populations exhibiting different caries experience.

Hypothesis

In order to explore the unusual phenomenon observed in Heliconia, and the absence of any obvious reason to account for it, it was postulated that the low incidence of dental caries is attributable to the absence of cariogenic organisms in the oral microflora or to the existence of factors interfering with the metabolism of cariogenic micro-organisms.

Methods of Investigation

A collaborative study was undertaken by the University of Antioquia, the Pan American Health Organization, and the Royal College of Surgeons of England, the actual study commencing in mid-1972.

Two hundred children in Heliconia (low caries) and two hundred in Don Matias (high caries) were dentally examined and divided into age groups 7 to 10 years and 11 to 14 years. Samples of dental plaque are to be obtained from each child and subjected to bacteriological and chemical analysis. Follow-up samples will be compared with samples originally taken. Where possible samples will be studied by electronhistochemical techniques. Samples of saliva will be studied from each group, and analyses of water from each

group will be carried out. The studies are directed towards identifying differences in the microbial composition and metabolism between the two groups.

Plaque was collected by a standardized method, the material being scraped from first molar teeth (where present), two hours after breakfast. Plaque was weighed after collection, the amount sought being of the order of 2 to 3 mgm. It was then dispersed in Todd-Hewitt broth in the approximate proportion of 1 mg to 1 ml. Serial dilutions of 10^{-4} and 10^{-6} were then made, and three different media (mitis salivarius agar, Rogosa's medium and DeMoor's medium) were inoculated and incubated anaerobically.

Fluorescent antibody studies were carried out on plaque samples which were suspended in a phosphate saline buffer and then spread on three separate slides. Fluorescent labelled antiserum was then applied and the smears viewed under ultraviolet light.

Acid production was investigated by collecting large samples of plaque into hollow trays, adding a measured amount of sucrose, and recording pH change with a small electrode.

Chemical analysis was carried out to determine the content of calcium, phosphorus, protein, and carbohydrate in plaque.

Results to date

It is obviously unsatisfactory to report on an incomplete investigation, but it can be said at this stage that several interesting possibilities have emerged from the first part of the study - although no single factor has been revealed which would suffice to account for the superior dental health of children in Heliconia.

Microbiology. So far as microbial populations are concerned it is evident that Streptococcus mutans, generally accepted as the organism principally concerned in cariogenesis, is substantially more abundant in the mouths of children from the high-caries community. The dental plaque of these children also contained more lactobacilli and more intracellular polysaccharide forming organisms. The degree to which Strep.mutans is present in different groups of children from the low-caries community is difficult to quantify more precisely, but this is one of the primary intentions of the study that it is hoped can be pursued further.

Limited studies by electronhistochemistry have shown more dividing cells and more intracellular carbohydrate in high-caries plaque, thus providing a sophisticated confirmation of the general observation made from culture methods. No new facts have emerged from studies of the microbial flora which would support the view that the caries-free state in Heliconia is due to hitherto unrecognized factors. Following the initial examination it was reported that plaque from Heliconia contained a profusion of amoebae, but this impression has not since been substantiated, nor has it been found possible to culture amoebae from plaque samples.

Whether the relatively low counts of Strep.mutans in samples from Heliconia are balanced by high counts of other organisms has yet to be determined. Clearly the possibility of non-cariogenic organisms competing for substrate is one that requires to be explored. Several investigators have postulated a positive correlation between high Strep.mutans counts and high caries activity, while conversely high counts of Strep.sanguis have been related to a low level of caries. Thus far, no clear-cut correlation has emerged from this study.

Table 1: Population of Microorganisms in Plaque (per mg wet weight)

	Don Matias (30 samples)	Heliconia (30 samples)
Total colonies on intracellular polysaccharide medium (10^6)	304	169
+ Intracellular polysaccharide forming organisms (10^6)	122.9	69.5
x Strep. mutans (non-selective medium) (10^6)	45.0	11.5
x Strep. sanguis (non-selective medium) (10^6)	17.5	5.5
Total streptococci on selective medium (10^6)	138.0	52.4
o Strep. mutans (on selective medium)(10^6)	118.0	27.5

+ Identified by iodine straining

x Identified by colony morphology

o Identified by colony morphology and ability to grow on this medium.

Dental Plaque. More than 400 samples of dental plaque have been collected, and it has been a regular finding that in the low-caries community it is often difficult to gather amounts sufficient for analysis. By contrast, plaque from the community with high caries is abundant and more cohesive; it is also more difficult to suspend in bacteriological media. The capacity of plaque to form acid was measured and although the indications are that plaque from Don Matias has a slightly greater capacity to lower the pH of sucrose solutions prepared in distilled water, there are some doubts as to the validity of this finding and these experiments are to be repeated using standard amounts of plaque.

