HYPERAMINO-ACIDURIA IN LIGNAC-FANCONI DISEASE, IN GALACTOSAEMIA AND IN AN OBSCURE SYNDROME

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The observation of various conditions with increased excretion of amino-acids in the urine has given rise to problems of differential diagnosis, many of which are still unsolved. This paper will compare and contrast three different diseases with increased amino-acid excretion. It is based on the description of the following cases; the first an example of Lignac-Fanconi disease, the second an infant suffering from galactosaemia, and the third a child with an obscure syndrome.

The detection of disturbances of amino-acid metabolism in a variety of diseases has been made possible since Consden, Gordon and Martin (1944) introduced paper chromatography. Dent (1947, 1948) first applied this new technique to the study of the amino-acid composition of biological fluids. It soon became clear that paper chromatography is a useful semi-quantitative method for investigating the urine for amino-acids and other substances, e.g., sugars.

Research of this kind has been systematically carried out in Great Britain by Dent (1949, 1950), Woolf (1951) and Bickel (1950, 1952). These and other workers have used paper chromatography to study hyperamino-aciduria in newborn and premature infants (Souchon, 1952), cystine-lysinuria (Dent and Rose, 1951), Lignac-Fanconi disease (Bickel, Smallwood, Smellie, Baar and Hickmans, 1953), phenylpyruvic oligophrenia (Woolf and Vulliamy, 1951; Meister, 1951), liver disease (Dent and Walshe, 1951; Walshe, 1951), idiopathic steatorrhoea and coeliac disease (Bickel, 1952), hepatolenticular degeneration (Uzman and Denny-Brown, 1948; de Verdier, 1950), galactosaemia (Holzel, Komrower and Wilson, 1952; Bickel and Hickmans, 1952), the Fanconi syndrome of adults (Dent, 1948; Milne, Stanbury and Thomson, 1952) and other diseases.

Lignac-Fanconi Disease

Case 1. The boy was the only child of healthy parents. Nothing abnormal was noticed until he was 10 months

old, when he began vomiting intermittently. At 13 months the vomiting became more frequent, his appetite failed and he lost weight. He developed severe thirst with polyuria.

On admission (aged 15 months) he was a small, thin, pale child with fair hair and sunken eyes (Fig. 1) and weighed 18 lb. 2 oz. He appeared ill and dehydrated, and was very fretful. Signs of rickets, such as a large fontanelle, slight bossing of the forehead and broadening of the epiphyses were noted. His muscles were hypotonic. The liver and spleen were not palpable. A radiograph of the wrists confirmed active rickets.

FINDINGS IN THE BLOOD AND URINE†. The blood chemistry is shown in Table 1. Haemoglobin was

FIG. 1.—Two children with hyperamino-aciduria and a healthy child. From left to right, Case 3 aged 5 years, healthy child aged 5½ years, Case 1 aged 5½ years.

11.5 g. %, and leucocytes 16,600 per c.mm. (polymorphs 34%, lymphocytes 66%). A reducing substance was found in the urine; it was identified by chromatography as glucose (Fig. 2), but the blood sugar levels were normal. An oral glucose tolerance test with 6.5 g. glucose gave

† For biochemical and chromatographic methods see Bickel and Hickmans (1953).

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half-hourly blood sugars of 138 (fasting) to 164, 170, 186, 120, 138 mg. per 100 ml.

The 24-hour urine volume, measured at the age of 16 and 17 months, was 1,010 ml. and 1,430 ml. (average normal for a child of 1 year being 300 to 600 ml.). The

FIG. 2.—One-dimensional sugar chromatogram with urines of Case 3 (column No. 2). Case 1 (column No. 3), and Case 2 (column No. 4), showing glycosuria in Case 1 and galactosuria in Case 2. Urine volume in all cases 100 μ l. For purposes of comparison pure lactose, galactose, glucose, fructose and xylose were run in the two outer columns.

specific gravity was 1,002-1,010, and albumin 10 mg. %. The centrifuge deposit showed an occasional pus cell, but no red cells or casts. The reaction of the urine varied, but was usually acid. The ammonia coefficient

$$\frac{\text{ammonia nitrogen} \times 100}{\text{total nitrogen}}$$

was slightly raised to 5.2-6.5% (normal 2%-4%).

