INVESTIGATIONS INTO THE PATHOGENESIS OF SCORBUTIC DYSTROPHY*

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In 1888 Sir Thomas Barlow definitely established the nature of infantile scurvy and after a long period of hesitation, the scorbutic character of this affection is now universally recognized. The same author, in 1904, drew attention to the 'formes frustes' of the disease and following this Alfred Hess gave a masterly description of these varieties. This latter author divided scurvy into an acute type, a subacute type and a third type characterized by a general disturbance of nutrition without manifest scorbutic lesions. This division corresponds exactly to what is observed in practice. It is not intended here to describe the history of scorbutic dystrophy, in the study of which a large number of authorities in different countries have collaborated. The essential features only will be described.

Apart from manifest scurvy, with its classical symptoms, there exists the variety of the disease in which the true nature of the uncharacteristic general disturbance is shown by the appearance of a specific tendency to haemorrhage with petechiae, haematuria, occult blood in the stools, tenderness over the bones and a positive tourniquet test. According to A. F. Hess the eyelids are puffy, the heart a little enlarged, the respiration rapid and the pulse rapid and variable. An antiscorbutic diet quickly causes the disappearance of these symptoms.

In prescorbutic dystrophy the confirmatory signs of the haemorrhagic tendency are absent. It is a state of chronic wasting, characterized by pallor, anorexia, change in character, a slowing-up of the increase in weight and also, rarely, of the increase in length. Sometimes chronic disturbances of digestion occur: other patients present an anaemia which only reacts to a combination of iron and vitamin C (Rohmer and Bindschedler¹). A persistent rise in temperature may be met with, explained usually by slight, recurring infections. It is accepted by most authorities that in this condition there exists, in fact, a special susceptibility to infections, which are more frequent, of longer duration and more serious than among normal children.

This dystrophic state can precede the appearance of manifest scurvy, which, however, also often supervenes without any premonitory signs in a state of apparent perfect health. The symptoms of manifest scurvy have

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been observed appearing suddenly in infants who have been in-patients in the clinic for several months, where they have received the standard diet and have not shown the least symptom of dystrophy. It has often been 'brought out' by an accidental cause, for example, by an infection such as whooping cough. In other instances, the major symptoms of scurvy do not occur at all. The scorbutic character of the dystrophy is only indicated by minor haemorrhagic symptoms which establish the diagnosis. In the majority of cases these minor diagnostic signs of a scorbutic affection are also absent. The commonplace state of wasting persists for a varying period and disappears gradually, generally when certain modifications are introduced into the child's diet as the age increases.

In experimental scurvy the guinea-pig, completely deprived of vitamin C, maintains at first the appearances of good health. In the early days the deficiency only shows itself by a slight diminution in appetite and in the increase in weight, until the moment when the manifest symptoms of the disease suddenly appear. It is equally possible to produce the syndrome of dystrophy in this animal if the intake of vitamin C is maintained for a definite period slightly below the necessary dose.

While the different varieties of experimental scurvy can be produced in the guinea-pig with an almost mathematical precision, it is quite otherwise in the child, in whom the pathogenesis remains obscure, obeying apparently very capricious rules, whose effect is unpredictable. It is certain that the requirements of infants for vitamin C vary enormously according to their age, their constitution, their general condition and their previous illnesses. This explains why, despite the fashion of sterilized milk which is still far from being always corrected by the addition of vitamin C, cases of scurvy are relatively rare. The frequency of prescorbutic dystrophy is unknown but it can be stated that this dystrophy only shows itself in a relatively small number of infants whose diet does not differ from that of the majority of babies who remain in good health and gain weight normally.

The recent discovery of the chemical nature of vitamin C—laevo-ascorbic acid containing a dienolic group ($-\text{COH} : \text{COH}-$), responsible primarily for its reducing properties—has not yet completely cleared up this difficulty.

In a communication made at the Third International Paediatric Congress (London, 1933), we announced our intention of studying the latent, prescorbutic state in infancy by means of certain methods, which one of us had introduced some years ago without their finding the widespread clinical application which they appeared to merit. In the first place we have used the method of testing the urine with monomolybdo-phosphotungstic acid. The following are briefly the details of procedure.

