AMINOACIDURIA in CHILDHOOD

A thesis presented by

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PREFACE.

While assisting Professor Fanconi in a study of two patients suffering from disorders of protein metabolism, I became aware of the many gaps in our knowledge of the urinary excretion of aminoacids in childhood. It occurred to me that a systematic investigation by paper chromatography of urine specimens from healthy children and from children with various metabolic disorders would help to clarify the following points:-

(1) The kind of aminoacids excreted in the urine of healthy children of various age-groups and their approximate concentration.

(2) The number and variety of metabolic disorders in which pathological aminoaciduria occurs.

(3) The aminoacid pattern of each disease and the extent to which it is specific and of diagnostic value. If various diseases were found to exhibit typical aminoacid patterns in the urine, then this would provide a new and valuable diagnostic aid which would be especially welcome in disorders of still ill-defined symptomatology, such as Lignac-Fanconi disease.

For the last two and a half years (October 1949-March 1952) I have undertaken a comprehensive study of the aminoacid pattern in the urine of children both in health and disease. The purpose of this study was to ascertain first the normal pattern and then its variations in pathological conditions. The following account is based on the evaluation of

over 5000 chromatograms prepared during this work. From experience gained during this study I am convinced that paper chromatography provides the clinician with a fascinating and powerful research tool. Its clinical application throws light on disturbances of the aminoacid metabolism which so far, due to lack of suitable methods, have remained largely obscure.

My thanks are due to Professor Smellie for his valuable material assistance and encouragement, and to Dr. C. Smallwood, Dr. H. S. Baar and Dr. J. Gerrard for their constructive criticism. I am indebted to other consultants of the Children's Hospital, Birmingham, the United Birmingham Hospitals and Hospitals of the Midland and other regions for providing me with urine and plasma specimens and for their generous permission to make use of their cases. I am also grateful to the medical and nursing staff of the Children's Hospital, without whose help and co-operation my work would have been impossible. Dr. C. E. Dent generously provided me with valuable technical advice and gave me access to his laboratory. Mr. J. G. Williamson, photographer to the Children's Hospital, kindly prepared the illustrations. Finally, my especial thanks are due to Dr. Evelyn M. Hickmans, to her assistants in the Biochemical Department, and to B. Rudd, technical assistant, whose tireless and reliable work has greatly assisted this study.

Kurt Michel

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AMINOACIDURIA in CHILDHOOD.

I. INTRODUCTION.

The first aminoaciduria was described nearly a hundred and fifty years ago when Wollaston in 1810 detected cystine as a component of a bladderstone. Since then many cases of cystinuria have been recorded where excessive cystine excretion was demonstrated by the cyanide-nitroprusside and Sullivan test or by the finding of cystine stones or crystals in the urine. The discovery of a second disorder with aminoaciduria, namely Lignac-Fanconi disease, was also due to the finding of cystine, which at the post mortem examination of the patients was seen by the naked eye in crystalline form in various organs (Abderhalden 1903, Lignac 1924). Lignac (1926), after finding cystine stones in the kidney and ureter of one of these patients, suggested that this disease too might exhibit cystinuria. In 1937 Fanconi and Freudenberg each independently confirmed Lignac's suggestion by demonstrating cystine in the urine of patients with Lignac-Fanconi disease.

These and many other reports concerning the two diseases dealt only with cystine, which, being the least soluble aminoacid, becomes visible in the

form of stonesor crystals in tissues or urine. Occasionally, however, mention was made of the fact that other aminoacids, such as tyrosine, leucine, lysine, etc., were simultaneously excreted in both diseases (Baumann and Udransky 1889, Percival 1902, Loewy and Neuberg 1904, Abderhalden and Schittenhelm 1905, Ackermann and Kutscher 1912, Sylla 1929, Fanconi 1942, 1946, van Creveld and Grünbaum 1941). Especially in Lignac-Fanconi disease, patients were observed (McCune, Mason and Clarke 1943, Fanconi 1946) whose total aminoacid excretion, measured by the method of Folin (1922) or by formol titration was excessive, even without any cystine excretion in their urine. In 1936 and 1942 Fanconi ventured to suggest that such patients were suffering from general aminoaciduria, not simply from cystinuria.

The history of cystinuria and Lignac-Fanconi disease illustrates how the lack of precise and specific analytical methods for the detection of aminoacids has, until recently, hampered this important branch of research. Thus, in spite of the vital importance of aminoacids in the metabolism, their concentration in blood and urine in health and disease has been but little investigated. Since more than twenty different aminoacids may be found in body fluids, real insight into structural changes of these fluids can only be obtained by determining not only one or two aminoacids or their total sum but the concentration of each individual aminoacid. This can vary greatly without any noticeable change in the total concentration.

For this purpose the traditional methods of testing single aminoacids, e.g. the cyanide-

nitroprusside or Millon's test, and the estimation of the total amino-nitrogen by Folin's method or by formol titration, are inadequate, as they fail to reveal changes in the aminoacid pattern. In recent years, however, two satisfactory new methods have been developed: paper chromatography and microbiological assay. Since their introduction into aminoacid research the knowledge of the behaviour of the various amino-acids in health and disease has rapidly advanced.

Paper chromatography was first described by Consden, Gordon & Martin (1944) and was later applied to the study of disease by Dent (1947) in an adult patient with so-called "Fanconi Syndrome". Since then, increasing use has been made of chromatography in the study of various forms of aminoaciduria (Dent, 1949, Fanconi and Bickel, 1949, Linder, Bull and Grayce, 1949, d'Avignon and Vahlquist 1949, Dent and Walshe, 1951; Crumpler, Dent and Lindan, 1950, Bickel, 1950a, b; Dent and Harris, 1951; Dent and Rose, 1951; Drablos, 1951; Meister, 1951; Walshe, 1951; Bickel, 1951a, b; Souchon 1952, Holzel, Komrower and Wilson, 1952; Spencer and Franglen, 1952; Milne, Stanbury and Thomson, 1952; Anderson, Miller and Kenny, 1952). Similarly, microbiological assay has been applied by an increasing number of workers to whose results frequent reference will be made.

This study is based principally upon investigations by paper chromatography, supplemented by microbiological assay in a few special cases. To ascertain the normal aminoacid patterns in urine, chromatography was applied to the urine of 243 children attending school and day nurseries, and 47 infants. The main diseases investigated were:

Table 1.

SURVEY of AMINOACID CHROMATOGRAMS.

(October 1949 - February 1952)

	No. of cases	No. of chromato-grams.
Total number		5100
Pure aminoacids		248
Normal urines	290	423
Cystine-lysinuria	30	184
Lignac-Fanconi disease	16	769
Phenylpyruvic oligophrenia	5	66
Liver disease (hepatitis, toxic liver damage, cirrhosis etc.)	51	147
Coeliac disease	34	106
Steatorrhoea	60	352
Galactosaemia	3	55
Hepatolenticular degeneration	4	74
Aminoaciduria in an obscure syndrome	1	55
Aminoaciduria in glycogen storage disease, acrodynia and osteopetrosis	4	20
Patients with burns	27	63
Kidney disorders without definite aminoaciduria (cystic kidney, nephritis, nephrosis, renal rickets, renal acidosis, renal calcification and calculi, renal glycosuria etc.)	57	168
Other conditions without aminoaciduria (adrenal tumour, 1 case; alkaptonuria, 2; congenital cataract, 1; cretin, 1; cystic bones, 1; diabetes insipidus, 2; diabetes mellitus, 4; gout 1; haemolytic disease of the newborn, 13; hyperpituitarism, 1; morbus Addison, 3; myelomatosis, 1; oligophrenia, 15; phosphatidosis, 1; porphyrinuria, 1; progressive muscular dystrophy, 1; pubertas praecox, 1; resistant rickets, 25; retinitis pigmentosa, 59; Tay Sachs, 1; thyrotoxicosis, 1 and others).		2730

Lignac-Fanconi disease.
Cystine-lysinuria.
Phenylpyruvic oligophrenia.
Liver cirrhosis and hepatitis.
Steatorrhoea.
Galactosaemia.
Wilson's disease.

A survey of the material is given in Table 1. In this thesis only those diseases will be discussed where sufficient cases and urine specimens could be investigated to form some basis for conclusions. A brief report will be included of an isolated case of a syndrome which appeared to be of special interest. The description of these aminoacidurias will deal principally with their chromatographic characteristics without giving a detailed account of the other biochemical findings or the accompanying clinical picture. As this research is still in an early stage the above list necessarily remains incomplete. Various new aminoacidurias will probably be discovered later in diseases not discussed in this paper. To illustrate the extent of this study, Table 1 includes a list of disorders such as various kidney diseases in which investigations have been carried out with negative results.

II. METHODS.

1. PAPER PARTITION CHROMATOGRAPHY.

Paper chromatography permits of the separation and identification of more than 20 aminoacids to be found in plasma and sometimes in urine. When a drop of one of these fluids is placed near one edge of a sheet of filter paper and a solvent such as phenol-water is irrigated over this spot, the aminoacids present in the drop are carried in the solvent across the paper at differing rates, and are thus separated. Further separation is achieved when a second solvent, e.g. collidine-lutidine-water, is then allowed to run across the paper at right angles to the original solvent. By this method the aminoacids are separated into a constant and characteristic pattern, which is made visible by spraying the paper with ninhydrin. The aminoacid deposits then appear as blue, purple, grey and yellow spots on the paper. The colour intensity of these spots depends partly on the quantity of acid present and also on its sensitivity to ninhydrin. The aminoacids can be identified by their position on the paper.

Preservation of specimens. Fresh urine specimens were collected, preserved with toluol or thymol crystals and kept at 4°C. Twenty-four hour urine collections are difficult with children and were used only occasionally. They were preserved with chloroform or merthiolate. Blood specimens were heparinised, centrifuged immediately and deproteinised by filtration through collodion sacs (Greenberg and Gunther 1929)

in a vessel surrounded by ice. The filtrate was kept at 4°C. Blood and urine specimens were always collected in the post-absorptive state.

Application. A urine volume containing 500 µg. nitrogen was pipetted on to a sheet of No.4 Whatman filter paper, unless a 24-hour urine collection was under examination, when the volume used was usually one millionth part of the total volume. For plasma estimations, 0.5 ml. of the heparinised, deproteinised and desalted (Consdon, Gordon and Martin 1947) specimen was taken. Urine and plasma were treated with ammonium molybdate and hydrogen peroxide on the paper, in order to oxidise methionine and cystine (see below), after which two-dimensional descending chromatography was carried out, employing phenol-water as the first and collidine-lutidine-water as the second solvent. Sodium cyanide and ammonium hydroxide were added to the phenol box, diethylamine to the collidine box. The temperature of the room was thermostatically controlled at 25°C. After the runs the papers were dried, sprayed with a 0.15% ninhydrin solution in butyl alcohol and dried again at a temperature not exceeding 40°C. Nearly all specimens were run in duplicate.

The position of the aminoacids on the paper. On a completed chromatogram each aminoacid occupies a typical position on the paper. The positions of more than 60 different aminoacids have been studied by Dent (1948) after preparing chromatograms with alcoholic solutions of pure crystalline aminoacids. He charted the position of these aminoacids on a "map". In 250 chromatograms with pure aminoacids I have

repeated Dent's procedure for 24 of the aminoacids found in urine and plasma in health and disease. An example of a chromatogram using pure aminoacids is given in Fig.1. My results confirm Dent's findings, which are reproduced in Fig.2.