INVESTIGATIONS OF AMINO-ACID METABOLISM. At the age of 16 months cystine crystals were found in the urinary deposit. Dr. C. E. Dent, University College Hospital, London, then demonstrated an excessive urinary excretion of some 10 to 15 amino-acids by means of paper chromatography, and this was confirmed by us in many subsequent specimens (Fig. 3).

The amino-acid nitrogen coefficient in the urine

$$\frac{\text{amino-acid nitrogen} \times 100}{\text{total nitrogen}}$$

was raised to 3.6% (normal up to 2%).

The amino-acid content of the plasma was tested by paper chromatography and the colour intensity of various amino-acids was found to be 50% or more above the normal range. The amino-acid pattern closely resembled that in the patient's urine. Paper chromatography is, however, a semi-quantitative method unsuitable for the assessment of slight changes of the amino-acid concentration in plasma.

The clinical and biochemical features of Case 1, especially the pattern of the hyperamino-aciduria and the glycosuria, suggested the diagnosis of Lignac-Fanconi disease. A search for cystine crystals in the eyes by slit-lamp revealed a typical distribution of masses of crystals in the cornea and conjunctiva. Bone-marrow films likewise showed cystine deposits, the crystallographic characteristics of which were recently discussed in detail by Baar and Bickel (1952).

The patient's mental development has been above that of the average child, but physical development has been much retarded. He is now 5½ years old and his height is 35 in. and weight 25½ lb. (Average normals for this age stand 43 in. high and weigh 43 lb.) At 2½ years old alkali treatment was started (sodium citrate 100, citric acid 140, water to 1,000 ml., 10 ml. five times daily by mouth), and also 'calciferol' (50,000 units, later reduced to 15,000 units, daily by mouth). During the following

FIG. 3.—Two-dimensional amino-acid chromatogram with urine of the patient with Lignac-Fanconi disease (Case 1). For all amino-acid chromatograms a urine volume=500 γ N₂ was taken.

AL.=alanine; AS.=aspartic acid; CY.=cystine as cysteic acid; GL. A.=glutamic acid; GLU.=glutamine; GLY.=glycine; LEU.=leucine + isoleucine; LY.=lysine; PRO.=proline; SE.=serine; TH.=threonine; TY.=tyrosine; VA.=valine. TT.5, 10 etc.=5 γ , 10 γ etc. test taurine, added to the chromatogram before the run. Figures beside amino-acids indicate colour intensity and size of spots as compared with taurine test spots (e.g., 10T=colour and size of spot equal to 10 γ taurine).

year on this treatment the condition greatly improved, the rickets healed, his appetite increased and the vomiting practically ceased. The child grew 2 in. and gained 3 lb. 4 oz. in weight. He continued to suffer from abnormal thirst and polyuria, but no further metabolic crises have been observed.

Galactosaemia

Case 2. A baby girl, aged 10 days, was admitted with vomiting and slight jaundice. She was a full-term baby from a normal delivery, weighing 8 lb. at birth. She seemed normal for the first week of life. Slight jaundice then appeared, which fluctuated in intensity. She began vomiting when 9 days old. The mother maintained that the child's symptoms were similar to those shown by her previous child, who died in 1948, aged 11 weeks. This sibling had had cirrhosis of the liver and a cataract in the right eye.

On admission the baby was pale and slightly jaundiced, weighing 6 lb. 11 oz. The liver was greatly enlarged, and the lower edge could be palpated $3\frac{1}{2}$ in. below the right costal margin. The umbilicus showed evidence of recent haemorrhage and sepsis which was at first thought to account for her symptoms. The eyes showed no sign of cataracts. The urine contained protein and a trace of sugar; bilirubin and urobilin were also present. After

found to contain 1.8% of a reducing substance which was identified by paper chromatography as galactose (Fig. 2). A galactose tolerance test provided further support for the diagnosis of galactosaemia. The fasting blood galactose was 14 mg. %, and after 5.25 g. of galactose by mouth (1.75 g./kg.) rose to 254 mg. % in 2 hours.