Detailed chemical analyses have been carried out on plaque from each community. No statistically significant differences were observed in the quantities of protein, soluble and insoluble hexose, soluble ketohexose, calcium or phosphorus. The only difference observed was that insoluble ketohexose was present in greater amount in plaque from the caries-free town, and this solitary difference is presumed to be attributable to a variation in bacterial metabolism. Because of the possibility that the sucrose could be inverted to glucose and fructose during the heating involved in the manufacturing process of panela, this too was subjected to chemical chromatographic analyses. It was found that little inversion had occurred, and that panela is composed almost exclusively of su crose.

Water. Although, as mentioned previously, the water in Heliconia had been analysed for trace elements - in particular, fluorine - microbiological studies drew further attention to the possibility that a water-borne factor might be in operation. When a standard strain of Strep.mutans was grown on sucrose-containing media made up with water from Heliconia, the polysaccharide formed by these organisms was much less adherent than that produced when water from London or Don Matias was used. The factor or factors present in Heliconia water which influence this particular action of Strep.mutans on sucrose have yet to be identified.

An interesting extension of this observation was carried out in an experimental system designed to produce caries-like lesions in vitro. Teeth were bisected and separate halves placed in acid gels prepared in Heliconia water and London tap water respectively, each buffered to pH 4.5 with lactic acid. The gels were then incubated for periods of 28 or 40 weeks, after which the teeth were sectioned and examined under polarized light. Extensive lesions developed in the London gels, while those in the Heliconia gels were minute by comparison.

Further detailed studies of Heliconia water are called for. There are three sources, known as Ceferino, Hatillo and Escuela respectively. The first two

are derived from streams, and the latter from a pond. Water from all three sources is more alkaline than Don Matias water, and each has substantially greater buffering capacity than water from Don Matias.

Table 2: pH and Buffering Capacity of Water

pH Water from Heliconia		pH Water from Don Matias	
School	7.9	School	7.4
Hatillo	8.1		
Ceferino	8.2		

Effect of adding 0.1 NHCl on pH of 20 ml of water

Heliconia		
School	+ 0.1 ml HCl	pH = 5.9; + 0.15 ml HCl pH = 4.0
Ceferino		+ 0.15 ml HCl pH = 5.5
Hatillo	+ 0.1 ml HCl	pH = 5.7; + 0.4 ml HCl pH = 3.9
Don Matias		
School	+ 0.1 ml HCl	pH = 3.3

Finally, it should be mentioned that Heliconia water appears, on the basis of preliminary analyses, to contain more iodine than Don Matias water.

An Investigation of Dental Caries in Two Colombian Communities

Summary

An investigation is being undertaken to seek for causes underlying an unusually low incidence of dental caries among the inhibitants of the village of Heliconia, a semi-isolated community situated in the mountains to the east of the city of Medellin in the province of Antioquia. The ubiquity of dental caries in most modern urban communities has long been attributed to the consumption of diets rich in fermentable carbohydrate - in particular sucrose. There is, however, solid evidence to show that sugar alone is incapable of destroying tooth substance. The deleterious effect is produced by mouth organisms fermenting dietary sugar to acids which, under particular conditions, are capable of initiating the breakdown of tooth enamel. The unique feature about Heliconia is that the apparent resistance to caries occurred in a population whose diet is particularly rich in sugar. A staple component of this diet is panela, prepared by heating crushed sugar cane until it crystallizes into a solid brick of sucrose. This is consumed not only at meal-times but also in drinks and between meals. Scientists from the University of Antioquia who were the first to identify the discrepancy between Heliconia and other villages in respect of dental caries, found that children in Heliconia averaged only one third as many carious teeth as their counterparts in other villages. No differences between Heliconia and four other towns could be found in respect of food consumption and food habits. Analyses of the water showed a content of less than 0.2 parts per million of fluorine which is less than one fifth of what is generally regarded as the optimum level. The water in Heliconia showed higher values of magnesium, calcium, molybdenum and vanadium, while the content of manganese, iron, and copper was higher in Don Matias, where caries is exceedingly common. No indications have been found to suggest genetic differences between populations exhibiting difference caries experience.

A collaborative study into the oral microflora was undertaken by the University of Antioquia, the Pan American Health Organization, and the