A strict localisation of an individual aminoacid on the paper according to its Rf-value in the two solvents (relation of the distance travelled by the aminoacid to the total distance travelled by the solvent) has been abandoned by Dent and has not been used by me, because the Rf-values vary considerably with different batches of solvents and with slight variations in the conditions of work. The individual aminoacids can be identified more accurately by their relationship to the whole spot-pattern and by their colour. This identification is further simplified by adding known test aminoacids to the chromatogram before the run is started.

To demonstrate methionine and cystine the filter paper has to be treated with perhydrol. This oxidises methionine, whose position overlaps leucine and isoleucine, to methionine sulphone, which latter occupies a characteristic position on the paper. Cystine does not give any ninhydrin reaction on the paper unless it is oxidised to cysteic acid, the position of which can easily be identified. Certain other aminoacids may be difficult to identify, for they sometimes overlap, e.g. leucine and isoleucine, citrulline and β-alanine, and histidine and β-amino-isobutyric acid, though the last four aminoacids can generally be differentiated by their colour.

The spots developed by ninhydrin nearly always

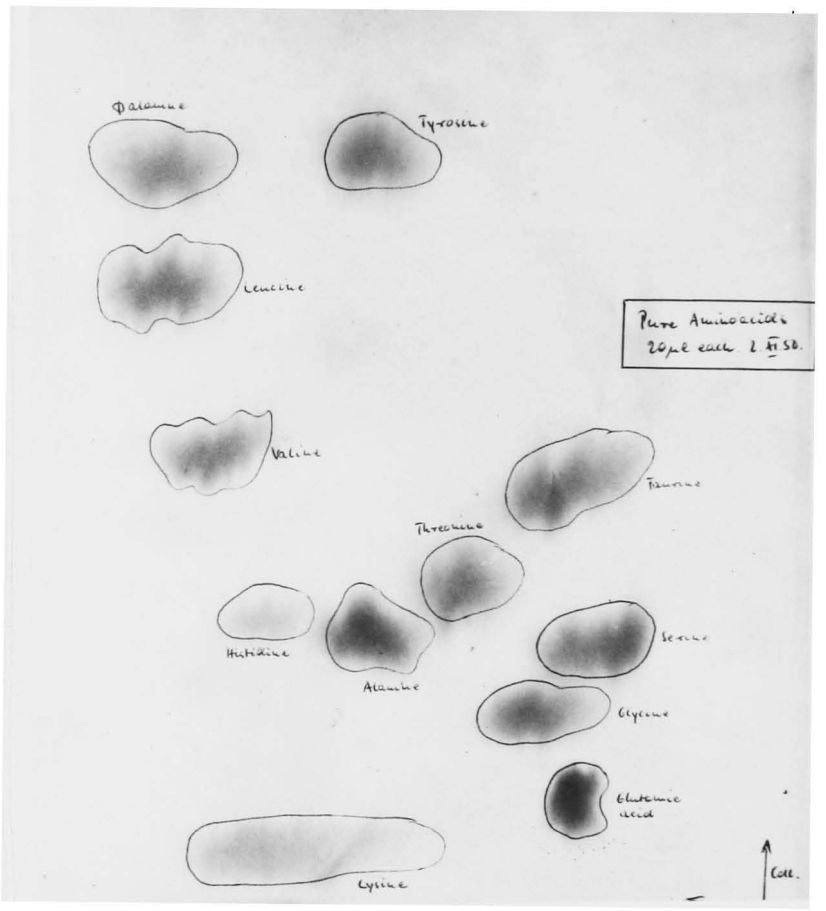
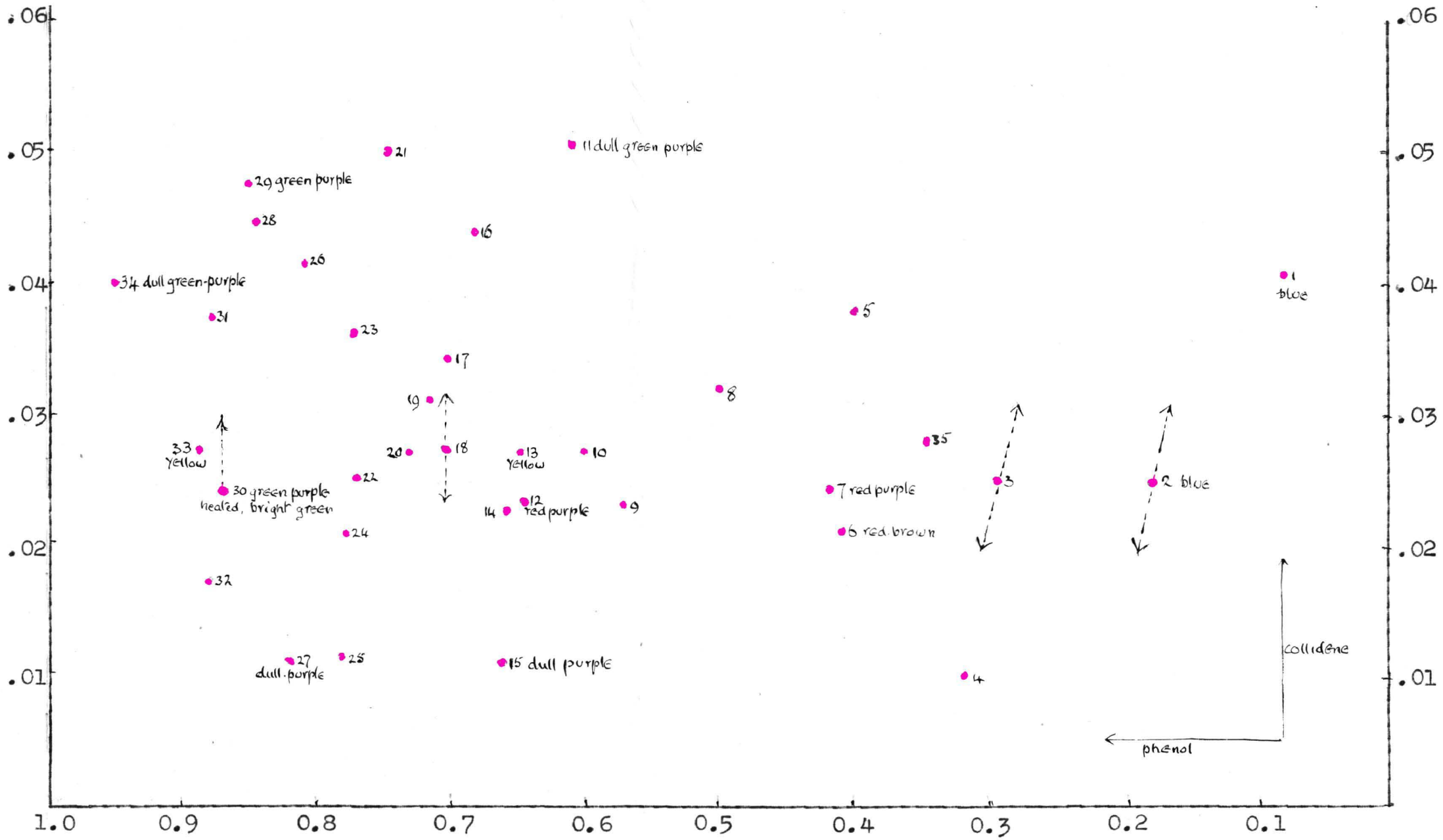


Fig. 1. Chromatogram of pure aminoacids.

Fig. 2.

MAP OF AMINOACID SPOTS (DENT 1948) CONFINED TO AMINOACIDS WHICH MAY OCCUR IN BIOLOGICAL FLUIDS.

(All spots are purple unless otherwise stated.)



1. Cysteic acid. 2. Aspartic acid. 3. Glutamic acid. 4. Ethanolamine-phosphoric acid. 5 Taurine.
6. Asparagine. 7. Glycine. 8. Threonine. 9. Glutamine. 10. α-alanine. 11. Tyrosine. 12. Citrulline.
13. Proline. 14. β alanine. 15. Hydroxy-lysine. 16. Glucosamine. 17. Methionine-sulphone. 18. Histidine.
19. α-amino-n-butyric acid. 20. β -amino-iso-butyric acid. 21. Tryptophane. 22. Methionine-sulphoxide.
23. Valine. 24. γ -amino-butyric acid. 25. Ornithine. 26. Methionine. 27. Lysine. 28. The leucines.
29. Phenylalanine. 30. Methyl-histidine. 31. Ethanolamine. 32. Arginine. 33. Proline. 34. Histamine.
35. Serine.

represent free aminoacids. Peptides and amines may, however, occasionally give a ninhydrin reaction (Dent 1947, Brenner and Kenten 1951). If, therefore, any spot appeared in an unusual position, the specimen was hydrolysed with hydrochloric acid and the run repeated. If this resulted in the disappearance of the spot it was presumed that it was originally due to a peptide. Confusion with amines is serious only in a few special areas of the paper, as pointed out by Brenner and Kenten (1951).

The quantitative evaluation of paper chromatograms. The colour intensity of the aminoacid spots was first recorded by using an arbitrary colour scale (Dent 1947) with ten shades, ranging from 1 for the weakest to 10 for the strongest colour. More recently the colour intensity has been compared with test spots of pure taurine, placed in five different positions and concentrations (5-10-20-40-60µg.) on the right hand edge of the paper and above the urine spot before starting the run. The final positions of these taurine test spots can be seen on some of the chromatograms reproduced (Fig. 12). This procedure was found to be preferable, as the intensity of the final ninhydrin colour reaction varies slightly from chromatogram to chromatogram, which renders a comparison with a fixed colour scale rather unsatisfactory.

An approximate quantitative estimation of any aminoacid spot can be made by comparing its size and colour intensity with those of the test aminoacid spots of known concentration which have been run and developed on the same paper under identical conditions.

Table 2.

SMALLEST AMOUNT OF VARIOUS AMINOACIDS DETECTABLE BY PAPER CHROMATOGRAPHY.

AMINOACID	μg.
Alanine	1
Alpha-amino-n-butyric acid	0.75
Arginine	4
Aspartic acid	0.5
Beta-alanine	0.5
Cystine as cysteic acid	2
Glutamic acid	0.5
Glutamine	1
Glycine	0.5
Histidine	10
Hydroxy-proline	15
Leucines	2.5
Lysine	6
Methionine as sulphone	5
Phenylalanine	10
Proline	15
Serine	1
Taurine	1.5
Threonine	4
Tryptophane	5
Tyrosine	10
Valine	1.5

Unfortunately the depth of colour produced by each aminoacid does not depend only on its concentration but also on its sensitivity to ninhydrin. This must be taken into account in every evaluation of a chromatogram. Table 2 lists the smallest amount detectable of various aminoacids and thus gives an idea of their varying ninhydrin sensitivity.

For clinical purposes, a comparison of the colour values of the aminoacid spots in pathological urine chromatograms with those of normal controls has generally proved sufficiently informative. At first I estimated the colour intensity of each amino-acid spot of the urine under test by using the arbitrary colour scale described above, latterly I have compared them with taurine test spots. Comparison of the results with those found in normal urines shows whether the colour intensity is within or above the normal range. The sum of the colour values of all the aminoacid spots of a chromatogram gives an approximate idea of its strength and enables one to see whether a generalised aminoaciduria is present or not. More refined techniques of eluting the aminoacid from the paper after the run (Boisonnes, 1950) or of washing out the ninhydrin colour from each spot and determining its strength colorimetrically (Fowden 1951) have not been tried as their quantitative reliability is not generally accepted.

