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A VITAMIN C STUDY OF MALAY SCHOOLBOYS
in the
Coastal Region of North Perak, Malaya.

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The diet of the Malays in the coastal region of North Perak is largely based on rice, which is a source of Vitamin C. The diet was therefore found to be affected to some extent by the influence of the Chinese and other immigrants, and the results of the study are discussed in the paper.

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A Vitamin C Study of Malay Schoolboys in
the Coastal Region of North Perak, Malaya.

by

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In a report on nutrition in the State of Kedah, Vickers and Strahan(1936), while considering that the subjects of their investigation were adequately supplied with vitamin C, noted that fruits and vegetables were lacking in the diets in the coastal areas. The question thus arises as to the sufficiency of vitamin C in the nutrition of the inhabitants of these areas. This is of particular interest in that, of the inhabited parts of Malaya, the maritime regions have probably been affected least by the process of industrialization of the country; the occupations have remained fishing and rice-cultivation throughout the centuries from the first settling of the Malays in the peninsula until today. The diet has therefore been unaffected to any extent by the influence of the Chinese and other incomers, and the vitamin C

nutrition of today affords an insight into that of the original inhabitants of the area.

The coastal region of Perak, the state to the south of Kedah, is similar to that of the latter country, and to it apply equally well the remarks quoted above. Consisting of low-lying land between the wide river deltas and the expansive hinterland of rice-fields, this region gives but a poor living to its large population of Malays and Chinese, of whom the former are in the majority. The topography of the area gives the clue to the diet, which is composed of rice and fish, with little variety and almost no seasonal change. The sources of vitamin C are green leaves, coconuts, and occasionally other fruits in small amounts. Green leaves are by far the most common source of the vitamin and may be eaten raw or after boiling. In either case further doubt thus arises as to vitamin C sufficiency owing to the possibly scanty absorption of ascorbic acid; for where the leaves are eaten raw much of the cell-wall of the leaves may resist the action

of the intestinal fluids and fail to break down, while if the leaves are boiled most of the vitamin may be destroyed.

Along with the primary object of determining the presence or absence of vitamin C deficiency, the opportunity was taken in the course of the investigation to attempt to ascertain what, if any, of the pathological conditions noted could be definitely ascribed to lack of this vitamin. It is well known that few of the signs of any one nutritional defect are specific for that defect, this being so particularly in the case of the various vitamin deficiencies. As during the present year there will be carried out in Perak a survey of nutrition in general, information on the differentiation of these signs is valuable and timely.

The basis of a vitamin C inquiry in the field must be urinary estimations, and the urine specimens for these can be collected in schools only, where the facilities are available; the inquiry was therefore confined to school-children, of whom only boys could be examined

without contravening native customs. As a certain degree of co-operation is required from the subjects of the tests, boys of a minimum age of 7 years were examined; the age of the oldest boys in the schools was generally 12 years. Although the scope of the investigation was thus limited, there was the advantage that in children the ill-effects of nutritional deficiency show readily. According to Harris (1939), children probably require twice as much vitamin C per unit of body-weight as do adults.

Scurvy does not occur, or at least does not come to medical notice, in the coastal region of North Perak, where the investigation took place. Frank scurvy was not therefore expected and in fact was not seen, the inquiry being into the possible presence of the milder vitamin C deficiencies. When it is considered that beri-beri is not a prevalent disease in Perak although the entire population lives on the verge of this condition (Baeza, 1939), there is reason to assume that deficiency of vitamin C might exist in the absence of cases of overt scurvy.

The examination was confined to those boys who were apparently in normal health as ample evidence has been brought forward to show that the vitamin C state of the body is disturbed in conditions of pyrexia(Falke,1939), toxæmia, and illness in general(Neuweiler, 1939). A preliminary inspection was made to exclude all but the average healthy subjects; the oral temperature was taken, the spleen examined for enlargement, and the presence of acute catarrhal or infectious conditions noted. Children showing obvious signs of other vitamin defects such as Bitot's spots, discoloration of the conjunctiva, or the tender calf-muscles of incipient beri-beri, were excluded to comply with the second object of the investigation, and one whole school could not be dealt with owing to the presence in the boys of scabies which masked any cutaneous pathological signs. Altogether, out of approximately 435 boys attending schools in the area, it was possible to fully examine only 220, roughly 50% of the male school population.