CHROMATOGRAPHIC INVESTIGATIONS. After three months on a galactose-free diet the urine was again tested by sugar chromatography but no sugar was detected. Amino-acid chromatograms were then carried out for the first time and were normal. Five months later the child was put for one day on a diet containing 600 ml. milk and galactose reappeared in the urine, but the amino-acid excretion remained normal. In two cases of galactosaemia studied before the elimination of lactose from the diet paper chromatography revealed hyperamino-aciduria (Fig. 4) which was also described in two patients by Holzel *et al.* (1952).

At the age of 5 weeks milk feeds were stopped, and a lactose-free regime was instituted. This was followed by marked improvement, reducing substances disappeared from the urine and the enlarged liver slowly returned to normal size. Solid food was gradually introduced into the diet at the normal age, and the child made normal progress with no evidence of mental retardation or the development of cataracts. At 11 months old there was no galactose in the fasting blood specimen, but after 18 g. of galactose by mouth, the half-hourly blood galactose levels were 50-117-167-234 mg. %. There was no hyperamino-aciduria during the test. At 19 months the child's height is now $28\frac{1}{2}$ in., and her weight is 21 lb. 12 oz. The galactose intolerance, however, remains unchanged.

Hyperamino-aciduria in an Obscure Syndrome

Case 3. The boy is the second child in a healthy family, and was delivered by forceps (birth weight 9 lb.). At 4 months he was noticed to have bilateral cataracts. From the second year onwards it became apparent that his mental and physical development was almost static. The joints could be bent like an acrobat's, and he developed senseless jerking movements of the limbs with frequent grimacing. His appetite was poor and he was often constipated, but there was no vomiting.

On admission (aged 4 years 5 months) he was dwarfed ($34\frac{1}{2}$ in., normal 39 in.) and weighed $27\frac{1}{2}$ lb. (Fig. 1). He looked pale and flabby, was very hypotonic, and displayed remarkable mannerisms in which he rapidly jerked his hyperflexible limbs and wagged his fingers in front of his eyes. He was almost blind, though he could distinguish light and darkness. There was considerable enophthalmos due to lack of periocular fat, and a coarse, searching nystagmus. The abdomen was protuberant, there was divarication of the recti and an umbilical hernia. Rickets was evident from frontal bossing, a slight rosary, enlarged wrists and knock knees. Radiographs showed gross active rickets, osteoporosis, and the suggestion of a pseudo-fracture of the left femur. Carpal ossification was equivalent to that of a boy of

FIG. 4.—Two-dimensional amino-acid chromatogram with urine of a case of galactosaemia. ME.SNE. = methionine as methionine sulphone; PH. = phenylalanine.

admission vomiting continued, and dehydration was corrected by parenteral fluids.

FINDINGS IN BLOOD AND URINE. At the age of 5 weeks the serum bilirubin was 0.66 mg. per 100 ml.; thymol turbidity 4.5 units; alkaline phosphatase 17 Kay units, albumin 4.0, globulin 5.0 g. %. The urine was

2 years. Ophthalmological examination revealed bilateral capsulolenticular cataracts.

FINDINGS IN BLOOD AND URINE. Normal values for red blood cells, haemoglobin, leucocytes, the differential count, prothrombin time and platelets were obtained. A differential count of the bone marrow showed low cellularity with an increase of immature white cells; no cystine crystals were seen in sections fixed in alcohol.

The blood chemistry is given in Table 1.

