Amino-Aciduria in a Case of Scurvy

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SCURVY can be regarded as one of the classic diseases, occurring with comparative rarity today. A most excellent account of human scurvy was given in 1753 by Lind, who detailed his experiences with thousands of cases, seen while he was a naval surgeon. This work has recently been republished,[1] a remarkable tribute to its merit.

Today there is a tendency for scurvy to occur in the extremes of life, and cases are becoming so uncommon that isolated cases are often considered worthy of publication.[2,3] Of the case reports published in more recent years, a high proportion of cases have occurred in the group of elderly men who live alone and neglect to maintain a sufficiently high dietary intake of vitamin C. In 1944, McMillan and Inglis[4] reported several such cases and applied the term "bachelor scurvy" to them. More recently, Cutforth[5] described eleven cases of adult scurvy, and it is worthy of note that seven of these were in elderly men, who could be assigned to the class covered by bachelor scurvy.

It has been known for many years, that scurvy is accompanied by disturbances of metabolism involving phenylalanine and tyrosine. As long ago as 1907, Stokvis[6] described the darkening of urine voided by a scorbutic patient, due to its abnormal phenylpyruvic acid content. More recently, Boscott and Cooke[7] have described a series of investigations which they made into cases of steatorrhoea and macrocytic anaemia, and they attributed the presence of hydroxyphenylacetic acid in the urine as being due to deranged tyrosine metabolism caused by deficiency of ascorbic acid.

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Amino-aciduria in scurvy has been studied quite extensively by Jonxis[8,9] in children, and in many instances in conjunction with a vitamin D deficiency. The following were the main facts demonstrated: (1) It was usual for about three weeks' treatment to have been applied before the amino acid pattern of the urine returned to normal. (2) In cases in which subperiosteal haematomas were present, the amino acid excretion became increased during the early stages of treatment. It was concluded that when such haematomas were absorbed, amino acids which had become bound to blood decomposition products were released. (3) The most pronounced increases in amino acid excretion were in taurine, threonine plus serine, glycine plus alanine, histidine and lysine plus β-alanine complexes.

In view of these interesting facts, it is rather surprising that in the many cases of adult scurvy reported, references are rarely made to chromatography of the urine, with a view to demonstrating amino-aciduria. When a patient with an advanced case of bachelor scurvy presented himself, we thought that in addition to determining the presence of the amino-aciduria (if any) by the two-dimensional technic of paper chromatography after electrolytic desalting of the urine, we might also apply a simple one-dimensional technic with which we had been experimenting. If it were successful in demonstrating amino-aciduria, we might offer it as a simple alternative method, which could be used when lack of time or facilities prevented a more complete chromatographic investigation. The conventional tests of ascorbic acid deficiency are not entirely satisfactory, the most usual test, the ascorbic acid saturation test, having suffered periodic criticism.[10,11] It would seem to us from the little experience so far obtained, that when

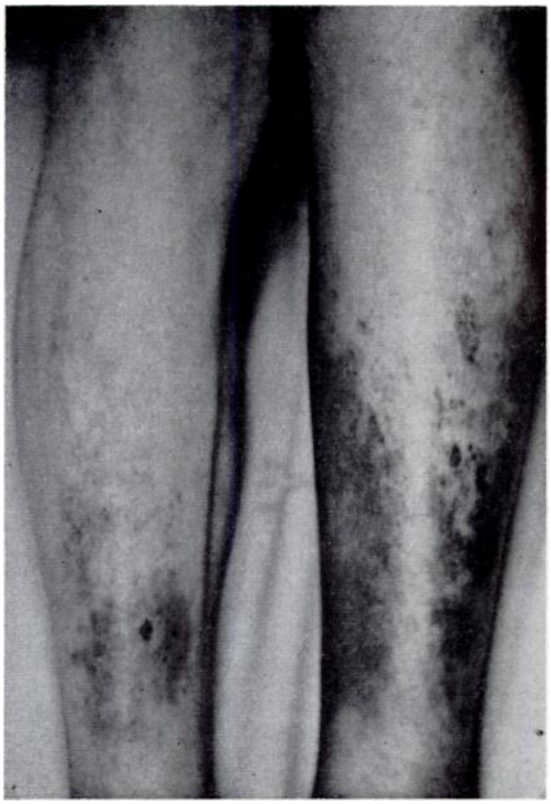

FIG. 1. Extensive hemorrhages of the left leg.