2. MICROBIOLOGICAL ASSAY.

This method was incorporated in my work 9 months ago, as it is of value in assessing some aminoacids, especially in plasma, more accurately than paper chromatography permits. I am indebted to Dr. K. Schreier

for his gift of strains of bacteria and for active help in establishing the technique; also to Dr. Baar for providing me with room facilities and, temporarily, with a technician. My technique is essentially that of Henderson and Snell (1948) and of Schreier and Pluckthun (1950). Leuconostoc mesenteroides P-60 was used for the assay of cystine, lysine, tyrosine and valine, lactobacillus arabinosus 17-5 for tryptophane and phenylalanine, streptococcus faecalis for arginine and threonine. In the final estimation of the acid formation I have measured the pH of the incubated final medium with a glass electrode. The results of this method are in accordance with those obtained by ionometric titration (Teeri and Josselyn 1949, Schreier and Pluckthun 1950). Two standard curves were added to each batch of assays and each urine specimen was assayed in triplicate, plasma in duplicate. In this thesis results of microbiological assay are only given where they help to elucidate questions which cannot be answered satisfactorily by paper chromatography alone.

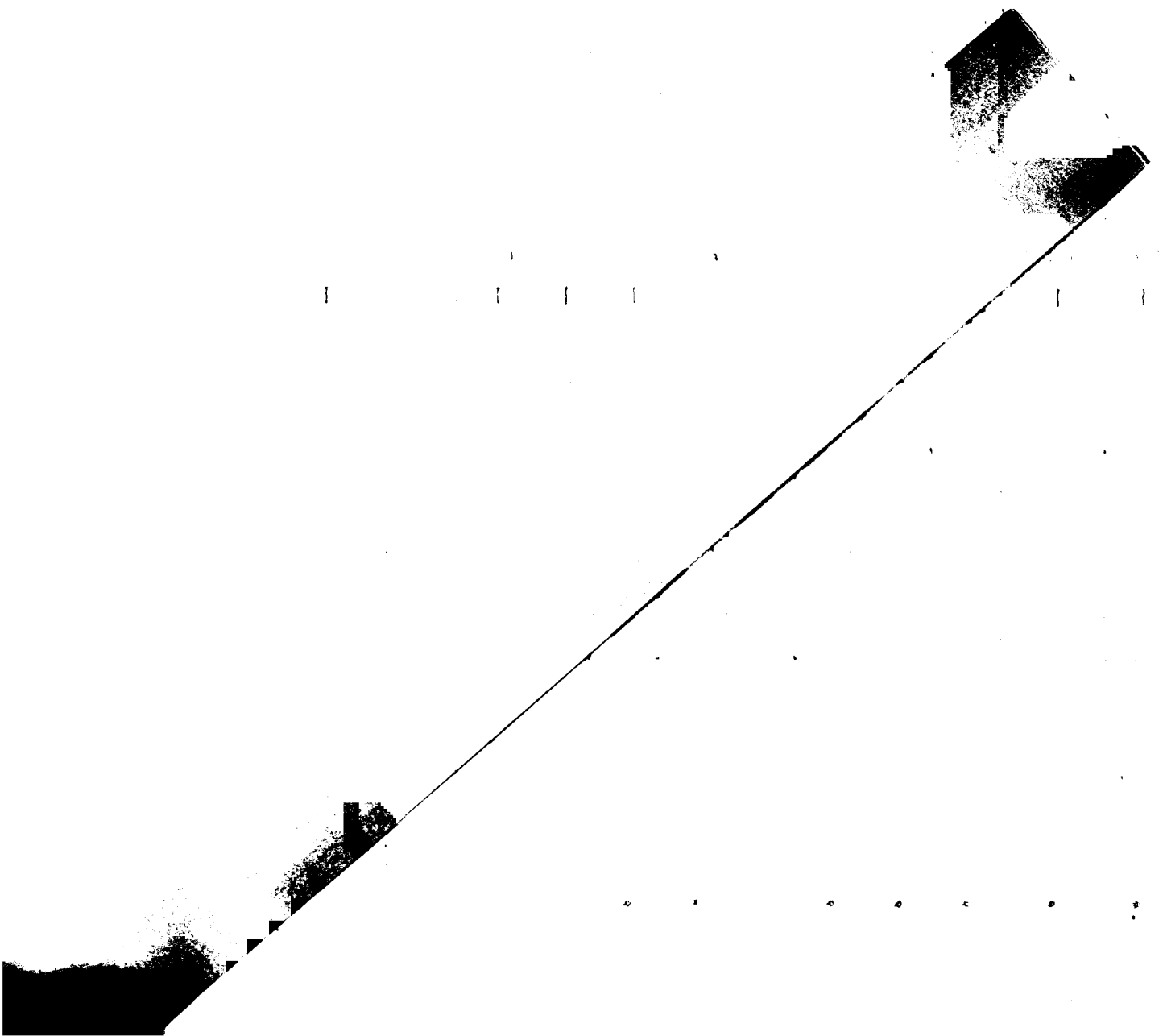


Table 3 .

URINE CHROMATOGRAM FINDINGS IN 200 HEALTHY CHILDREN:

(a) 100 urines evaluated by test taurine standard.

	Frequency of occurence	Range in T-values	Average T-value
Glycine	78%	0–>60 T	10.4 T
Glutamic acid	73%	0– 20 T	4.4 T
Alanine	68%	0– 15 T	3.7 T
Glutamine	64%	0– 20 T	4.0 T
Histidine	37%	0– 25 T	2.7 T
Cystine	21%	0– 15 T	0.8 T
β-amino-iso-butyric acid	11%	0– 20 T	0.9 T
Taurine	9%	0– 10 T	0.4 T
Serine	8%	0– 5 T	0.4 T
Valine	5%	0– 5 T	0.15T
Tyrosine	4%	0–< 5 T	0.08T
Lysine	3%	0– 15 T	0.3 T
Methyl-histidine	2%	0– 2.5T	0.06T
The leucines	-		
Phenylalanine	-		
Total colour per chromatogram		0– 80T	28 T

(b) 100 urines evaluated by arbitrary colour scale.

Frequency of occurence	Range in standard fig.	Average in standard fig.
97%	0–>10	6
68%	0– 6	3
74%	0– 8	2.7
42%	0– 9	1.8
54%	0– 9	2.2
24%	0– 6	0.5
11%	0– 10	0.5
33%	0– 10	1.1
17%	0– 8	0.5
11%	0– 6	0.25
11%	0– 4	0.2
3%	0– 10	0.05
-	-	-
7%	0– 5	0.1
4%	0– 1	0.04
	3– 55	19

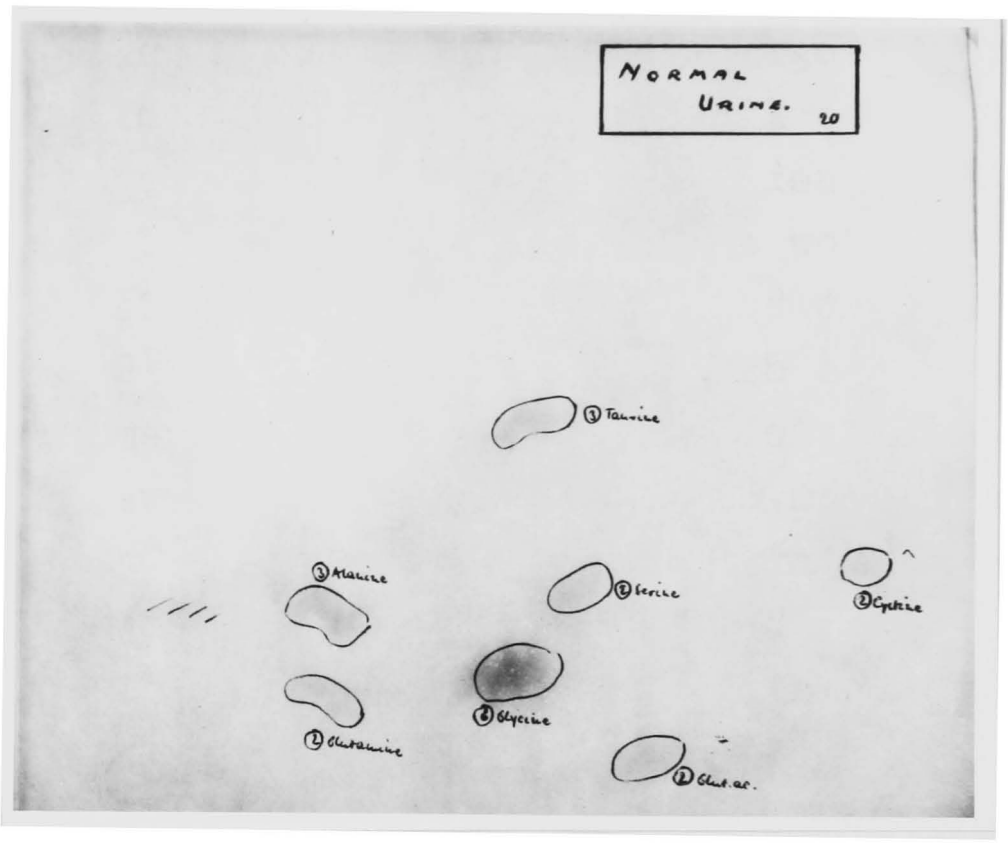


Fig. 3. Chromatogram of urine of a schoolchild. Total colour sum 20.

III. THE AMINOACID CONTENT OF NORMAL URINE AND PLASMA.

1. URINE.

No systematic chromatographic investigations of the urine of healthy children have yet been published. The purpose of this study of normal urines was to provide a basis for the evaluation of pathological specimens. 243 urines of children attending school and day nursery, and 47 urines of infants were tested by chromatography. There was no significant difference between the findings in the school children and those in the nursery, nor did infants after the first month of life show a noteworthy increase of the aminoacid output, but babies during the first five days of life exhibited a definite aminoaciduria. The results are summarised in Tables 3 and 4. An example of a urine chromatogram of a school child is given in Fig.3, of a newborn infant in Fig. 4.

It may be seen from Table 3 that nearly every urine chromatogram of a healthy child shows some faint aminoacid spots, if a urine volume containing 500µg. nitrogen is used. Glycine was found in 88% of the 200 children, alanine and glutamic acid in about 70%, glutamine and histidine in about 50%, cystine and taurine in about 20%, β-amino-isobutyric acid and serine in about 12%, valine and tyrosine in 8%, methylhistidine, the leucines and phenylalanine in 5% or less. When the colour intensity of the aminoacid spots was compared with taurine test spots (as described above), the strongest glycine spot seen in normal urine roughly equalled that produced by 60µg. taurine. The colour intensity of glutamine, glutamic acid, histidine and β-amino-isobutyric acid never exceeded

Table 4.

AMINOACID EXCRETION IN THE URINE OF NEWBORN INFANTS AND IN LATER INFANCY, EXPRESSED IN COLOUR UNITS, 1 FOR THE WEAKEST, >10 FOR THE STRONGEST COLOUR.