The cholesterol and bilirubin levels and thymol turbidity were normal. The 24-hour urine volume was 300 to 600 ml. The urine contained 40-90 mg. % protein. Benedict's test and chromatography were negative for sugar (Fig. 2) except in two specimens, where traces of glucose and galactose were detected. The centrifuged urine deposit was often found to contain some granular casts and tyrosine crystals. The urine pH varied between 7.8 and 5.7. Bicarbonate excretion was 2.3 (pH 6.6) and 0.8 mEq./l. (pH 5.7). The ammonia coefficient was 4%, 10% and 10%.

GALACTOSE TOLERANCE TESTS. These were performed because of the clinical resemblance between this patient and cases with galactosaemia. (a) Within four hours after ingestion of 30 g. galactose, only 0.3 mg. and, in a repeat, 0.5 mg. galactose, were excreted in the urine. (b) Blood galactose levels were estimated after ingestion of 20 g. galactose. The half-hourly blood galactose levels were nil (fasting) to 17, 86, 134, 17 mg. per 100 ml. Repeating the test the levels were nil to 17, 84, 134, 17 mg. per 100 ml. According to Peters and Van Slyke (1946), the tests under (a) were normal, but the blood tolerance curve of test (b) was somewhat high and prolonged.

HIPPURIC ACID TEST. Only 37% of 3 g. ingested sodium benzoate was recovered in the urine as hippuric acid within four hours (normal above 50%).

LIVER BIOPSY. The liver architecture was normal. Glycogen was abundant, though not excessive in all liver cells. There was no suggestion of glycogen storage

FIG. 5.—Electrolytes in plasma (left) and urine (right) of Case 3 as compared with average normal values for plasma and urine. OA = organic acids, estimated by subtracting the other anions from the total bases.

disease. No cystine crystals were found. Some nuclei contained periodic-acid-Schiff-positive material, but similar changes have also been found in other conditions and probably represent nuclear glycogen deposits despite their resistance to short salivary digestion.

KIDNEY FUNCTION TESTS. The average urea clearance corrected for surface area was 91%. Urography showed normal diodone concentration and no structural abnormalities of renal pelves or ureters. In a water concentration test the specific gravity of the urine rose to 1,024 after 16 hours' deprivation of water.

CALCIUM-PHOSPHORUS BALANCES. The results were essentially normal, but they were probably influenced by a single dose of 100,000 units vitamin D before admission, three weeks before the balance.

ELECTROLYTIC FINDINGS. In the plasma there were hypophosphataemia and a moderately decreased bicarbonate level (Fig. 5). The plasma organic acids, estimated by difference between total bases and the measured anions, were 12 mEq./l. which is at the upper limit of normal. The 24-hour urine specimen was alkaline (pH 7.68), with a high bicarbonate and low ammonia content. The excretion of phosphate and sulphate was small, that of organic acids (estimated as in plasma but taking titratable acidity into account) was raised to 2.0 mEq./kg./24 hours. The urine was collected under toluene with thymol crystals added.

INVESTIGATIONS OF AMINO-ACID METABOLISM. Chromatograms of numerous urine specimens showed a

constant and considerable hyperamino-aciduria (Fig. 6). The total colour intensity of the chromatograms was often five times that of normal urine. The amino-acid nitrogen coefficient was 4.5%.

The sum of the glutamine and glutamic acid in the plasma was raised to 13.8 and 14.0 mg. per 100 ml. (normal 7-10 mg. per 100 ml.).* The daily urine excretion of these amino-acids was 535 mg. (normal up to 100 mg. per day). Chromatographic investigation of two plasma specimens gave no conclusive evidence of an increased amino-acid concentration, though the level of various amino-acids, especially of cystine, lysine, threonine, tyrosine and phenylalanine seemed to be above the upper limit of normal.