The investigation proper took the form of a clinical examination followed by a series of special tests. The former, which was to determine the significance of the abnormal signs present, consisted of a full inspection of the skin, the mouth, teeth and oral region generally; the latter, which was to provide an answer to the main object of the inquiry, included the intradermal dye test, the capillary fragility test and urinary estimations.

The results only of the urinary estimations will be given now; the methods used and discussion are best left until the clinical signs have been dealt with. At this stage it is sufficient to state that out of 220 boys, 17 (7.7%) showed vitamin C deficiency. These figures being known, the clinical signs may be assessed with reference to them. The number of conditions which are now ascribed to lack of vitamin C is so great that only the more important of these can be discussed and references to authors must be kept at a minimum.

The defects of the skin found may be shown in tabular form:

<u>1.</u>	<u>2.</u>	<u>3.</u>	<u>4.</u>	<u>5.</u>	<u>6.</u>
	Total.	In boys with no deficiency	%age of 1 in 3.	In boys deficient in vit C.	%age of 1 in 5.
Haemorrhagic conditions.	3	3	1.4	-	-
Ulceration.	24	24	10.9	-	-
Phrynodermia.	22	15	6.8	7	41.2
Epidermi- phytoses.	7	2	0.9	5	29.4

Hellier(1938) has stated that severe vitamin C deficiency may cause purpura, but the correct view seems to be that of Parsons(1938) who, basing his remarks on the pathology of haemorrhagic conditions, contends that vitamin C has no relationship with any such conditions except those associated with scurvy. In the present series, 3 cases were seen with petechiae on the limbs, but in each case there were no other deficiency signs and the urinary examinations gave satisfactory results. Odd cases showing haemorrhagic signs cannot thus be said to have a causal relationship to vitamin C deficiency.

No active ulceration of the skin was noted, but in 24 cases which showed signs of

recent ulceration the output of ascorbic acid in the urine was normal. In the cases under review this condition did not therefore show any correlation with deficiency of the vitamin.

Phrynodermia is indicative of vitamin deficiency, but may occur in various states of malnutrition(Hutchison,1929). It is ascribed to vitamin C deficiency by Hellier(1938) who states, however, that it may also be associated with defect of vitamin A. Later writers have agreed in general with these views. In the present series the condition was noted in 22 instances, of which 7 were later found to be deficient in vitamin C according to the urinary tests. Thus almost half of the vitamin C-deficient cases showed phrynodermia. It could therefore be accepted as a sign of lack of the vitamin, since other marked nutritional deficiencies had been excluded.

Peck(1939), in a study of fungous infections of the skin, analysed perspiration and found ascorbic acid to be one of the acids present. He suggested that lack of perspiration

or too small a concentration of the contained acids might be a factor in the growth of epidermiphytoses due to the absence of the normal fungicidal action of these acids. This suggestion appears to be supported by the findings of the present series where, of the 7 cases showing this condition, 5 were in boys who had deficiency of ascorbic acid in the urine. It is possible, then, that lack of the vitamin may encourage the growth of the dermal mycoses. The fact that there are other acids present in perspiration, however, makes such an influence a possibility only.

Pathological conditions of the oral region were as follows:

1.	2.	3.	4.	5.	6.
	Total	In boys	%age	In boys	%age
		with no	of 1	deficient	of 1
		deficiency	in 3	in vit C.	in 5
Angular					
stomatitis.	19	18	8.8	1	5.9
Glossitis.	25	23	11.3	2	10.8
Gingivitis.	33	33	100.0	-	-
Pyorrhoea.	3	3	100.0	-	-
Dental	79	77	33.0	2	10.8
caries.					

Although angular stomatitis as a sign of nutritional deficiency occupies much the same position as does phrynoderma, in that it is believed to be common to several vitamin deficiencies including that of vitamin C, its occurrence in the present investigation does not show any correlation with the vitamin C state of the subjects.

Glossitis was fairly common, 25 cases being noted. In 2 instances it was seen in boys who later responded badly to the urinary test, but in the remaining cases the boys had a normal excretion of ascorbic acid. This result does not suggest a causal relationship between lack of vitamin C and glossitis. The occurrence of this sign apart from this deficiency has lately been recorded by Katzenellenbogen(1939), who in a series of over 100 cases of glossitis found the vitamin C state to be normal in every case.