amino-aciduria does occur in scurvy it manifests a magnitude in keeping with the severity of the disease. This does not always seem to be true with the ascorbic acid saturation test. The patient to be described was quite ill, and had amino-aciduria which was quite easy to demonstrate. However, in other milder cases of scurvy which we have seen in both children and adults, amino-aciduria was either not present or slight.

For the purpose of investigation we took urine samples daily and prepared two chromatograms from each daily sample. (1) One was a two-dimensional chromatogram, using an electrically desalted urine sample, and employing phenol for the first run, and tetrahydro-furfuryl alcohol for the second. (2) The other was a one-dimensional chromatogram, employing the technic to be described later. We found that by using this one-dimensional technic we could demonstrate and follow the amino-aciduria with little trouble, although it was not as accurate as the more time consuming two-dimensional technic.

CASE REPORT

The patient was a seventy-three year old man who had been a widower for twelve years. Living alone, he had, during the latter part of this period in particular, failed to maintain a diet which provided an adequate amount of vitamin C. On close questioning regarding his diet, it was found that he had lived mainly on chips and fish, with bread and margarine. He consumed a moderate quantity of alcohol as draught beer. The only food which he cooked for himself was vegetable stew which he always boiled for long periods of time. It is interesting to note that a proprietary brand of fortified margarine satisfied his basic requirements of vitamins A and D. The patient could not remember the last occasion on which he ate fresh fruit, but was certain that it was many years ago.

When he was first seen in the outpatient's department he complained of constant pain and swelling of the left leg, so severe as to prevent walking or even moving the leg. He also complained of infrequent pains in his right thigh, and he was breathless and pale.

On examination, his left leg was swollen and indurated, and there were extensive haemorrhages extending along the anterior part of the leg, passing up the thigh and reaching the buttocks. Similar haemorrhages with induration were present in the back of the right thigh (Fig. 1). The blood pressure was 160/90 mm. Hg. Nothing abnormal was felt in his abdomen, and the central nervous system was normal. He was edentulous, and there were no lesions to be seen in the mouth. From the clinical signs, and in view of the history, a diagnosis of scurvy was made and he was admitted to the hospital immediately.

Laboratory Findings

The laboratory findings were as follows: Serum proteins: Total protein was 5.0 gm./100 ml.; albumin, 3.2 gm./100 ml.; and globulin, 1.8 gm./100 ml. Vitamin A, 60 μg./100 ml. (normal) and carotenes 30 μg./100 ml. Vitamin B_1 (pyruvate metabolism test) blood pyruvate, first specimen 1.0 mg./100 ml.; second specimen, one hour after the administration of 50 gm. glucose, 1.25 mg./100 ml.; third specimen, two hours after the administration of 50 gm. glucose, 1.15 mg./100 ml. (suggests a mild deficit of vitamin B_1). Vitamin C (serum) under 0.4 mg./100 ml. Haemoglobin 44 per cent (6.4 gm./100 ml.), white cells 5,100 per cu. mm., differential and morphology normal, platelets 200,000 per cu. mm., red cells normochromic, some anisocytosis and a few poikilytic forms, reticulocytes 2 per cent. Stools, dark in color, occult blood test positive, barium meal showed no abnormality. Urine, first specimen (before treatment) gave some slight reduction of dichlorophenolindophenol reagent, vitamin C content probably nil. Subsequent specimens taken at daily intervals, after treatment had commenced, gave similar results up to the sixth day, when vitamin C was first positively identified in the urine, at a level of 10 mg./100 ml. Further daily specimens gave vitamin C levels of 8.3 mg./100 ml., 8.3 mg./100 ml., 4.4 mg./

FIG. 2. One-dimensional chromatograms, run for thirty hours ascending, and using the butanol-formic acid-water solvent. First specimen. Before treatment: increased excretion of phenylalanine and leucine (top 2 bands) and the smaller molecular weight amino acids, alanine, threonine, glycine and serine (middle bands). Second specimen. Haematoma resolving: further increase in excretion of threonine and glycine. Third specimen. After six days of treatment: amino acid pattern about normal.