Royal College of Surgeons of England, the actual study commencing in mid-1972. Two hundred children in Heliconia (low caries) and two hundred in Don Matias (high caries) were selected for dental examination. Samples of dental plaque were obtained from each child for bacteriological and chemical anaylsis. Samples of saliva were studied from each group, and analyses of water from each town will be carried out. Several possibilities have emerged from the first part of the study, although no single factor has been revealed which would suffice to account for the superior dental health of children in Heliconia. So far as microbial populations are concerned it it evident that Streptococcus mutans, generally accepted as the organism principally concerned in cariogenesis, is substantially more abundant in the mouths of children from the high-caries community. In comparing the two populations it has been found that dental plaque is more abundant in children from the high-caries town, that it is more cohesive, and that it is more difficult to suspend in culture media; it appears, in addition, to have a greater capacity for reducing the pH of sucrose solution than does plaque from the low-caries town. Minor differences have been detected between samples of saliva taken from the two groups. Distinctive features have been observed in the physical nature of polysaccharide formed by Streptococcus mutans when grown on sucrose-containing media made up with water from Heliconia, Don Matias, and London. When the culture medium was made up with Heliconia water the degree of adherence of the polysaccharide appears to be substantially less than is the case when the media are prepared with water from the other two sources. This suggests that elements are present in Heliconia water which influence the formation of polysaccharide from sucrose by the streptococcus. Analyses of the water have shown that Heliconia water is more alkaline than the water of Don Matias and that it also has a considerably greater buffering capacity. This feature is not thought likely to be sufficient to account for the effects observed during the culture of streptococci, nor is it likely to be entirely responsible for the abnormally low caries incidence in Heliconia.

Pan American Health Organization

FOURTEENTH MEETING OF THE
ADVISORY COMMITTEE ON MEDICAL RESEARCH

Headquarters Building
Conference Room B
Pan American Health Organization
525 Twenty-third Street, N.W.
Washington, D.C.

7-10 July 1975

AGENDA

Monday
7 July

9:00 a.m.	1.	Opening of the meeting - J. C. Waterlow
9:05	2.	Introductory statement - H. R. Acuña
9:15	3.	Present status of Venezuelan encephalitis research - W. F. Scherer
9:35		Discussion
9:50	4.	Ecology of arboviruses and their diseases in French Guiana - J.-P. Digoutte
10:10		Discussion
10:25	5.	Observations on the seroepidemiology of typhus in Bolivia and the status of the attenuated vaccine field trials - C. L. Wisseman, Jr.
10:45		Discussion
11:00		C o f f e e
11:15	6.	Field trials of oral vaccines against typhoid - J. M. Borgoño
11:35		Discussion

11:50 a.m.	7.	Impact of malaria on economic development: a case study - G. N. Conly
12:10 p.m.		Discussion
13:30		L u n c h
2:00	8.	Organization and development of a multidisciplinary research program in the Trans-Amazon area - C. H. Llewellyn
2:20		Discussion
2:35	9.	Progress report from the PAHO/WHO International Center for Training and Research in Leprosy and Related Diseases - J. Convit
2:55		Discussion
3:10	10.	Progress report on the Caribbean Epidemiology Centre - P. J. S. Hamilton
3:30		Discussion
3:45		C o f f e e
4:00	11.	Collaborative studies on hepatitis B virus - L. Spence and M. C. Williams
4:20		Discussion

Tuesday
8 July

9:00 a.m.	12.	Preservation of nonhuman primates and their utilization for biomedical research - R. W. Thorington
9:20		Discussion
9:35	13.	Effect of maternal nutrition on fetal growth and infant development - R. Klein
9:55		Discussion
10:10		C o f f e e

10:30 a.m.	14.	Evaluation of nutrition intervention programs - M. Gueri
10:50		Discussion
11:05	15.	Interaction between nutrition and productivity of agricultural laborers - F. E. Viteri
11:25		Discussion
11:45	16.	Stress and the mechanism of the diabetogenic action of pituitary growth hormone - L. Vargas
12:05		Discussion
12:20		L u n c h
2:00	17.	Correlative morphologic and biochemical studies on experimental epilepsy - A. Feria-Velasco
2:20		Discussion
2:35	18.	PAHO guidelines and review system for the protection of human rights in medical investigations - J. Osuna
2:55		Discussion
3:10	19.	Application of new educational technology in the development of human resources on health - L. Carlos Lobo
3:30		Discussion
3:45		C o f f e e
4:00	20.	System for evaluating hospital supporting services - J. Peña and J. Ortiz
4:20		Discussion

Wednesday
9 July

9:00 a.m.	21.	Reports of scientific meetings and symposia
	21.1	Third international conference on the mycoses - M. Silva-Hutner

9:20 a.m.		Discussion
9:35	21.2	Research and control of onchocerciasis in the Western Hemisphere - D. H. Connor
9:55		Discussion
10:10		C o f f e e
10:25	21.3	New approaches in American trypanosomiasis research - Z. Brener
10:45		Discussion
11:00	21.4	Diabetes mellitus: A challenge to the countries of the Region - J. Litvak
11:20		Discussion
11:35		L u n c h
1:30 p.m. to 5:00	22.	Review of the role of the PAHO/ACMR

Thursday
10 July

9:00 a.m. to 12:00 noon	23.	Preparation by the rapporteurs of the meeting's report
12:30 p.m.		L u n c h
2:00	24.	Committee's recommendations and selection of the topic of the Special Session for the XVth Meeting
4:30	25.	Closure of the meeting

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FOURTEENTH MEETING OF THE PAHO ADVISORY COMMITTEE ON MEDICAL RESEARCH

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