	1st day of life													2nd day			3rd day				4th day			5th day				
Glycine	>10	>10	>10	7	9	9	>10	10	>10	7	6	10	>10	>10	>10	>10	>10	>10	10	9	10	8	7	9	>10	>10	10	9
Serine	8	10	8	5	7	5	5	7		6	4	1	3	5	10	8	8	8	8	7	7	7	6	7	2	6	9	5
Glutamic acid	9	10	9	4	7	4	5	6	5	2	9	8	8	9	5	9	7	7	6	6	4	7	4	7	3	5	8	6
Alanine	7	5	5	5	7	5	2	7	5	2	4	8	8	7	5	7	8	8	8	6	4	6	5	6	6	8	7	5
Cystine	7	9	6	10	7	4	5	6	5	8	4	10	6	6	7	6	7	6	5	4	4	5	5	6	6	6	6	4
Aspartic acid			3			4		5	2			7		2	4	5		4		3		5		2		5	5	4
Glutamine	7	4	8		5	4		2		2	3		10		8	5	4	9			5	4	4		7	9	5	4
Taurine	9	>10	9	9	10	10	9	10	10	>10	10	>10	10	5	>10	8	6	7	7	2	4	10		2		5	9	2
Histidine			5		7	6		5			6	8		8	5	8	7		7	7	7		9	8	4	7	6	5
Valine		10	4	2	1		2		4					2		6			4		1					2	3	2
Hydroxy-proline			5											5			3	5	5		5				10	5	5
Lysine	5	9	5	1		4	6		7		2	>10	2	3			5				5					2		
Threonine					2	1	4							6			2				3				2			
The leucines		6		2		3												4			1							
Arginine			4																									2
β amino-iso-butyric acid																												
Tyrosine																					1							
Proline			5				5																					
Aethanolamine				5				5																				
Total colour	67	93	91	50	62	59	58	63	53	42	48	80	62	73	74	77	72	73	60	44	61	52	40	47	40	78	75	53

	6th day		7th day	8th day	9th day	10th day		2nd month					4th month					6th month		10th month		Later childhood frequency	range
Glycine	8	10	10	>10	10	10		6	6	9	10	6	8	9	9	5	9	8	9	6		88%	0->10
Serine	7	7	7	8	9						7	2		7	3	2			6			12%	0- 8
Glutamic acid	4	8		3	10	5		8	6	4	9		3	10	8	2	2	7				70%	0- 6
Alanine	6	8	5	9	9	7		5			6	1		7	2			6				71%	0- 8
Cystine	3	5		7	4	5		3			1			4				5	7	4		22%	0- 6
Aspartic acid					3	7									6								
Glutamine	6													5				5	4			53%	0- 9
Taurine					7					8	9						8					21%	0- 10
Histidine	6			9	3	7		5			7	2										45%	0- 9
Valine						2								4				3				8%	0- 6
Hydroxy-proline	5	5				5																	
Lysine				7																		3%	0- 10
Threonine																							
The leucines																						3%	0- 5
Arginine																							
β amino-iso-butyric acid			7										5									11%	0- 10
Tyrosine																						7%	0- 4
Proline																							
Aethanolamine																							
Total colour	45	43	29	58	55	48		27	12	21	49	11	16	46	28	9	19	34	26	10			

Note. For the total colour >10 is calculated as 15.

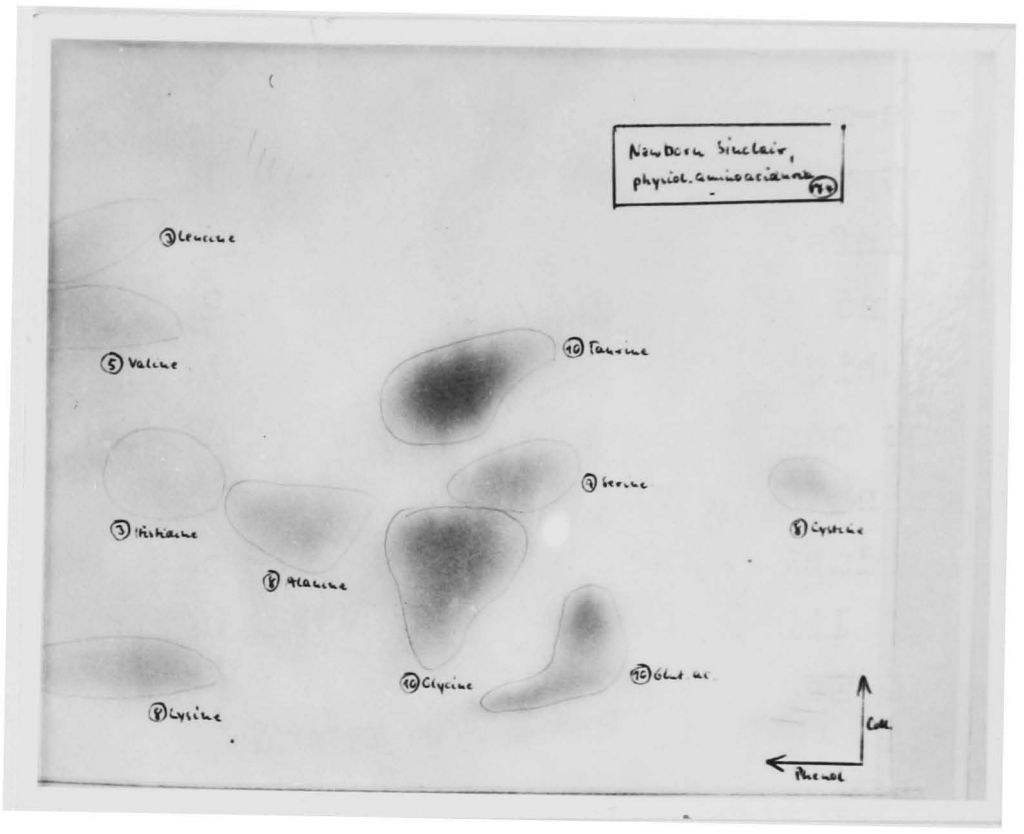


Fig. 4. Chromatogram of urine of a healthy newborn infant, showing physiological aminoaciduria. Note the increased taurine, cystine and especially lysine.
Total colour sum 74.

the colour produced by 25µg. taurine, the colour intensity of cystine, lysine, alanine, taurine never exceeded 15µg. taurine, that of the other aminoacids mentioned was less than 10µg. taurine. The colour values of the average chromatogram equalled that of 30µg. taurine and ranged from 0 to 80µg. taurine.

The urine chromatograms of newborn infants in the first week of life showed an increased excretion of the above-mentioned aminoacids and there was also a raised output of aspartic acid and lysine in about 50% of the infants. of valine and hydroxyproline in about 30%, and of threonine in about 20%. Among the aminoacids which are also found in later childhood the frequent occurrence in the first days of life of strong taurine and cystine spots was characteristic. The aminoaciduria seems to decrease soon after the fifth day of life, though this point has not been investigated in any detail.

Discussion. As this study of normal urines was undertaken for purposes of comparison with pathological specimens, the method of collecting normal urine specimens was the same as for the pathological urines, namely, single morning urine specimens, and the urine volume used equalled 500µg. nitrogen in every chromatogram. Thus the results do not reflect the aminoacid excretion in 24 hours, but the relation of aminoacid nitrogen to total nitrogen. A very similar chromatographic pattern was, however, produced by ten 24-hour urine specimens of three healthy children.

Microbiological aminoacid estimations in the urine of healthy children have recently been published

by Schreier and Plückthun (1950a, b). Most of their values fell within the range of similar estimations in adults quoted by Schreier. According to him the aminoacids excreted were, in the order of strength, histidine, glycine, cystine, serine, valine, tyrosine, arginine, tryptophane, lysine, threonine, phenylalanine and traces of others. The histidine and glycine excretion of 86 and 81 mg/24 hrs. was by far the highest, the cystine excretion was 51, the serine excretion 25, the other aminoacids fell below 20 mg/24 hrs. The excretion of glutamine, glutamic acid, β-amino-isobutyric acid and taurine was not estimated. Schreier's results tally well with mine, if allowance is made for the different ninhydrin sensitivity of the various aminoacids. Such a correction would raise the histidine output above that of glycine, and also explains why arginine, tryptophane and threonine have not been encountered in this investigation; they have a low ninhydrin sensitivity and would only be revealed in a higher concentration. Schreier and Plückthun were also the first to report on normal aminoacid levels in serum and urine of infants after the newborn period. Compared with the values in later childhood and adults they found no increase of the levels in infants. This tallies with my urine chromatograms in healthy infants.

The finding of aminoaciduria in newborn infants was not unexpected, as an increased amino-nitrogen content of the urine of newborns was reported long ago (Simon 1911, Goebel 1923). Paper chromatography permits the differentiation of the various aminoacids which make up this aminoaciduria. Its pattern is characterised by the strong excretion of serine,

cystine and taurine and frequently also of lysine, aspartic acid, valine and hydroxyproline. As the number of my investigations on newborns is small, a recent and more detailed study by paper chromatography of the same subject by Souchon (1952) is of special interest. Souchon's results confirm my findings of a frequent and strong cystine, serine and taurine excretion; he also found proline, hydroxyproline and aspartic acid to be above the normal range, though less constant than the former aminoacids. By contrast, Souchon found no lysine, but frequently methionine, which I could not detect in my chromatograms. Souchon, like me, noticed that the aminoacid output in infants of 7 days often already equals that of later infancy.

The mechanism of this aminoaciduria is still obscure but might be clarified by a study of the aminoacid content of the blood. Christensen and Streicher (1948) and Crumpler, Dent and Lindan (1950) have now confirmed the earlier view that foetal plasma collected directly after birth shows a raised aminoacid nitrogen level. Chromatographic investigations of the plasma by the last mentioned authors show that all the aminoacids present in maternal plasma are present in the same relation but in higher concentrations in the foetal plasma and, as these are the aminoacids required as protein builders, Crumpler, Dent and Lindan argue that the raised plasma level serves to facilitate protein synthesis in the foetus. In plasma specimens of two foeti, however, they found an increased taurine and γ-aminobutyric acid level and these substances are presumably not required as protein builders. Schreier and Stieg (1950) in their microbiological study of

various aminoacids in cord blood found an increase of all the aminoacids tested (arginine, leucine, isoleucine, lysine, methionine, phenylalanine, threonine, tryptophane, tyrosine and valine), if compared with older infants or adults. The increase, however, was not the same for all aminoacids but varied from 24% in leucine to 182% in lysine and 113% in histidine.

The results obtained by Crumpler et al. and Schreier and Stieg both point to an overflow from a high aminoacid blood level into the urine as the mechanism of the aminoaciduria in newborn infants. In the foetus the increased blood level may be explained on the hypothesis that the placenta acts as an aminoacid pump, as Crumpler, Dent and Lindan (1950) found a simultaneous decrease of the aminoacid nitrogen in the maternal plasma. This theory, while providing an explanation of the aminoaciduria on the first and perhaps second day of life, does not explain the increased aminoacid output during the following days. This may be related to the immaturity of the liver and kidney function in the newborn baby.

2. PLASMA.

Paper chromatography is not sufficiently quantitative to be of great value for the determination of the plasma level of various aminoacids. Definite increases of 30 and even 40 per cent might escape detection, especially if they concern all the aminoacids equally, so that the aminoacid pattern itself is unchanged. The marked increase of a few aminoacids, however, can easily be recognised, as it

Table 5.

PLASMA CHROMATOGRAM FINDINGS IN 30 HEALTHY STUDENTS, EXPRESSED IN UNITS OF AN ARBITRARY COLOUR SCALE, 1 FOR THE WEAKEST, 10 FOR THE STRONGEST COLOUR.