100 ml. and 4.0 mg./100 ml., after which examination was not made daily, but only occasionally as a precautionary check.

Urinary Amino Acids

Specimens 1, 3 and 5 were selected for the one-way amino acid chromatograms shown in the illustration (Fig. 2). Specimen 1 showed amino-aciduria with the following amino acids excreted in abnormal amounts: phenylalanine, leucine and isoleucine, methionine, alanine, serine, glycine, threonine, arginine, and possibly aspartic acid. Specimen 3 showed that the amino-aciduria had become more marked in the course of two days, and specimen 5 showed a practically normal amino acid pattern after six days of treatment.

It will be noted that the tests indicated a slight vitamin B_1 deficiency, but that vitamin A was not deficient. On these grounds, and in view of the patient's diet which had included a sufficiency of margarine, it was

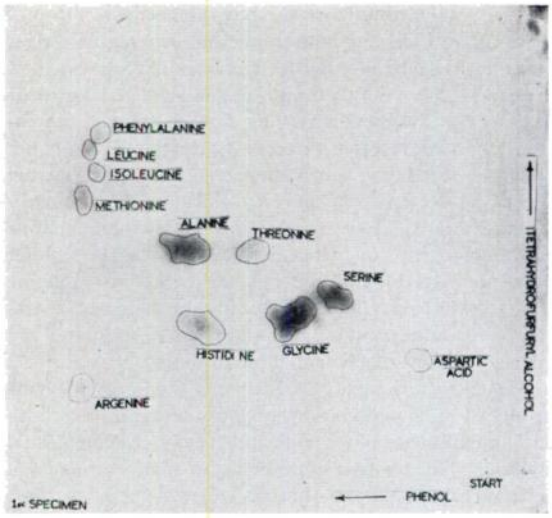

FIG. 3. Two-dimensional chromatogram of the first urine specimen.

considered that vitamin D was probably not deficient either. This case could therefore be regarded as primarily one of vitamin C deficiency.

The anaemia was in accordance with the findings of other workers,[12] who report that 70 per cent of scurvy cases are complicated by anaemia.

The patient responded well to treatment with 500 mg. of ascorbic acid administered parenterally twice daily, and 5 mg. of ferrous gluconate administered twice daily. In five days he showed a reticulocyte response of 15 per cent. Within a month of admission his haemoglobin had risen to 70 per cent (10.2 gm./100 ml.) and the haemorrhages in his legs gradually disappeared. One month after admission the right leg still showed some slight evidence of haemorrhage, while the left leg showed only slight discolouration with desquamation. He could walk about the ward without any dyspnoea, and with no swelling of the ankles.

Chromatographic Findings

The progress as followed by the one-dimensional paper chromatograms of the urinary amino acids was very interesting. Three of the chromatograms are shown, along with a normal control for comparison (Fig. 2). Of these, the first, which is from the patient's urine on admission, shows an abnormal pattern as compared with the normal control. At the top of the strip increases in phenylalanine and leucine are apparent, while lower down in descending order, the bands representing alanine, glutamine-threonine, glycine, and asparagine-serine are increased. These increases were confirmed by the two-dimensional chromatogram for which an electrically desalted urine was used. In this case the following amino acids were shown to be in excess (Fig. 3): phenylalanine, leucine, isoleucine, methionine, alanine, serine, glycine, threonine, arginine, and possibly, aspartic acid.

It is a shortcoming of the one-dimensional chromatogram that threonine-glutamine and asparagine-serine, occur as single bands, so that increases in threonine and in serine cannot be distinguished as such but occur as increases in the corresponding dual band, i.e., an increase in serine causes a deeper asparagine-serine band.