TABLE 1
BIOCHEMICAL FINDINGS IN CASES 1 AND 3

Biochemical Finding	Case 1 Range	Case 3 Range	Normal Range
Serum calcium	10.3-10.8	9.1-11.6*	9.0-11.5 mg. %
Serum phosphorus	1.8-5.2*	2.1-2.8	4.5-5.5 mg. %
Serum alkali phosphate	30.2*-50.0	40.4-46.2	8-15 Kay units
CO ₂ -combining power	13.5-27.9†	18.8-22.1†	24-30 mEq./l.
Blood urea	16.5-75.0‡	22.6-36.1	20-40 mg. %
Serum albumin	4.6		4.5 g. %
Serum globulin	2.6		2-2.5 g. %
Plasma potassium	3.3	3.8-4.4	3.8-5.0 mEq./l.
Plasma sodium		137	137-145 mEq./l.
Plasma chlorides		103	96-110 mEq./l.
Fasting blood sugar	84-138	80	75-110 mg. %

* During massive vitamin-D therapy.

† During alkali therapy.

‡ During dehydration.

Treatment with 8 g. sodium citrate and 5,000 units 'calciferol' daily did not produce marked improvement, and the rickets remained active. Large doses of 50,000 units of 'calciferol' daily were not well tolerated and produced a moderate elevation of the serum calcium level to 11.6 mg. per 100 ml. A lactose-free diet also failed to bring about any improvement in the clinical or biochemical picture.

Discussion

Case 3 shows a peculiar syndrome characterized by bilateral cataracts, enophthalmos, hypophosphataemic rickets and dwarfing. The patient is backward in his mental and physical development, and shows severe muscular hypotonia. In the blood the CO₂-combining power is reduced, and in the urine there is slight albuminuria and a strong hyperamino-aciduria. The special interest of this syndrome lies in its partial resemblance to Lignac-Fanconi disease and galactosaemia, inborn errors of amino-acid and galactose metabolism.

Lignac-Fanconi Disease. This name is applied here to a condition which has been described under various names, such as cystine storage disease or Lignac's disease, nephrotic-glycosuric dwarfism with

* We are indebted to Professor H. A. Krebs for carrying out this estimation.

FIG. 6.—Two-dimensional amino-acid chromatogram with urine of the patient with the obscure syndrome (Case 3). AR.=arginine; HI.=histidine; TA.=taurine; TRY.=tryptophane.

hypophosphataemic rickets, de Toni-Debré-Fanconi syndrome, cystine rickets, amino-acid diabetes, etc. It seems doubtful whether all these cases correspond to one clinical entity. The name 'Fanconi's syndrome' in particular has been applied rather indiscriminately to conditions characterized by hypophosphataemic rickets or osteomalacia, hyperamino-aciduria, perhaps with glycosuria, acidaemia, with or without dwarfing, in children and adults. The one term may thus conceal quite different diseases, for which a common name is misleading, as it invites generalizations. If, however, cystine storage is regarded as an essential feature of the disease, then such cases form a well defined clinical entity which for purposes of clear distinction we propose to call Lignac-Fanconi disease (Lignac, 1924; Fanconi, 1936). It is our contention that in many cases of Fanconi's syndrome reported in the literature, cystine storage has been overlooked during life and even at necropsy. Slit-lamp investigations for cystine crystals in the cornea and conjunctiva are difficult in young children, and cystine crystals in bone-marrow smears are often scanty and easily dissolved in acid stains or water.

Case 1 represents a typical example of Lignac-Fanconi disease as we have observed it in 17 further patients (Bickel *et al.*, 1953). In this case anorexia, vomiting, polyuria and dehydration were the presenting symptoms, beginning characteristically in the second six months of life. The finding of glycosuria at first suggested the diagnosis of diabetes but the blood sugar level was normal. The child was dwarfed, and this became more striking as he grew older, though a small measure of growth was achieved by treatment with sodium citrate-citric acid solutions and massive doses of 'calciferol'. This treatment cured the rickets, produced a remarkable general improvement and led to the cessation of the hyperamino-aciduria*. Although the hyperamino-aciduria varied considerably from day to day it was always of a pattern characteristic of the disease. No quantitative amino-acid blood tests were carried out, but in six other cases investigations by microbiological assay by Dr. K. Schreier, and by the glutaminase method by Professor H. A. Krebs, showed a raised plasma level of various amino-acids (Bickel and Hickmans, 1953; Philpott, Harvey and Finch, 1953). The demonstration of cystine storage in eyes and bone-marrow established the diagnosis, and was a regular feature of all our other cases.