Preparation of standards.—The violet coloration which is given by monomolybdo-phosphotungstic acid with hydroquinone, pyrocatechin or vitamin C attains its maximum intensity when three molecules of reagent react with one molecule of this substance. This detail is important since certain other polyphenols, frequently present in organic substances, such as tannin, also contain a dienolic group which can give this reaction. But with

these the reaction is only produced in the presence of a large excess of reagent and is preceded by a yellowish-brown colour. It is therefore essential to gauge the amount of reagent so as to add sufficient but at the same time to avoid all excess. The reaction is given equally by vitamin C and by its immediate break-down product which still retains the dienolic group (probably a diénol-hexosic acid). Elsewhere² a method of differentiating these two substances has been described. For the purposes of the present investigation this differentiation is unnecessary.

As a standard hydroquinone has been used with the provision that in equal molar concentration the violet reaction of vitamin C is about 12 per cent. more feeble than that given by hydroquinone. This disparity is corrected by using in the preparation of the standard, 100 mgm. of hydroquinone instead of 110 mgm. which would correspond exactly with the molecular weight. The 100 mgm. of hydroquinone are dissolved in 100 c.c. of water and 1 c.c. of this solution is freshly diluted to 100 c.c. Of this second solution 1 c.c., 1.5 c.c. and 2 c.c. are placed in three test-tubes of colourless glass, marked to show 5 c.c. and 10 c.c. To each of the three tubes is added one drop of the reagent and 5 per cent. sulphuric acid is then added to the 10 c.c. mark. The reagent is prepared by dissolving 7.5 gm. of monomolybdo-phosphotungstic acid in sufficient dilute sulphuric acid (5 per cent. by volume) to make 100 c.c. (Another solution containing only 3.75 gm. of reagent is also prepared.) The reagent solution is kept in dropping bottles made of brown glass, giving 15 drops per c.c. If the standards are protected against the direct effect of light they maintain their colour without any notable change for two or three days.

The intensity of colour of the standard containing 1 c.c. of the solution of hydroquinone is equal to that produced by a solution of 10^{-5} N. ascorbic acid, containing 1.76 mgm. of this substance per litre. This colorimetric equality is indicated by the symbol 'U.H.' ('unité hydroquinol'). The intensity of the colour of the standards in the second and third tubes corresponds to 1.5 and to 2 U.H. respectively, that is to solutions of vitamin C of approximately 2.6 and 3.5 mgm. per litre.

Preparation of human urine for analysis.—The urine is first cleared. For this purpose the following clearing agent is utilized: 100 gm. of crystalline neutral lead acetate and 100 c.c. of glacial acetic acid are dissolved in distilled water to make 1 litre. To 20 c.c. of urine 12 c.c. of this clearing agent are added. Two minutes afterwards, without filtration, there should be added 8 c.c. of a solution containing 200 gm. of crystallized sodium sulphate ($\text{Na}_2\text{SO}_4 \cdot 12\text{H}_2\text{O}$) per litre. This mixture is rapidly shaken and filtered through filter paper. The filtrate ought to be absolutely clear. Of this cleared urine 10 c.c. are placed in a test-tube exactly similar to those used for the standards and one drop of reagent is added. The total time for these procedures ought not to exceed twenty minutes.

Reading the results—If the urine tested is absolutely colourless after the clearing process and if the colour obtained with one drop of the reagent (7.5 per cent.) is equal to that in the third standard tube, this is equivalent to 2 U.H. The quantity of reagent contained in one drop is sufficient to produce a colour change equivalent to 3.5 U.H. so that in the example given there is sufficient reagent used but, on the other hand, not too much. The reading can therefore be regarded as correct. Since the urine was diluted by half in the clearing process the figure of 2 U.H. must be multiplied by two. This urine therefore contains, as vitamin C or its immediate break-down product, four times 10^{-5} N, which equals 7 mgm. of ascorbic acid per litre.

If the colour obtained is deeper than that in the third standard tube the urine must be diluted with equal parts of distilled water and test repeated. It frequently happens, with the urine of young, healthy infants, that the

urine has to be diluted eight times or even sixteen times. For such dilute urines it is preferable to use the weaker reagent (3.75 per cent.). The intensity of colour change finally obtained should not exceed that of the second standard tube, that is corresponding to 1.5 U.H. or 2.6 mgm. ascorbic acid per litre. This figure is multiplied according to the degree of dilution.