	Frequency	Range	Average
Glycine	100%	3 - 10	7
Glutamic acid	100%	1 - 6	4
Alanine	100%	4 - 10	8
Glutamine	100%	2 - 10	8
Valine	100%	3 - 9	6
The leucines	100%	2 - 7	5
Serine	80%	0 - 6	3
Proline	70%	0 - 10	5
Lysine	50%	0 - 7	2
Cystine	40%	0 - 6	1
Taurine	40%	0 - 2	0.7
Histidine	30%	0 - 7	1
Arginine	20%	0 - 4	0.7
Phenylalanine	20%	0 - 3	0.3
Tyrosine	20%	0 - 2	0.2
Threonine	10%	0 - 2	0.2
α-amino-n-butyric acid	10%	0 - 2	0.2
Total colour per chromatogram		28 - 78	52

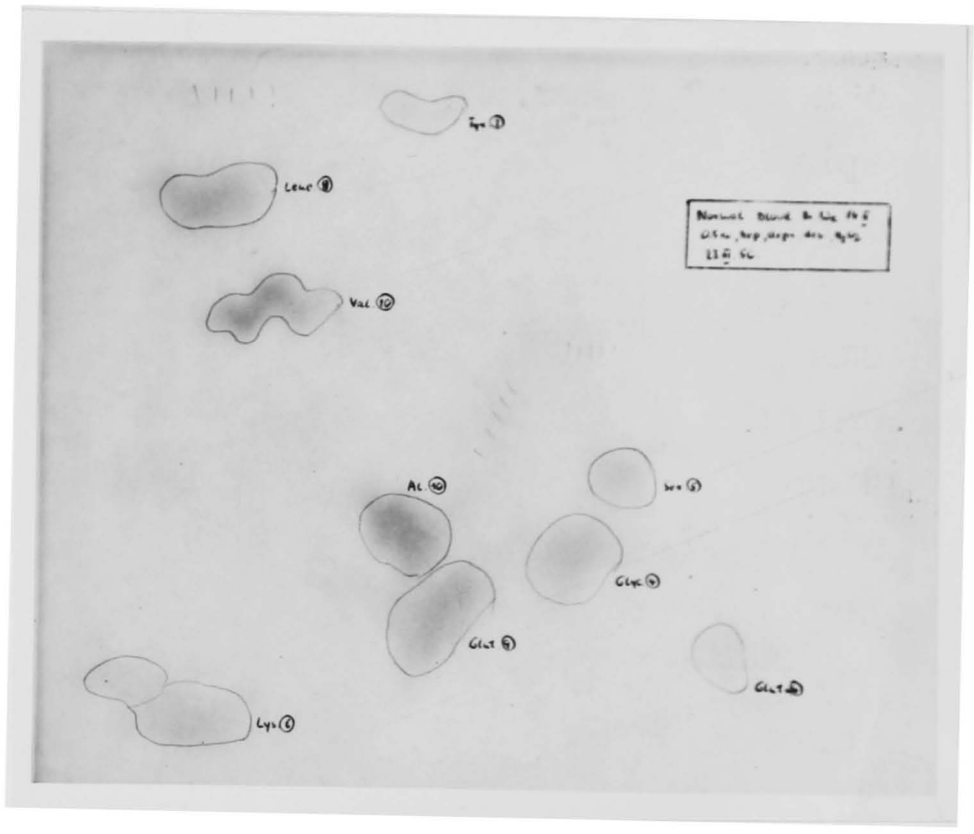


Fig. 5. Chromatogram of plasma of healthy adult.
Total colour sum 58.

results in an alteration of the normal aminoacid pattern. An even increase of all the aminoacids in the plasma of 50 per cent and more should also be demonstrable by this method. Finally, chromatography is of value in revealing some aminoacids in the blood which are not easily traceable by other methods.

In this study plasma chromatography has been employed to a much lesser degree than urine chromatography. Reference will, however, be made later on to plasma chromatograms in various patients and mention is, therefore, made here of investigations on the plasma of 30 healthy, fasting students who kindly volunteered. The results are listed in Table 5 and an example of a normal plasma chromatogram is given in Fig. 5. It is evident that the aminoacid plasma pattern differs considerably from that of normal urine. The aminoacid spots which are most frequently encountered and which are strongest in their colour intensity are alanine, glutamine, glycine, valine, the leucines, and glutamic acid. Slightly less common and of weaker coloration are serine, proline and lysine, still rarer cystine, taurine, histidine, arginine, phenylalanine and tyrosine. Only occasionally have threonine and α-amino-n-butyric acid been encountered. These represent free aminoacids as found in 0.5 ml. of deproteinised and desalted plasma filtrate. The findings refer to adults but no significant differences are to be expected in children, as has been shown recently by Schreier and Plückthun (1950a, b) for children between 1 and 14 years and for infants after the newborn period.

Krebs (1950) has recently reviewed the average levels for various aminoacids in normal plasma obtained by recognised workers employing chemical and microbiological methods. He lists in order of strength glutamine (5.8 mg. per 100 ml.), alanine (4.0), leucine and isoleucine (3.5), glutamic acid (3.4), lysine (3.0), valine (2.8), arginine (2.3), threonine (2.0), glycine (1.8), tyrosine (1.5), histidine (1.4), phenylalanine (1.4), tryptophane (1.1), methionine (0.9) and citrulline (0.5). Values for cystine, serine, proline and taurine are not given. This list agrees well with my findings, if again due allowance is made for the different ninhydrin sensitivity of the various aminoacids, especially for the high sensitivity of glycine, which in chromatograms produces a comparatively strong colour reaction; the colour of lysine, histidine, tyrosine, phenylalanine, threonine, tryptophane and arginine is comparatively weak and the concentration of these aminoacids may be underestimated. In addition approximately 50% arginine and 30% histidine are lost during the desalting procedure (Stein and Moore, 1951).

IV. AMINOACIDURIA in CYSTINE-LYSINURIA.

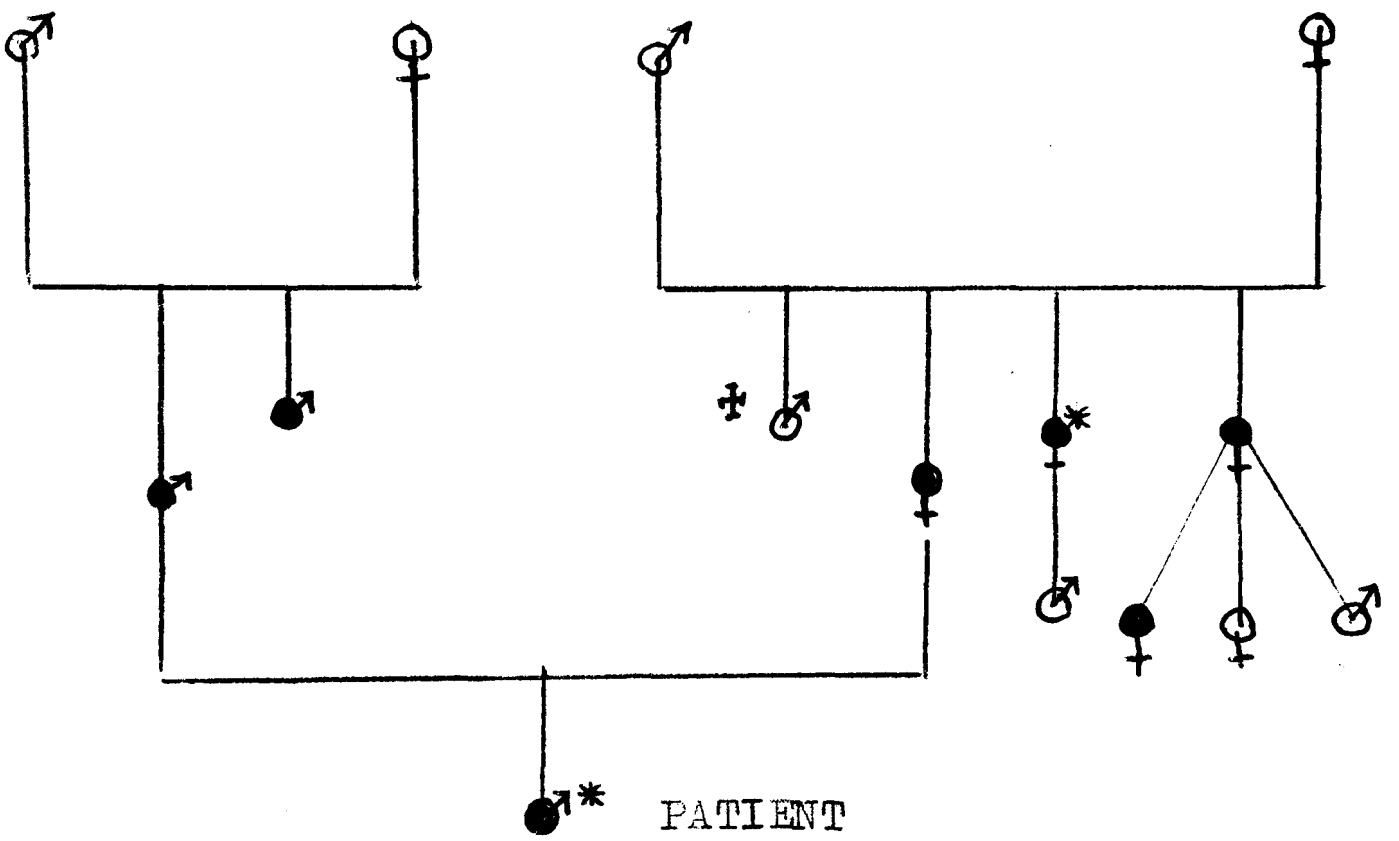
General considerations.

In 1810 Wollaston, in his study of various urinary calculi, described a new and rare bladder stone detected in two patients and concluded "since both the calculi have been taken from the bladder, it may be convenient to give it (the new substance in the calculus) the name of cystic oxide, which will serve to distinguish it from other calculi". Wollaston was the first to discover a case of cystinuria and to describe an aminoacid concerned in metabolism-"cystic oxide", or, as we now term it, "cystine".

Since Wollaston's brilliant discovery more than 300 cases of cystinuria have been reported and valuable new information has been collected, though a real understanding of this disorder was delayed by practical difficulties in the methods of demonstrating aminoacids. The clinical picture of the disease is usually benign, and as a rule cystinuria remains undetected throughout life and does not lead to any complaints. This has been clearly shown by Lewis (1932) in a large scale survey of about 11,000 normal people. Occasionally cystine stone formation occurs in the urinary tract without obvious reason and these stones can lead to clinical symptoms and even to kidney destruction with final uraemia (Russell and Barrie, 1936).

During the past thirty months I have studied 30 cases of cystinuria in 18 different families. The disorder cannot, therefore, be so rare as is normally believed, and this has recently been confirmed by Dent and Harris (1951), who described in detail the findings in 11 original cases and 11 further cases discovered

Fig. 6. FAMILY TREE OF CYSTINURIC PATIENT (R.T.)



● Cystine-lysinuria shown in chromatogram.

○ Normal chromatogram.

* Presence of stones proved.

during family investigations. Nine of our 30 cases were children, of whom four showed their first cystine stones in the first, second or third year of life. In all these children cystine storage in bone marrow and/or eyes was excluded; their development and well-being continued undisturbed, as kidney damage by stone formation was prevented by timely removal of the stones. Two of these children have now been alkalinised for 2 years and their urine pH has been kept between 7.8 and 8. During this time there has been no fresh stone formation.

Cystinuria is a hereditary disorder, though the exact mode of inheritance is not yet clear (Dent and Harris, 1951). Both dominant and recessive inheritance and the presence of a heterozygous condition with irregular manifestation have been suggested. In most of our cases, the same condition was found in some of the patients' siblings as well as in previous generations, and the family tree of one such family is given in Fig.6. In some families, however, no further cases could be detected despite extensive urinary analyses.