* A recurrence of the hyperamino-aciduria and rickets has recently been observed in a boy with Lignac-Fanconi disease whose 'calciferol' treatment had been stopped but who was satisfactorily alkalinized. This suggests that vitamin D therapy, not alkalization, leads to the cessation of the hyperamino-aciduria.

Hypopotassaemia is another important biochemical finding in Lignac-Fanconi disease, though it may not be observed in the presence of dehydration, a common occurrence in such patients.

Cases 1 and 3 showed many points of resemblance, but at the same time certain important differences. Dwarfing, rickets with a low serum phosphorus and high phosphatase, hyperamino-aciduria and reduced CO₂-combining power, were seen in both. On the other hand, cystine storage, polyuria and polydipsia, which were marked in Case 1, were absent in Case 3. Case 3 also differed from the case of Lignac-Fanconi disease in being mentally retarded and suffering from enophthalmos and cataracts. Finally, the relative amounts of various amino-acids in the urine, such as the strong excretion of histidine, tyrosine and phenylalanine, differentiated Case 3 from Case 1. Case 3 could not thus be classified under the diagnosis of Lignac-Fanconi disease. The retarded mental development and bilateral cataracts drew attention to another possibility, galactosaemia, particularly as a trace of galactose was found in the sugar chromatogram of the urine on two occasions.

Galactosaemia. When milk feeds were started in the neonatal period Case 2 showed the characteristic features of this condition, i.e., transient jaundice, hepatomegaly, albuminuria and galactosuria. Galactose tolerance tests and the improvement on the lactose-free formula confirmed the diagnosis. The sibling who died untreated four years previously had cataracts and cirrhosis of the liver. Older untreated cases are usually mentally retarded. The assessment of the most important diagnostic feature, galactosuria, may prove difficult. A patient has been described by Townsend, Mason and Strong (1951) and another by Bray, Isaac and Watkins (1952) who, though proved or probable cases of galactosaemia, could at the age of 7 years tolerate a milk intake of 200 and 500 ml. respectively without showing galactosuria. On the other hand, galactosuria has been observed in other conditions, such as liver cirrhosis, liver atrophy, and in newborn infants (Rapaport, 1950). Thus intolerance of galactose after a test dose may simply be a sign of impaired liver function. Despite these limitations, the demonstration of galactosuria and impaired galactose tolerance are still important factors in establishing the diagnosis. Sugar chromatography is a specific, sensitive, and relatively simple method of demonstrating galactose in the urine, especially when it occurs only in traces or together with other sugars.

Another biochemical abnormality found in galactosaemia is hyperamino-aciduria, which has been observed independently by Holzel *et al.* (1952)

and by Bickel and Hickmans (1952). A striking feature of the hyperamino-aciduria in both of Holzel's patients was its disappearance with the omission of galactose from the diet and reappearance when galactose was again given. Two of our patients, however, continued to show a definite though reduced hyperamino-aciduria for months after the institution of a galactose-free diet. This discrepancy may depend on the extent to which the liver or kidney damage leading to the hyperamino-aciduria remains reversible. Case 2 in this paper was diagnosed and treated exceptionally early, and showed no hyperamino-aciduria even during galactose ingestion. We do not know if there is a characteristic amino-acid pattern in galactosaemia, as there is in Lignac-Fanconi disease.

Despite cataracts and retarded development, there seems sufficient evidence to exclude the diagnosis of galactosaemia in Case 3. The liver was not markedly enlarged, and its normal architecture in the biopsy contrasted with the cirrhotic and necrotic changes found in galactosaemia. The results of the galactose tolerance and hippuric acid tests point to some liver dysfunction, but the galactose intolerance does not seem severe enough to justify the assumption of an inborn error of the galactose metabolism. There was no clear-cut galactosuria in the chromatograms, and a galactose-free diet did not prove beneficial.