In frank scurvy a considerable effect of the disease falls on the gums; in lesser vitamin C deficiencies it might therefore be

expected that gum signs would be prominent. Experimentally, in monkeys, it has been shown that lack of this vitamin may cause gingivitis but that lack of other vitamins may give a like result (Topping and Fraser, 1939). In the present series, all of the 33 instances of this sign occurred in boys whose ascorbic acid nutrition proved to be normal, and only in those cases having dental caries was gingivitis seen. The condition thus appeared to be related to the state of the teeth and not to an independent vitamin deficiency.

Only 3 instances of definite pyorrhoea were seen. Boyle (1938) states that abnormal vitamin C nutrition may play a part in the etiology of this disease, since the vitamin is necessary for the health of the peri-odontal tissues, but in the present cases the condition did not occur in any of the boys with deficiency.

In the case of the teeth the problem is most difficult. The experimental work has been carried out in guinea-pigs, and much of the

difficulty in drawing conclusions from the experimental work is due to the fact that in these animals the teeth are peculiarly sensitive to withdrawal of vitamin C from the diet(Nicholls,1938); this author, however, gives dental decay in the human subject as a sequel to vitamin C deficiency. Similarly, Bucher(1937) ascribes dental caries to the same cause, although at the same time stating that in the test animal the effect of vitamin C deficiency was noticeable on the teeth before any other. In the human subject, Shourie(1939) in the tropics, found dental caries conspicuously absent from a large series of cases with definite vitamin C deficiency.

In the present investigation, out of 79 instances of dental decay noted, only 2 were in boys deficient in the vitamin. There seems, therefore, to be no ground for linking dental caries with lack of vitamin C.

No investigation of the blood-state as influenced by vitamin C was undertaken, since abnormal conditions of the blood might have

been present due to previous malaria, to other nutritional deficiencies, or to helminthiasis.

Of the special tests, the detection of capillary fragility was first attempted. This test is said to be of value if other diseases are excluded, and Bourne(1938) was able to disclose vitamin C deficiency by means of it. There is undoubtedly a pathological basis for the test, as in vitamin C deficiency there is an early loss of strength of the capillary walls due to the malnutrition of the cement substance binding the walls. The latter thus become permeable to an abnormal extent(Pribram,1939). Although the original procedure of the test has been modified by G8thlin(1937), who is the originator of the test, it still remains too long and too complicated for mass examination. The method used in the present series was the stasis of the blood-flow in one arm by means of a sphygmomanometer band, a pressure of 80 mm.Hg. being sustained for 5 minutes. This is a relatively severe test in children. The band was placed above the antecubital fossa of the

left arm, and the skin of this area was examined before and after the test through a lens of +5 dioptries. In the 3 instances where petechiae were primarily present, no testing was done.

In 4 instances petechiae appeared within 5 minutes, but in these cases there existed no vitamin C defect in the urine. There was thus no correlation between the vitamin C state and the results described. The control of capillary fragility by vitamin C has been questioned by the work of Jersild(1938) and more recently by that of Scarborough and Stewart(1939), who have demonstrated regulation of capillary permeability by vitamin P in the absence of vitamin C abnormality; and by the work of Dam and Glavind(1938) on vitamin K, as lately applied to the adult by Kark and Lozner (1939). The test may soon have to be discarded as a means of diagnosing vitamin C deficiency.

The intradermal test of Rotter(1937) was applied in each case. The dye solution was made up after the method of Banerjee and Guha (1939), that is, by dissolving 2 mg. of 2:6

dichlorophenolindophenol in 4.9 c.cm. of water; a further modification was introduced by diluting the solution with water to one tenth of the strength used by these workers, so that the test-dose might be 0.1 c.cm. instead of 0.01 c.cm., an amount with which it is very difficult to work in dark-skinned peoples.

There were certain factors which seemed to require controlling, owing to their possible influence on the results. One factor was that of perspiration, or rather the number of sweat-glands in the area of skin used for the test, since ascorbic acid is excreted in perspiration. In a site bearing many such glands, as in the forearm, ascorbic acid may be withdrawn from the deeper layers of the skin before it can reach the injected dye. A second consideration was the amount of movement which goes on under the test site; the forearm, the muscles of which are constantly being exercised, is very well supplied with blood-vessels as compared to the upper arm where muscular movement is less and the vessels in the skin overlying the

muscles are fewer. The cutaneous circulation, then, is less likely to be as brisk in the upper arm as in the forearm. Assuming that ascorbic acid is deposited by the blood-stream in all parts of the skin at the same rate, the amounts of the acid in the skin of the upper and fore-arm respectively might be appreciably different. The results of the test in the upper arm would, in theory, differ from those in the forearm by the combined effects of perspiration and muscular movement on the ascorbic acid content of the skin.