In the literature mention is rarely made of the increased excretion of aminoacids other than cystine. Lysinuria together with cystinuria was observed by Ackermann and Kutscher (1912) and Hoppe-Seyler (1933). Yeh, Franke, Dunn, Parker, Hughes and Gyorgy (1947), using microbiological assay, also found an excess of arginine in the urine. Since then the systematic application of paper chromatography in more than 50 cases (Dent's and my material together) has revealed in every instance at least cystine and lysine, so that it is probable that lysine is always present and was overlooked in earlier studies. In this study the

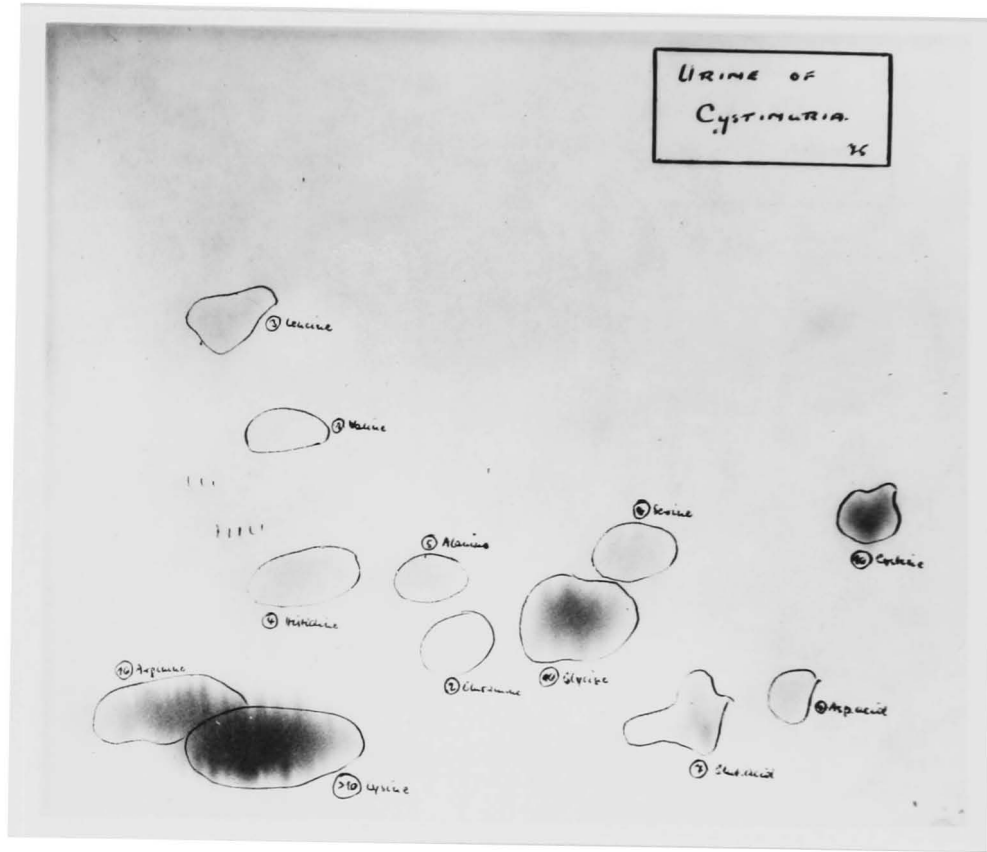


Fig. 7. Urine chromatogram of a patient with cystine-lysine-argininuria (R.T.). Total colour sum 75.

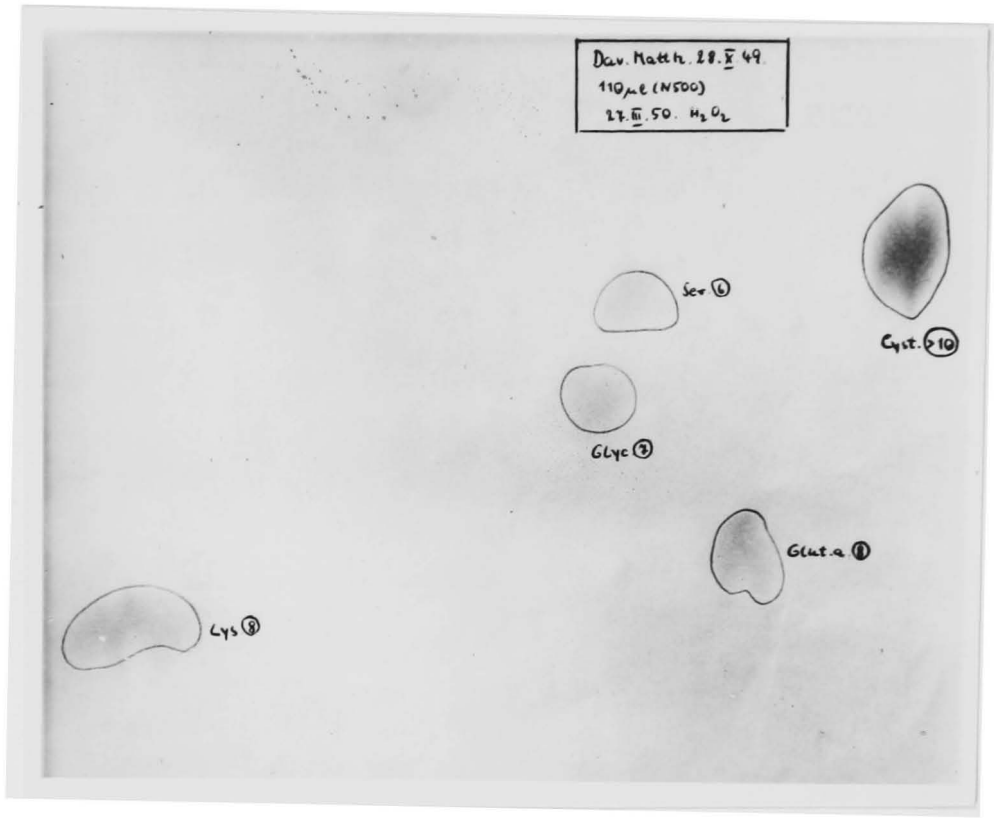


Fig. 8. Urine chromatogram of a patient with cystine-lysinuria (D.M.).
Total colour sum 45 (>10 calculated as 15).

term "cystine-lysinuria" will, therefore, be applied to this condition to differentiate it from other forms of aminoaciduria with cystine excretion which have often been confused with classical cystinuria, e.g. aminoaciduria in Lignac-Fanconi disease (see later).

Cystine-lysinuria has generally been regarded as an inborn error of the metabolism of the sulphur containing aminoacids (Garrod 1923), but in 1941 Brown, et al. and more recently Dent and Rose (1951) and Fowler (1952), reported normal plasma cystine levels and attributed the cystinuria to a low renal threshold for cystine rather than to a prerenal metabolic error. Such an explanation had been suggested before (Brand, Harris and Biloon, 1930, and other authors) though without substantial evidence.

Results of chromatographic and microbiological studies.

The chromatographic pattern of the urine of the 30 patients studied was so characteristic that the diagnosis was established by one glance at the developed paper. Fig. 7 shows the more common type, namely the excessive excretion of cystine, lysine and arginine (Case R.T.), Fig. 8 shows the other type, namely the cystine and lysine excess without arginine (D.M.); the brother and mother of this patient also showed only cystine and lysine without arginine. Both R.T. and D.M. suffered from extensive stone formation in their second and third year of life. In numerous further urine chromatograms the aminoacid pattern of these children was constant though not always of the same strength. The other aminoacids in the urine are generally excreted in normal concentration, though in the chromatogram of R.T. (Fig.7) the faint aspartic acid spot is unusual and in another

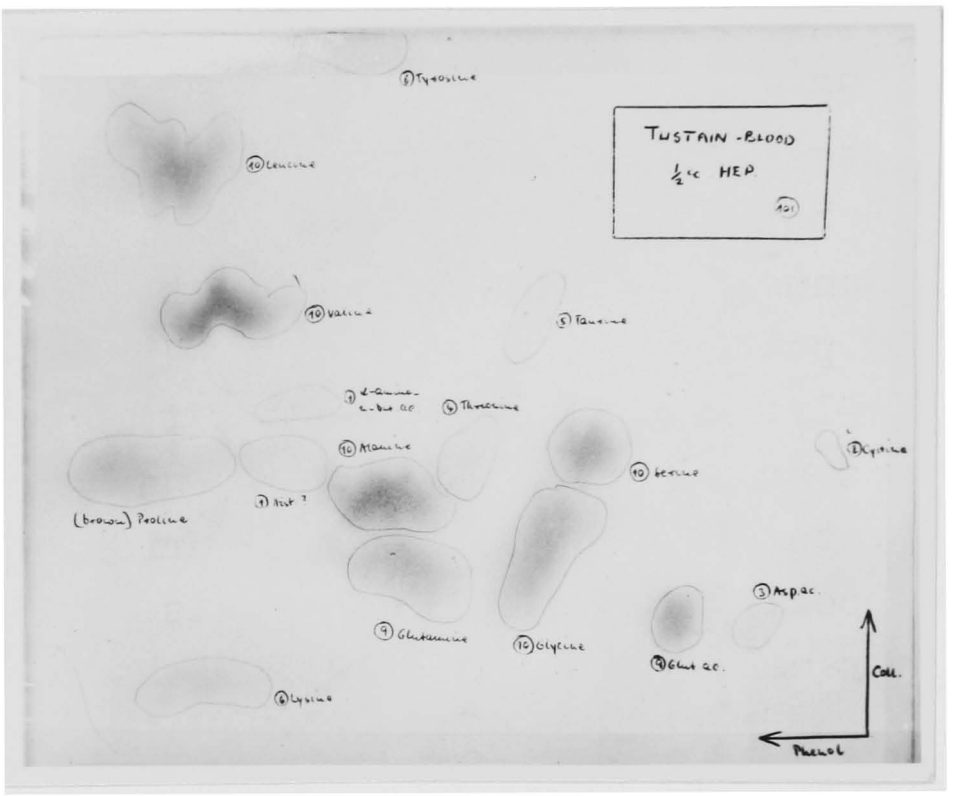


Fig. 9. Plasma chromatogram of a patient with cystine-lysine-argininuria (R.T.).
Note normal level of cystine and lysine, and absence of arginine.
Total colour sum 101 (slightly raised).

case of cystinuria which I investigated for Professor Linneweh (1951) β-alanine and/or citrulline was observed. This may have been due to decomposition during the long transport in the post, though the urine arrived sterile. The greatest quantity of lysine and arginine was found in the urine of the youngest case of cystinuria I have examined and which I owe to Drs. Dreadon, Davison and Latner. The child is now 2 years old and perfectly well, weighing 28 lbs., though at the age of eleven months he had bilateral hydronephrosis and anuria caused by cystine stones in both ureters.

To summarise the findings in the urine chromatograms of our 30 patients, 23 had cystine-lysine-arginuria and 7 cystine-lysinuria. The concentration of these aminoacids was always considerably raised and was generally about ten times greater than that in normal urine.

Of special interest in this disease are the plasma chromatograms which might indicate whether the excessive excretion of cystine, lysine and arginine is due to a high blood level. Plasma chromatograms have been prepared repeatedly in five of our cases. None of these patients showed a cystine, lysine or arginine level above that of normal plasma. It was, however, noticed that in three instances the other aminoacids, compared with normal plasma, showed rather a high concentration. The total colour value of these chromatograms was 101 (R.T.), 114 (D.M.) and 82 (Mo.) (normal range 28-78, average 52 colour units). This might have been a chance finding. The plasma chromatogram of R.T. is reproduced in Fig.9.