It may happen that after clearance the urine retains a slight colour-tint of its own which gives the final reaction a mauve shade. In this case it is necessary to use a four-chambered comparator. In two chambers are placed, one behind the other, a tube containing urine plus reagent and a second tube containing water. In the two other chambers are placed a standard tube and another containing cleared urine but without the addition of reagent. It should be possible to obtain matching of equal tint and intensity between the two sides.

Results.

When this method is applied to urine from young healthy infants, receiving human milk or artificial feeds which are well balanced and sufficiently rich in vitamin C, the figure generally obtained is at least 8 U.H. It is from this fact that an attempt was made to determine the requirements of vitamin C for healthy infants in the early months of life by substituting, during two periods of twenty-four hours, a diet strictly free from vitamin C for their usual food. Surprisingly it was found that in contrast to what occurs in older children and in adults, the intensity of the reaction, instead of falling to zero, was increased in infants between two and nine months. Between the ages of nine and eleven months there was a certain variability. In one of the two infants in this group the elimination of vitamin C increased during the second day of the experiment. This infant behaved, therefore, like those of the younger age period. In the other infant, the figure for U.H. fell towards the end of the second day. With infants over one year in age (fourteen to thirty-three months) the elimination of vitamin C fell to zero by the end of the first day or at the latest by the end of the second or beginning of the third day³.

These experiments have shown for the first time in a strict sense that the healthy and normal human infant is capable of synthesizing vitamin C. This function tends to decrease towards the eleventh month and is definitely lost after the end of the first year. In later experiments it has been shown that the synthesis of vitamin C is carried out physiologically by the infant independent of the vitamin C taken in with the food. The quantities of this vitamin which are utilized, in fact, appear greatly to exceed the amounts taken into the body even under the best conditions. For example, an infant of fifteen days ingested 2,990 U.H. in twenty-four hours and excreted 6,050 U.H. Another, aged two months, took in 2,400 U.H. in twenty-four hours and passed out 6,496 U.H. A third, aged two months, ingested on the first occasion 1,990 U.H. and excreted 3,288 U.H. Four days later the figures were 470 and 4,638 U.H. respectively. This case is a fine example of the fact that the 'consumption' of vitamin C greatly exceeds the quantity of this substance available in the food of the young infant and that it remains the same whatever the vitamin C content of the diet. If the food contains little of the vitamin, the infant's body produces a greater quantity.

By further experiments it has been shown that the same synthesis of vitamin C is going on in the case of the milch cow and that the vitamin C content of the milk is independent of the cow's food intake. It is well recognized that summer milk is richer in vitamin C than winter milk and this

difference is not entirely due to variations in the vitamin C content of the diet (Florence, Macleod). The synthesis of vitamin C by the milch cow had already also been demonstrated in guinea-pig tests by Hughes, Fitch, Cave and Riddell⁴. It has been shown by us⁵ by the colorimetric method, which allows much more frequent estimations, that there exists in the milch cow an annual cycle in the sense that the vitamin C content of the milk remains low during the winter, and goes up sharply in March, well before the grazing season and at a period when the feed of the animals has not yet undergone any essential change. The vitamin C content remains high until November when it falls abruptly to the winter level which does not vary until March.

There are, therefore, extra-alimentary factors which regulate the vitamin C content of the milk. It is only possible to put forward suppositions as to their nature: solar radiations, temperature, certain cyclical hormonal influences can be taken into consideration.

Discussion.

To return to the pathogenesis of scorbutic dystrophy it will be seen, in the light of the experiments just quoted, that it is no longer possible to maintain the theory which explains its occurrence by the simple lack of vitamin C in the infant's diet. The infant does not become scorbutic because his food is lacking in vitamin C, but because the inherent function controlling the synthesis of this vitamin has become feeble or has disappeared. This fact also explains the capricious and irregular manner in which this disorder occurs in the infant. The problem therefore arises to what are the conditions governing the total or partial inhibition of the synthesis of vitamin C in the infant. A priori, two possibilities must be considered: the infant's diet might be lacking in the dietetic factors from which the synthesis of ascorbic acid takes place or even this function itself could be diminished or completely inhibited by some pathological influence.