On the third day, when the haematoma was showing signs of resolving, the urinary amino acid chromatogram showed a decreasing excretion of phenylalanine and leucine, but an increasing excretion of the smaller molecular weight amino acids, the bands of threonine and glycine in particular, denoting an increased excretion of these amino acids. The two-dimensional chromatograms confirmed these findings.

Finally, after six days' treatment, the patient's urine produced a virtually normal chromatogram on both the one-dimensional strip and the two-dimensional square. The photographs, being in monochrome, fail to give full justice to the changes reflected, as these were very apparent indeed on the original strips.

The method employed for producing the one-dimensional strip was a modification of that by Awapara and Sato.[13] The original method involved desalting the urine by employing an exchange resin, Dowex 2. This technic we found rather time consuming and never really very satisfactory. Certainly it could not compare with electrolytic desalting. Therefore we made a trial with untreated urines, and in spite of the adverse criticism of this method by the authors, we were pleased with the results, and had been using it along with the better known one-dimensional technic of Dent[15] (using phenol) as a routine screening test, prior to two-dimensional chromatography.

When the method was applied to the urine specimens from our scorbutic patient, the results were so gratifying that it was thought it might be offered as a simple method which could be applied in similar cases.

Method for One-Dimensional Chromatography

The paper used was No. 1 Whatman, strips of 18 inch length, being cut from a 2 inch wide roll. The start line was drawn $1^1/_2$ inches from one end, and the urine applied with a finely drawn out pipette in a narrow band, the aim being to keep the band of applied fluid as narrow as possible. Each application was allowed to dry before the next was applied.

The amount of urine used was 0.1 ml. and in this case, since the patient's urine was concentrated, it was adjusted to the same specific gravity as the control. A determination of nitrogen would have been too time consuming to justify inclusion in a simple method. The amount of application may appear to be rather large, being in the nature of four times the usual amount, but it must be remembered that the application is right across the 2 inch wide strip, and we found that smaller applications were not successful. It has in fact been stated that a moderate excess of a normal urine will not produce an abnormal amino acid chromatogram.[14]

The strips were then suspended vertically with about $1^1/_4$ inch of the end dipping into the solvent, so that the level of the top surface of the solvent was about $^1/_4$ inch from the start line. The solvent used was monophasic, and does not appear to have had wide publication. It is: butanol, 75 parts; formic acid, 15 parts; and water, 10 parts. It suffers from two minor disadvantages: (1) it tends to esterify, so that prolonged runs of three days are not satisfactory, and (2) it appears to work best at temperatures around 22°C. and it will not work well in cold weather if the temperature is allowed to fall to around 16°C. However, it was found that a run of about thirty-six hours maximum, was quite satisfactory, and in very cold weather, the temperature problem can be solved by resorting to such devices as leaving a hot plate (or a Bunsen burner) on in a fume cupboard.

The strips were dried at 37°C. in an incubator (heating above this temperature should be avoided) and, when dry, dipped in a 0.5 per cent solution of ninhydrin in acetone, and returned to hang in the 37°C. incubator. After an hour or two the staining becomes fairly intense, and seems to achieve a maximum after about twelve hours. The strips can then be placed alongside the normal control, with the start lines opposite and comparisons made with the normal. It should be borne in mind that *small* increases in certain amino acids (glycine, alanine, serine, taurine, glutamic acid) have no clinical significance.

SUMMARY

A case of "bachelor scurvy" is described in which the patient is in an advanced state of vitamin C deficiency.

An attempt was made to determine the presence of amino-aciduria, and it was found that this case was not markedly different from those already described. During the period when haematoma was being resolved the amino-aciduria increased. Increased excretion of threonine and glycine was particularly well marked.

This patient responded almost dramatically to treatment, and the rapid physical changes were reflected by the extent of the amino-aciduria. The return to a normal amino acid excretion was only a matter of about one week, as compared to three weeks reported in children.

A simple method for one-dimensional chromatography is given, by which it was possible to follow the course of the amino-aciduria.

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