Microbiological assay carried out with plasma from two patients (R.T. and D.M.) showed normal or slightly decreased plasma levels for cystine, lysine and arginine (expressed as μg/ml).

		lysine	arginine	cystine
2 normal controls*		15-22	15-18	9-12
R.T.	15.11.51	20	14	8
	30.11.51	8	10	5
D.M.	24.10.51	10		
	12.1.52	15	12	5
	16.1.52	15		11
	19.1.52	14		7

* plasma of 2 healthy adults assayed at the same time as plasma of patients.

Discussion.

The cystine-lysinuria described above seems to be a clearly defined disorder arising from an inborn familial defect of the tubular reabsorption of cystine, lysine and often of arginine also. The normal plasma levels of lysine, arginine and cystine found in several of our patients by chromatographic and microbiological analysis support this hypothesis. Dent and Rose (1951) have come to the same conclusion after chromatographic studies and also microbiological estimations of cystine in the plasma of two patients, who showed a plasma level of 15 and 8 µg/ml. compared with 10 µg/ml. for normal plasma. Further investigations of cystine in the plasma have recently been reported by Fowler, Harris and Warren (1952) using a polarographic method, also giving normal results.

Cystine-lysinuria is usually of a benign nature and this in itself differentiates it from cystine storage disease (Lignac-Fanconi disease), a severe metabolic disorder which is discussed later. In the past, cystinuria and cystine storage disease have often been attributed to a common underlying defect of the cystine metabolism. Several theories have been advanced to explain why some patients develop cystine storage whereas others have symptomless cystinuria all their life. Cystine storage was attributed to the toxic effect of the excessive cystine excretion which was thought gradually to render the glomeruli unable to filter it. This is not borne out by my observations, as the children with cystine storage whom I have investigated were still able to excrete large amounts of cystine (see Fig.10) and showed no other signs of glomerular insufficiency; moreover, our patients with cystine-lysinuria have not developed cystine storage. More recently Freudenberg (1949) imputed cystine storage to the inability of the infantile kidney to excrete cystine efficiently, basing his theory on the assumption that cystinuria occurred only in adults and older children. Dent and Rose (1951) rightly opposed this view, which is also disproved by our finding of cystine-lysinuria in infancy and by the physiological cystinuria of the newborn infant.

The clinical picture and the biochemistry of cystine-lysinuria and those of cystine storage disease reveal little common ground. It is, however, still possible that the two diseases, though not encountered in one and the same patient, are genetically related and occur in the same family. This is suggested by Abderhalden's case (1903) of cystine storage disease,

where the father and grandfather exhibited cystinuria, and also by a patient of ours who died of cystine storage disease in early childhood and whose father exhibited typical symptomless cystine-lysinuria. Dent and Harris (1951) recorded a cystine-lysinuria in the family of a case of the so-called Fanconi Syndrome of adult life (Stowers and Dent 1947), but as cystine storage has not yet been found in such patients, this may well be a disease altogether different from Lignac-Fanconi disease of childhood described below. Dent and Harris pointed out that the association of the two diseases might be a chance finding, as in a survey of approximately 500 urines taken at random, they came across one with the cystine-lysine pattern, while Lewis (1932) found 1 in 600 of a college student population to be cystinuric. This may well be true of our cases also, as 138 urines of other relatives of children with cystine storage disease were investigated with negative results. Moreover, during our chromatographic urine investigations on 200 school children one child showed a typical cystine-lysinuria, which shows that such chance findings may well occur. Nevertheless, it is too early to claim that a genetical relationship between cystine storage disease and cystine-lysinuria does not exist.

V. AMINOACIDURIA in LIGNAC-FANCONI DISEASE.

General considerations.

Rickets in association with kidney dysfunction was first described by Lucas nearly 70 years ago. Since then the disorder has been reported so many times that in 1930 Mitchell was able to refer to 76 examples of "renal rickets" in the literature. The excellent studies of Mitchell (1930), Hamperl and Wallis (1933) and Kaiyser (1940), together with Parsons' (1927) and Teall's (1928) classical description of the bone changes, reveal "renal rickets" as a clinical and pathological condition which is both variable and complex and, in my opinion, is without any single aetiology or pathogenesis.

In 1924 and 1926 Lignac described the results of his detailed study of 3 children who showed dwarfism, severe rickety deformities, albuminuria, glycosuria, polydipsia, polyuria, anorexia, constipation, vomiting and repeated attacks of fever. All succumbed to minor infection and in many tissues, including the liver, spleen, lymph nodes and kidneys, was found a crystalline substance which was identified as cystine. While some of this deposit was obvious on naked eye examination, some was demonstrated only after careful microscopic investigation. Cystine stones in the urinary tract of one of his patients led Lignac to suggest that an excessive cystine excretion as well as cystine storage was present. He also noted the familial incidence of the disease, as a sister of one of his patients was affected with it. He referred to the publications of Abderhalden (1903) and

Kaufmann (1922), who had described, though not very fully, the first case of cystine storage disease. One feature of Lignac's cases which he appears to have overlooked was their similarity to some examples of renal rickets.

In 1931 in his paper on "non-diabetic glycosuria in childhood" and during the years which followed, Fanconi studied a syndrome in young children which resembled renal rickets but at the same time differed from it in several respects and to which the cases described by de Toni in 1933 and by Debré et al. in 1934 clearly belonged. This disorder was noted to be familial but its pathogenesis was obscure, and in his account written in 1936 Fanconi chose the purely descriptive name "nephrotic glycosuric dwarfing with hypophosphataemic rickets in early childhood". Further contributions to the literature followed and were reviewed in the excellent paper of McCune, Mason and Clarke (1943). Both Fanconi and McCune observed excessive aminoaciduria in their patients.

A year after Fanconi's original publication in 1936, Beumer and Wepler drew attention to the close clinical similarity between Fanconi's cases and Lignac's cystine storage disease. This is borne out in all the twenty-two cases of cystine storage disease in the literature, which showed a clinical picture indistinguishable from that of Fanconi's cases. Furthermore, since Bürki and Esser (1941) described the demonstration of cystine storage in vivo in eyes and bone marrow, Fanconi himself has detected cystine storage in two patients with "nephrotic glycosuric dwarfism" (1946, 1949) and believed the syndrome described by him to be identical with cystine storage disease. The discovery of cystine storage in each

of our 16 cases of "nephrotic glycosuric dwarfism" strongly supports this viewpoint and has led me to name this disorder "Lignac-Fanconi disease".

The original publications refer exclusively to children, but lately an adult form of Fanconi's Syndrome has been reported (Stowers and Dent 1947, Milne, Stanbury and Thomson, 1952), showing as salient features osteomalacia, hypophosphataemia, acidosis, renal glycosuria and aminoaciduria. In the present state of our knowledge it is impossible to decide whether all conditions exhibiting the above symptoms are one and the same disease or are even interrelated. In my opinion Lignac-Fanconi disease is a well-defined clinical entity (Fanconi 1946, Fanconi and Bickel 1949, d'Avignon and Vahlquist 1949, Bickel 1951, Baar 1951, further publications in progress) which cannot at present readily be identified with other forms of Fanconi's syndrome without cystine storage, and particularly not with that seen in adults. The findings in Lignac-Fanconi disease should thus not be generalised as applicable to other forms of Fanconi's syndrome and vice versa.

The clinical picture of Lignac-Fanconi disease, as observed in our patients, may be summarised as follows:

A. Characteristic clinical findings.

(a) Dwarfing.
(b) Cystine storage in eyes and bone marrow.
(c) Photophobia.
(d) Resistant rickets, bony deformities, pathological fractures.

B. Non-specific clinical findings.

(a) Thirst, polyuria.
(b) Failure to thrive and to gain weight.
(c) Anorexia and attacks of vomiting.
(d) Susceptibility to infection, unexplained fever, metabolic crises.
(e) Muscular weakness, delayed standing and walking.
(f) Onset between 6 and 12 months of age, but later in the chronic form.
(g) Affection of other siblings.
(h) Latent and manifest tetany in the late stages.

C. Chemical findings (none of which are constant).

(a) Albuminuria, polyuria, scanty casts and cellular elements in the deposit.
(b) Aminoaciduria and glycosuria.
(c) Bicarbonate low in plasma, high in urine with alkaline urine.
(d) Plasma phosphorus low or raised. Phosphatase normal or raised.
(e) Plasma potassium low, sodium and chloride lowered slightly.
(f) Cholesterol normal or raised.

As regards the possibility of treating this profound metabolic disturbance, Fanconi's recent review (1950) was pessimistic. So far no case of Lignac-Fanconi disease has been observed beyond the years of puberty. In the acute form death occurs early, usually in a metabolic crisis with acidosis and probably hypopotassaemia (Drabløs 1951). In the chronic form the later stages are characterised by progressive kidney destruction, and the more advanced this destruction, the smaller the chance of any successful therapy.

Experience in this series of cases suggests, however, that the disease is in fact amenable to treatment and time may show that a fatal outcome is not inevitable. The three main points of treatment are (1) therapy of the rickets with massive doses of vitamin D (up to

Table 6 .

CHROMATOGRAPHIC FINDINGS FOR URINE IN 12 CASES OF LIGNAC-FANCONI DISEASE, EXPRESSED IN UNITS OF AN ARBITRARY COLOUR SCALE, 1 FOR THE WEAKEST, ≫10 FOR THE STRONGEST COLOUR.

	Normal		Cases											
	Aver.	range	1 K.C.	2 P.R.	5 J.N.	6 D.S.	7 O.R.	8 M.R.	9 M.B.	10 K.L.	11 D.L.	12 A.M.	13 M.L.	14 J.S.	Average
Alanine	2.7	0 - 6	> 10	≫10	> 10	> 10	10	≫10	> 10	8	> 10	10	≫ 10	≫10	> 10
α-amino-n-butyric acid	0	0	0	0	0	5	0	1	1	0	0	2	0	0	7.5
Arginine	0	0	0	7	0	2	2	5	7	0	0	0	2	0	2
Aspartic acid	0•3	0 - 3	10	> 10	9	9	0	2	4	3	4	0	2	5	5
β-alanine or citrulline	0	0	9	9	2	0	0	0	8	0	0	4	0	0	3.5
Cystine as cysteic acid	0.6	0 - 4	10	> 10	10	8	7	6	8	5	1	6	1	5	7
Glutamine	1•8	0 - 7	≫10	10	6	5	8	≫10	> 10	6	5	≫10	≫ 10	8	> 10
Glutamic acid	3	0 - 8	> 10	≫10	10	10	4	10	8	> 10	> 10	9	> 10	5	> 10
Glycine	6	0 - 10	8	> 10	10	10	10	≫10	9	> 10	10	8	≫ 10	> 10	> 10
Histidine	2•2	0 - 7	4	4	2	0	7	5	3	7	1	2	2	2	3
Leucine and iso-leucine	0.1	0 - 2	≫10	> 10	10	9	2	8	9	8	6	10	≫ 10	8	> 10
Lysine	0.05	0 - 1	10	> 10	3	7	2	6	9	7	4	8	> 10	8	8
Methionine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Phenylalanine	0	0	6	7	0	2	1	4	0	1	1	3	6	0	2.5
Proline and hydroxyproline	0	0	+	++	++	-	-	+	+	++	++	+	++	+	+
Serine	0.5	0 - 5	8	> 10	9	1	5	10	8	6	3	5	5	5	7
Taurine	1.1	0 - 6	0	0	0	0	5	0	0	0	0	0	0	0	0.5
Threonine	0	0	2	9	0	3	0	4	5	2	0	2	1	0	2.5
Tyrosine	0•2	0 - 2	8	9	1	4	1	6	2	4	1	3	2	6	4
Valine	0•2	0 - 4	≫10	≫10	10	10	2	9	10	7	8	10	> 10	10	> 10
Total	18.7	0 - 65	> 125	>145	> 92	> 95	66	>106	>111	> 84	> 64	>92	> 91	>82	>113.5

Note. Volume of urine used ≡ 500μg.N

500,000 units daily, later a maintenance dose of 10-30,000 units daily), (2) therapy of the acidosis with a sodium citrate-citric acid-water mixture (up to 20 grams of sodium citrate daily by mouth), (3) therapy of the hypopotassaemia with 2-3 grams of potassium chloride daily.