The former of these possibilities must first be faced. It definitely occurs under certain conditions. It is well-known that vitamin C itself is not stored in the body. In animals unable to manufacture it or in man from the second year onwards it only requires the elimination of the vitamin from the diet for two days to produce complete disappearance of the urinary excretion of the vitamin. It has been shown that the human infant, during the first year of life, is independent of the dietetic supply of vitamin C since he can manufacture it for himself. Consequently it was surprising to find in infants aged from one to eight months, fed on a commercial milk preparation, that the urinary elimination of vitamin C disappeared after four to five days of this diet. The preparation used was particularly poor in vitamin C, but the infants, who were healthy, should have been able to compensate for this deficiency by an increase in their production of the vitamin. It must therefore be admitted that this particular food is lacking in a substance which contributes to the synthesis of vitamin C.

Does it lack a 'provitamin'? This is not probable for the following reasons. It is difficult at present to be precise about the unstable bodies which form the steps in the synthesis of the vitamin. It can, however, be said that 2-keto-gulonic (or *l*-sorburonic) acid and 2-keto-galactonic acid

appear to be the only stable and sufficiently characterized precursors which precede the formation of vitamin C. It is probable that these substances play the part of 'provitamin C.' It is not yet proved that these acids or in general the substances designated as 'provitamin C' are normally present in the food and in the tissues of the higher animals, but there is nothing chemically to oppose the view that these bodies can be formed by biological processes from material which is widely distributed in living matter. This is the reason why it is necessary to accept that it is not the lack of 'provitamin C' which appears to be the dominant cause of the cessation of the synthesis of vitamin C in the animal.

This synthesis is, in fact, still controlled by other factors which are necessary to produce the passage—difficult from the chemical point of view—of the provitamin to the vitamin. Waldmann has recently shown⁶ that the urine of rats and birds ceased to give the violet reaction of Bezssonoff when these animals (who are manufacturers of vitamin C) are submitted for several days to a diet of decorticated rice. If bran or yeast is added to the diet, the reaction reappears but it remains indefinite and abnormally feeble. If the further addition is made of cod-liver oil, the reaction is greatly intensified and becomes normal. If the diet of decorticated rice is supplemented only by cod-liver oil the violet reaction does not appear.

The interest of these findings lies in the demonstration that vitamins A and D and probably the vitamin B complex, have a decisive action upon the biological synthesis of vitamin C. Our own researches have independently confirmed those of Waldmann, namely that the intake of fat-soluble vitamins plays a definite part in the accomplishment of the synthesis of ascorbic acid in the animal body.

In the infants mentioned above the intake of vitamins A and D was augmented without changing the amount of vitamin C in the diet. Five of the nine infants were each given half of the yolk of a raw egg daily, to the four remaining infants there was given 0.03 c.c. of an extract of fish oil, representing in vitamins A and D, the equivalent of about 1.2 c.c. of a good cod-liver oil. After seven days of this treatment the urinary excretion of vitamin C reappeared in four infants of the former group and, after eight days, in all the infants of the second group. The intensity of the violet reaction in the urines was, respectively, 8, 7, 6, 4, 4, 4, 3 and 2 U.H. Thus the addition of fat-soluble vitamins to a diet lacking in vitamin C re-established the synthesis of this vitamin in the infant's body in eight out of nine instances. Certain considerations permit the view that the action of vitamin A predominates in this connection, since according to H. de Euler⁷ the biological evolution of this vitamin is related to that of vitamin C. This is, moreover, a point which it will not be difficult to verify experimentally.

From these observations it may be concluded that even if the healthy infant is, during the early months of life, independent of the supply of vitamin C in the diet because he is able to synthesize it for himself, the quality of the diet cannot altogether be ignored. This must be well balanced: the lack of other vitamins, and in particular vitamin A, can lead to a diminution of the synthesis of vitamin C and thus take part in the pathogenesis of scorbutic dystrophy.

The second possibility for explaining the etiology of scorbutic dystrophy is that the synthesis of vitamin C is inhibited as a result of a pathological condition. Such a condition ought to show itself in infants maintained on a rational diet by the disappearance of the violet reaction in the urine. Our experience has shown that this is quite a frequent happening.