There was a striking resemblance of Case 3 to three patients recently described by Lowe, Terrey and MacLachlan (1952). These children showed mental retardation, flabby musculature with an abundance of subcutaneous fat, a peculiar, high-pitched cry, intermittent fever, glaucoma and cataracts. Active rickets was demonstrated in two of them, one of whom suffered from multiple fractures. Biochemical features included hyperamino-aciduria, organic-aciduria, reduced CO_2 -combining power and, in two cases, slight glycosuria. The ammonia production following the administration of ammonium chloride was decreased.

There are, however, certain differences between Lowe's patients and Case 3. Glaucoma, the peculiar cry and intermittent fever were not features of our patient, and his ammonia production seemed normal, though this was not estimated during ammonium chloride feeding. The pattern of the hyperamino-aciduria in Lowe's cases was similar to that in Case 3, though our patient excreted in addition arginine, tyrosine and phenylalanine. The sum of glutamine and glutamic acid in the plasma of Case 3 provides evidence that the blood levels of at least these amino-acids are raised and that the hyperamino-aciduria is not entirely renal in origin, as suggested in Lowe's cases.

Lowe defined his patients' disease as 'organic-aciduria', as their organic acid excretion was too high to be accounted for solely by amino-acid increase. Our Case 3 showed an increase of the organic acid excretion to 2.0 mEq./kg./24 hrs. (normal less than 1 mEq./kg./24 hrs.) when estimated by the indirect method of subtracting the known anions from total bases. According to Dent (1953) and Linder, Bull and Grayce (1949), this is at present the only practicable method in the presence of hyperamino-aciduria, as the amino-acids interfere with the end-point determination of Van Slyke and Palmer's titration method used by Lowe. The moderate increase of organic acids in Case 3 is probably satisfactorily explained by the approximately threefold increase of the amino-acid content in the chromatogram of this urine specimen, which in terms of milliequivalents would amount to about 1.6 mEq./kg./24 hrs.

In conclusion, though not showing all the features of the syndrome described by Lowe, Terrey and MacLachlan, the disease of Case 3 certainly bears a closer resemblance to that syndrome than to either Lignac-Fanconi disease or galactosaemia. The differences mentioned above may be due not to a fundamentally different disorder but to individual variations in the features of the disease.

Summary

Various diseases with hyperamino-aciduria are enumerated, some of which present difficulties in differential diagnosis. Representative cases of three such conditions, Lignac-Fanconi disease, galactosaemia and an obscure syndrome, are described in detail.

Lignac-Fanconi disease represents a clearly defined clinical entity if cystine storage is regarded as an essential feature of the condition. The hyperamino-aciduria is of a characteristic pattern involving the increase of some 10 to 20 amino-acids. Glycosuria may be minimal and traceable only by sugar chromatography.

For the diagnosis of galactosaemia the demonstration of galactosuria and galactose intolerance is fundamental, though not conclusive. The significance of the hyperamino-aciduria recently demonstrated in this disease is still unknown.

An obscure syndrome consisting of hyperamino-aciduria, mental and physical retardation, peculiar motility and mannerisms, cataracts, enophthalmos, hypophosphataemic rickets, muscular hypotonia, reduced CO_2 -combining power of the blood and albuminuria is described in a 4½-year-old boy. Despite certain similarities to Lignac-Fanconi disease

and galactosaemia both diagnoses were excluded. There was, however, a considerable resemblance to a syndrome recently described by Lowe, Terrey and MacLachlan, though an increase in the urine of organic acids other than amino-acids was not a feature of our case.