In the test, therefore, 0.1 c.cm. of the dye solution was injected intradermally in the front and the back of the forearm, and the outer side and the back of the upper arm. The readings were made in the four sites after 5, and after 10 minutes. Owing to the number of boys being examined at one time, it was not possible to note the exact time taken for decolorization in each case. The results were disappointing in that all the tests cleared within 5 minutes except in six boys who were

clear, however, within 10 minutes. The findings had a rather negative value, but it may be that the skin test is less sensitive than the urinary estimations. Certainly Jennings and Glazebrook (1938) found that in their cases of recovering scurvy the intradermal test became normal while there still existed vitamin C deficiency according to the other biochemical tests. Or the test may give erratic results, as suggested by Poncher and Stubenrauch(1939).

No significant difference was shown by the results at the four sites; the test apparently does not demonstrate the slight variations which perspirations and movement might make. Generally, from these results, it may be possible to conclude that the intradermal test lacks the exactness of the urinary estimations.

When it is not practicable to determine the ascorbic acid content of the blood, the urinary content becomes the basis of any inquiry into the vitamin C state. Such urinary estimation may take the form of determining the average amount of ascorbic acid excreted by the urine

in 24 hours, which indicates the "resting-level" of this substance in the tissues. The vitamin C state may also be shown, probably more accurately, by the response in the urine to large doses of the vitamin given by mouth, on the grounds that the body, if fully supplied with the vitamin, will quickly excrete the surplus ascorbic acid. All the boys were tested by the first method and 74 were in addition tested by the second - the "saturation" test.

As it was not possible to obtain the 24 hour urines of the boys, the modified procedure of Harris and Abbasy(1937) for use in routine surveys of schoolchildren, was adopted. Each school was visited on two successive days; each morning, when the school assembled at 8.30 a.m., the boys were instructed to empty the bladder into the bottle provided. At 11.30 a.m. the urines were again collected into bottles, these latter specimens being the amounts of urine excreted in 3 hours. The mean of the 3 hour quantity on the two days is taken as being approximately one eighth part of the 24 hour

urine, and the total amount of ascorbic acid excreted in a day is thus calculated. the preliminary specimen collected at 8.30 a.m. was not used for the purpose of this calculation but will be discussed later.

On the days of the tests, the boys were not allowed to take part in games, as, although Wright and MacLenathen(1939) state that the loss of ascorbic acid through even excessive perspiration is very slight, yet increased utilization of vitamin C might be expected due to the increased metabolic rate associated with vigorous games.

In two of the schools it was also possible to carry out the saturation test on the pupils. The bladder was emptied as on previous days at 8.30 a.m. and each of the boys was then given 150 mg. of synthetic ascorbic acid in the form of 3 tablets of Redoxon. The urines were collected at 12 noon, giving a $3\frac{1}{2}$ hour quantity of urine; this period was the maximum time available. Redoxon tablets were used as the local children are accustomed to take medicine

in tablet form, and also since the tablets may be easily carried. With the test-dose, each boy was given 100 c.cm. of water to drink, to give similar dilutions of urine as far as possible.

The amount of 150 mg. of ascorbic acid was arrived at in two ways: a test-dose of 70 mg. per stone of body weight was suggested by Abbasy and others(1935), and the average weight of the boys was just under 3 stones; and normal adults were found to have deficiencies of 500 mg. and even more by Portnoy and Wilkinson(1938), so that it could be safely assumed that those boys who gave a good response to 150 mg. would be definitely "saturated" with vitamin C.

In the case of all the specimens the procedure after collection was the same. The urines were brought to the laboratory as quickly as possible in dark bottles, glacial acetic acid being added to each specimen in a proportion of 10% by volume, and then titrated against a 2% solution of 2:6 dichlorophenolindophenol; in the calculation of the results allowance was

made for the acetic acid added.