It must first be recalled that vitamin C deficiency shows itself in the older child and in adults simply by the disappearance of the violet reaction. In these cases if the urine is examined by means of the monomolybdo-phosphotungstic reagent there is no reaction. It is interesting to note that this simple absence of reaction is uncommon in the young infant. We have only observed it twice and only transitorily in some thousands of analyses. At this age the disappearance of the violet reaction is invariably followed by pathological reactions, showing themselves either by a yellowish brown colour or by the formation of a whiteish-grey precipitate ('cloudy reaction'), the supernatant liquid remaining colourless. Sometimes a mauve tint is obtained which, when it is not due to the yellow colour of the urine, results from a super-imposition of yellow and violet ('mixed reaction'). Observation of a large number of cases has shown that the yellow reaction is always the index of a pathological process. From the chemical point of view it is difficult to explain for there are many substances which might produce it. Thus this reaction can be obtained with pyrogallol, with tannin and especially with different alkaloids: it is accompanied by the formation of a precipitate when the concentration of the alkaloid reaches a certain limit. Skatole gives a yellow reaction when its concentration reaches 10 mgm. per litre. In greater concentration (above 100 mgm. per litre) a precipitate occurs in an acid medium in the presence of excess of the reagent: a reddish-brown precipitate is formed by four to five molecule of skatole with one molecule of reagent and the supernatant liquid is coloured violet.

The 'cloudy reaction' is, in general, due to the fact that very different nitrogenous bases form complex combinations with the reagent, which are only slightly soluble. Cretinine is precipitated in the presence of the reagent when its concentration reaches 320 mgm. per litre. Precipitation of guanine occurs when its concentration in distilled water approaches saturation (at 20° C.). The precipitation of skatole has already been mentioned. The cloudy reaction is readily observed in the adult following a diet rich in meat, for example. It is present only in pathological cases in infants. Its significance remains doubtful. It is possible that it indicates the complete disappearance of vitamin C, involving, in infants who are very sensitive to this deficiency, a disturbance of nitrogenous metabolism showing itself by the excretion of substances which produce the yellow or the cloudy reaction. But it is also possible that there exist combinations of these substances with ascorbic acid and that the pathological reactions only indicate the absence of free ascorbic acid from the urine, showing that the quantity of vitamin C prepared by the body is insufficient in the presence of an exaggerated pathological requirements. It can therefore be asserted that the disappearance of the violet reaction in the urine and the appearance of one of the other reactions, always represents a certain degree of vitamin C deficiency.

Clinical results.

To determine the frequency of deficiency states in young infants, investigations have been carried out on two hundred and forty-six children in the hospital. Amongst these, healthy infants or those who could be regarded as such at the end of their convalescence were naturally exceptional.

Amongst the latter the violet reaction was the rule, being found in one hundred and ten instances. Among the infants suffering from a chronic disease process the yellow reaction was found in one hundred and thirty-six instances. To determine the distribution of the two reactions among the infants admitted to the department for whatever reason, fifty-eight unselected children have been examined, all of whom were receiving a normal and balanced diet. The violet reaction was found eleven times and the yellow reaction forty-seven times.

In order to study more closely the distribution of the different pathological reactions in relation to the disorders of the infants, the one hundred and twelve patients who did not give the violet reaction have been utilized. The yellow reaction was given by seventy-eight of these cases, the mixed reaction by seven, the cloudy reaction by twenty-six and a negative result by one. This last infant had lost the violet reaction the day before and by next day the urine gave a yellow reaction. As a general rule it was found that the yellow reaction was obtained in infants with chronic disease, in premature infants, in those designated as dystrophic, hypoparathyroid, and hypotrophic, in dyspeptic conditions, in coeliac disease, in habitual vomiting and in pyloric stenosis, in rickets and spasmophilia, in anaemia, eczema, tuberculosis, the non-febrile stages of whooping cough and various other chronic wasting affections. In rare instances a mixed reaction was obtained. The cloudy reaction appeared principally and regularly in infections. Very often the appearance of this reaction allowed the prediction of the onset of an infectious disorder which appeared later.

Conclusions.

Our investigations have reached this point. They show that with the aid of the reaction with monomolybdo-phosphotungstic acid it is possible to detect states of deficiency in vitamin C at a stage when they do not show themselves by any other characteristic sign. On the one hand, this method permits the study of the conditions under which the synthesis of vitamin C in the infant's body is inhibited and allows the following up from the onset of the repercussions of the vitamin C deficiency on the organism. On the other hand, the disappearance of the violet reaction and the appearance of the yellow reaction indicate the moment when it is necessary to begin the administration of vitamin C, which should be increased until the appearance of the violet reaction in the urine and continued as long as this reaction shows any tendency to become feeble or to disappear. There is thus available a simple method by which in the future the occurrence of scorbutic dystrophy can be prevented.

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