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REFERENCES

- Baar, H. S. and Bickel, H. (1952). *Acta paediat., Uppsala*, 42, Suppl. 90, p. 171.
- Bickel, H. (1950). *Archives of Disease in Childhood*, 25, 208.
- (1952). Ph.D. Thesis, University of Birmingham.
- and Hickmans, E. M. (1952a). *Archives of Disease in Childhood*, 27, 348.
- (1952b). *Acta paediat., Uppsala*, 42, Suppl. 90, p. 137.
- , Smallwood, W. C., Smellie, J. M., Baar, H. S. and Hickmans, E. M. (1953). *Ibid.*, Suppl. 90, p. 9.
- Bray, P. T., Isaac, R. J. and Watkins, A. G. (1952). *Archives of Disease in Childhood*, 27, 341.
- Consden, R., Gordon, A. H. and Martin, A. J. P. (1944). *Biochem. J.*, 38, 224.
- Dent, C. E. (1947). *Ibid.*, 41, 240.
- (1948). *Ibid.*, 43, 169.
- (1949). *Biochem. Soc. Symposia*, 3, 34.
- (1950). *Schweiz. med. Wschr.*, 80, 752.
- (1953). Personal communication.
- and Rose, G. A. (1951). *Quart. J. Med.*, n.s., 20, 205.
- and Walshe, J. M. (1951). Ciba Symposium: *Liver Disease*, ed. Sherlock, S. and Wolstenholme, G. E. W. London.
- Fanconi, G. (1936). *Jb. Kinderheilk.*, 147, 299.
- Holzel, A., Komrower, G. M. and Wilson, V. K. (1952). *Brit. med. J.*, 1, 194.
- Lignac, G. O. E. (1924). *Dtsch. Arch. klin. Med.*, 145, 139.
- Linder, G. C., Bull, G. M. and Grayce, I. (1949). *Clin. Proc.*, 8, 1.
- Lowe, C. U., Terrey, M. and MacLachlan, E. A. (1952). *Amer. J. Dis. Child.*, 83, 164.
- Meister, P. (1951). *Helv. paediat. Acta*, 6, 504.
- Milne, M. D., Stanbury, S. W. and Thomson, A. E. (1952). *Quart. J. Med.*, n.s., 21, 61.
- Peters, J. P. and Van Slyke, D. D. (1946). *Quantitative Clinical Chemistry Interpretations*, Vol. I, 2nd ed. London.
- Philpott, M. G., Harvey, C. C. and Finch, E. (1952). *Acta paediat., Uppsala*, Suppl. 90, p. 114.
- Rapoport, M. (1950). In *Mitchell-Nelson Textbook of Paediatrics*, 5th ed., p. 862. Philadelphia.
- Souchon, F. (1952). *Z. ges. exp. Med.*, 118, 219.
- Townsend, E. H., Mason, H. H. and Strong, P. S. (1951). *Pediatrics*, 7, 760.
- Uzman, L. and Denny-Brown, D. (1948). *Amer. J. med. Sci.*, 215, 599.
- Verdier, C. H. de (1950). *Acta med. scand.*, 138, 344.
- Walshe, J. M. (1951). *Quart. J. Med.*, n.s., 20, 421.
- Woolf, L. I. (1951). *Gt Ormond Str. J.*, No. 2, p. 61.
- and Vulliamy, D. G. (1951). *Archives of Disease in Childhood*, 26, 487.

Addendum

Since this paper was written we have had the opportunity of studying the development of hyperamino-aciduria in Lignac-Fanconi disease and galactosaemia since birth. The baby with Lignac-Fanconi disease was perfectly healthy until the age of 5 months, when hyperamino-aciduria and glycosuria slowly developed together with certain typical symptoms, thirst, anorexia, dehydration and failure to gain weight. At the same time the first few cystine crystals appeared in the cornea and bone marrow, while a lymph-gland biopsy showed abundant crystals.

In a newborn infant with galactosaemia no hyperamino-aciduria was present on the third, fourth and fifth days of life, despite massive galactosuria. When re-examined at 3 months of age hyperamino-aciduria was found, together with a raised alpha-amino-nitrogen in the plasma of 8.8 and 8.3 mg. per 100 ml. (gasometric ninhydrin method). When the child was given a galactose-free diet the hyperamino-aciduria ceased and did not return during two subsequent galactose tolerance tests.