Objections to the titration procedure have been collected and well assessed by the Health Organization of the League of Nations (1937), but the present view is that the test, if done carefully, reaching the end-point within two minutes, overcomes most of its defects. Where it is carried out in a large number of cases in similar circumstances the results are usually accepted as being accurate.

The optimum daily excretion of ascorbic acid is given by Harris and Ray(1935) among many others, as 13 mg. for an adult and 1-2 mg. for an infant, with children showing intermediate values. From the boys' weights, as given by their medical inspection cards, it was possible to calculate the optimum daily excretions of ascorbic acid, and to compare these with the actual 24 hour amounts deduced from the urine specimens.

On the basis of this standard, 17 boys out of the total 220 gave an excretion below the optimum, and were therefore classed as

deficient in vitamin C.

The calculation of the results of the saturation test was after the simplified method of Gander and Niederberger(1936) who found that in the case of adults the tissues became saturated with ascorbic acid when the urinary concentration of the acid rose to 5 mg.%, especially if the concentration before the administration of vitamin C had been half this amount or less. These concentrations were, of course, dependent on the urinary output being within the limits of normality. No statistics are available for the average 24 hour urines in the Malays of the rural districts, but using the European standards for children of 7-12 years(25-35 oz.), a concentration of 2 mg.% would signify vitamin C saturation by this method. Finally, to take into account the greater concentration of the urine in the tropics, it was decided to regard the minimum concentration for saturation as 3 mg.%. Of the 74 boys examined thus, 11 were found to be deficient. The ordinary test had previously

shown 10 of them to be deficient in vitamin C so that discrepancy occurred in one case only. As the urine from this case might have been overdiluted with fluids from the morning meal, this result was regarded as doubtful. The saturation test was therefore of value in controlling the results of the ordinary test.

The specimen of urine collected at 8.30 a.m. at the commencement of the other tests, was also titrated against the dye solution for the concentration of ascorbic acid. Although the concentrations of isolated specimens are generally regarded as being of no value, in this case they gave a fairly close relationship to the results of the ordinary tests as follows:

1.	2.	3.	4.	5.
mg. %	Satis- factory	Unsatis- factory	(By ordinary test)	
			Satis.	Unsatis.
2+	91) 177		203	
1-2	86) (80.4%)		(92.3%)	
0.5-1		37) 43		17
0-0.5		6) (19.6%)		(7.7%)

The standard of 1-2 mg. % as a satisfactory concentration of ascorbic acid in

ordinary urine specimens is suggested by Harris and Ray(1935) but they are referring to the mean concentration of many specimens from the same subject. The same authors state that the variations in the output of ascorbic acid in the urine depend on "the immediate dietary intake and also on the past nutritional history". In the present series, the subjects have a diet which varies but slightly, and as the specimen was collected in the early morning before the boys had perspired greatly, and also since it was too soon for the food taken in the morning meal to affect the ascorbic acid content of the urine, the single specimen probably gives information on the past nutritional history only. There must be variations in results as the amount of water drunk in the early morning is an unknown quantity, but in a large number of estimations done on a population on an unvarying diet, the average concentration of such single morning specimens would probably give a rapid and fairly exact clue to the vitamin C state of the community.

It is not easy, nor practical, to draw conclusions from an investigation of limited scope; but the following comments apply, it must be remembered, to that proportion of the inhabitants of Malaya in which the possibility of vitamin C deficiency is greatest.

This deficiency was present in a small proportion(7.7%) of the boys examined, and in these cases the deficiency was assessed on very severe standards. It may thus generally be concluded that vitamin C deficiency in the subjects of the investigation was present in only slight degree.

Of the clinical signs found, phrynodermia might be accepted as a sequel to vitamin C deficiency, and possibly the growth of epidermiphytoses is aided by such deficiency. There was definitely no relationship between the vitamin C state and the condition of the teeth, gums or mouth. The capillary fragility test failed to disclose deficiency, while the intradermal test was apparently inaccurate in the estimation of slight deficiency.

Summary.

Malay schoolboys in the coastal region of Perak were investigated as to the presence of vitamin C deficiency.

Although the subjects were drawn from that part of the population of Malaya most prone to this deficiency, only a few showed subnormal excretions of ascorbic acid in the urine.

Few of the abnormal clinical signs noted in the boys could be attributed to lack of the vitamin.

Special tests for detecting vitamin C deficiency did not give satisfactory results.

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