There is no doubt that this treatment has an important influence on the course of the disease. The alkali reserve and potassium level in the blood return to normal, the rickets heal and one may, in future, hope to prevent the severe crippling bone deformities of the chronically ill patients. These changes are accompanied by a considerable clinical improvement. No more metabolic crises have been observed, vomiting has ceased, the children are livelier and stronger and three are now walking for the first time. Their appetite has improved, though gains in weight and height are still retarded.

Of special interest in this disease is the aminoaciduria, which together with cystine storage is the cardinal symptom of Lignac-Fanconi disease. Paper chromatography has made possible a detailed study of this aminoaciduria and the results are given below.

Results of chromatographic and microbiological studies.

During the last 2 years 629 urine and 136 plasma chromatograms of 16 patients with Lignac-Fanconi disease have been carried out. Table 6 gives the colour intensity of urine chromatograms of 12 cases of this disease. For every patient a characteristic chromatogram was chosen and the colour intensity of

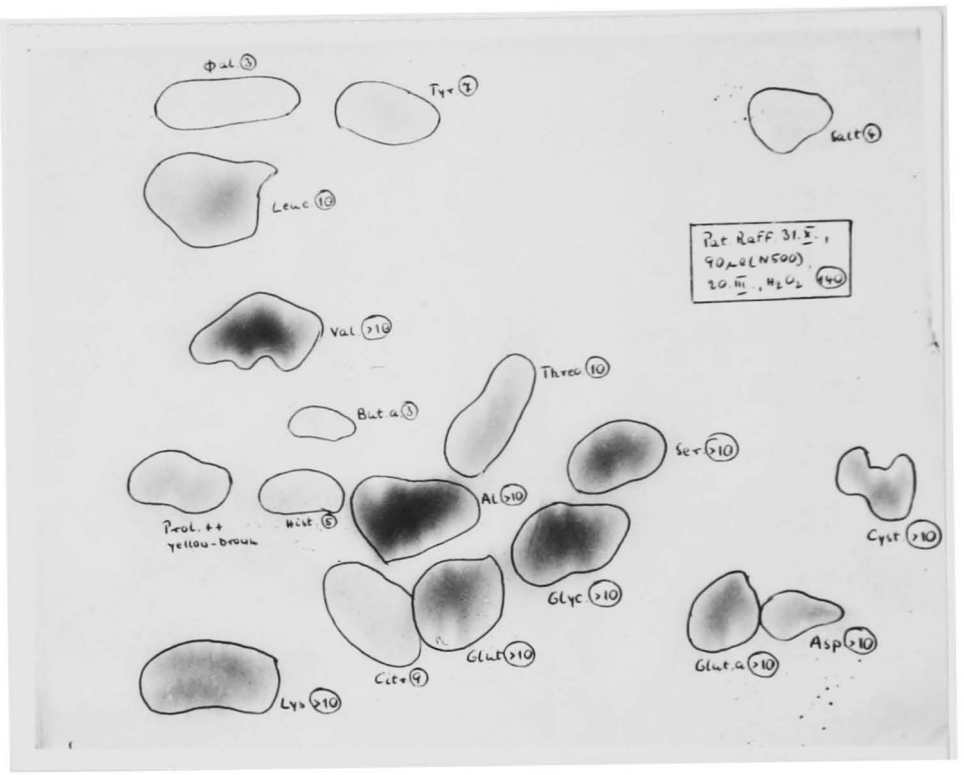


Fig. 10. Urine chromatogram of a patient with Lignac-Fanconi disease.
Note the generalised aminoaciduria (17 aminoacids).
Total colour sum 182 (>10 calculated as 15).

the spots compared with an arbitrary colour scale as described above. In Lignac-Fanconi disease, the aminoacid pattern was distinctly different from that of normal urines, with an increase of some 10-20 aminoacids, especially of the leucines, valine, lysine, proline, serine, cystine, aspartic acid, tyrosine, phenylalanine and threonine, but also of the amino-acids commonly found in normal urine, with the exception of histidine and taurine. Many of these aminoacids showed a more than tenfold increase of their colour intensity. The total colour sum of the chromatograms of the 12 patients ranged from values just above normal (66) to values considerably over 100. A photograph of a typical urine chromatogram is given in Fig.10.

The aminoacid pattern seen in this photograph resembles that of normal plasma, especially with the strong concentration of valine, the leucines and proline, which are rarely found in other aminoacidurias. Cystine (as cysteic acid) is only one of 15 aminoacids excreted in increased amounts and the name "cystinuria", sometimes applied to this aminoaciduria, is as narrow a conception as would be, for instance, "valinuria".

Plasma chromatograms of 9 patients with Lignac-Fanconi disease are summarised in Table 7. When compared with normal plasma their pattern did not show any striking deviation from the normal, nor any excessive increase of one or two special aminoacids, as, for example, in phenylketonuria, where the phenylalanine spot is very strong (see later). An accurate quantitative comparison of the colour spots in these chromatograms with those of normals is difficult, as the variations of the aminoacid

Table 7.

CHROMATOGRAPHIC FINDINGS FOR PLASMA IN 9 CASES OF LIGNAC-FANCONI DISEASE, EXPRESSED IN UNITS OF AN ARBITRARY COLOUR SCALE, 1 FOR THE WEAKEST, >10 FOR THE STRONGEST COLOUR.

	Normal		Cases									
	Aver.	range	1 K.C.	2 P.R.	5 J.N.	6 D.S.	8 M.R.	9 M.B.	10 K.L.	11 D.L.	13 M.L.	Average
Alanine	8	4 - 10	10	10	8	>10	>10	>10	10	10	>10	>10
γ-amino-n-butyric acid	0.2	0 - 2	0	0	0	0	0	0	1	1	0	0.2
Arginine	0.7	0 - 4	0	0	0	0	0	3	0	1	0	0.4
Aspartic acid	0	0	5	>10	2	10	3	0	1	6	0	5
β-alanine or citrulline	0	0	0	6	2	6	2	8	2	1	0	3
Cystine as cysteic acid	1	0 - 6	1	3	7	3	1	7	0	0	0	2.5
Glutamine	8	2 - 10	8	10	6	8	>10	>10	>10	10	10	>10
Glutamic acid	4	1 - 6	9	>10	9	>10	10	7	8	10	6	10
Glycine	7	3 - 10	6	7	8	10	10	9	8	10	10	9
Histidine	1	0 - 7	2	0	0	6	1	0	0	1	0	1
The leucines	5	2 - 7	8	>10	9	8	5	10	8	9	10	9
Lysine	2	0 - 7	3	9	2	6	3	7	6	5	2	5
Methionine	0	0	0	1	0	0	0	0	0	0	0	0.1
Phenylalanine	0.3	0 - 3	2	8	0	2	0	1	0	1	1	2
Proline	+	±	+	++	-	+	+	+	++	++	-	+
Serine	3	0 - 6	6	10	6	9	8	7	5	7	3	7
Taurine	0.7	0 - 2	3	0	6	6	4	1	1	1	1	2.5
Threonine	0.2	0 - 2	1	9	0	1	0	1	0	1	0	1.5
Tyrosine	0.2	0 - 2	2	7	0	4	0	2	0	1	0	2
Valine	6	3 - 9	8	10	10	9	7	>10	7	8	8	9
Total	52	28 - 78	74	>120	75	>108	>74	>93	>67	>83	>61	>89

Note. Plasma was deproteinised, volume used 0.5 ml.
The normal values are based on a study of plasma from 30 healthy students.

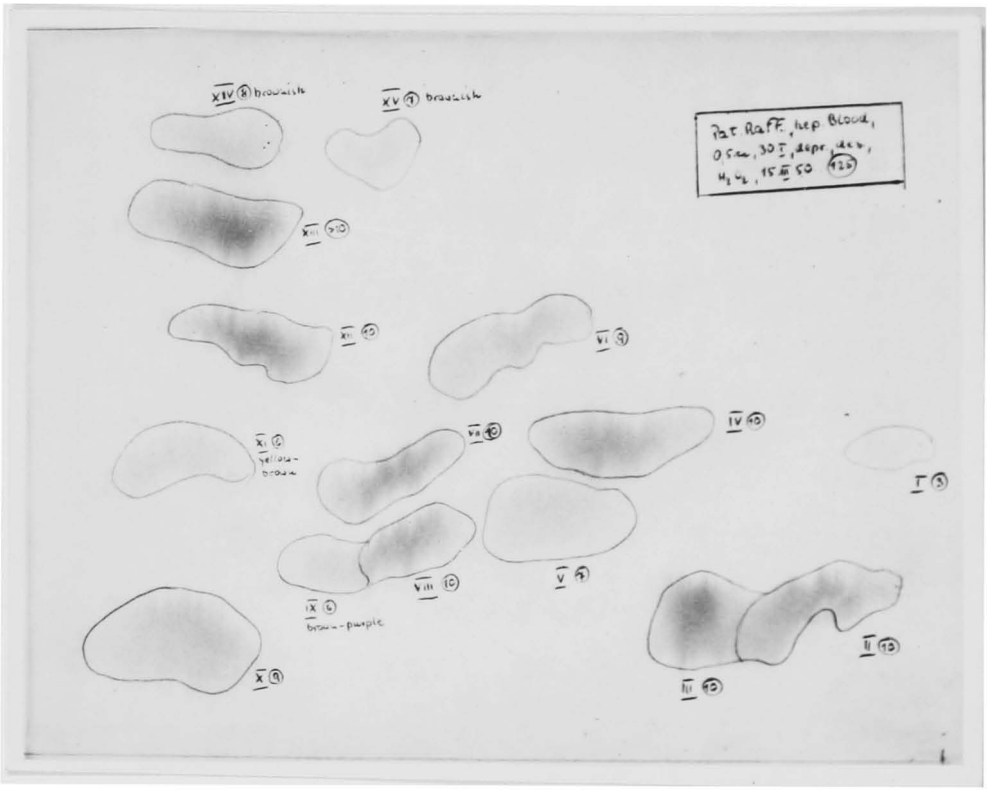


Fig. 11. Plasma chromatogram of the same patient showing hyperaminoacidaemia.
Total colour sum 124 (normal up to 78).
I cystine as cysteic acid, II aspartic acid, III glutamic acid, IV serine, V glycine, VI threonine + taurine, VII alanine, VIII glutamine, IX citrulline, X lysine, XI proline, XII valine, XIII the leucines, XIV phenylalanine, XV tyrosine.

concentration in plasma is very much smaller than in the urine of these patients. I nevertheless felt that in several plasma specimens the colour intensity of valine, the leucines, proline, alanine, glutamine, serine, aspartic acid and β-alanine and/or citrulline, perhaps also of tyrosine, was above that to be expected in normal plasma. There was no definite increase of cystine. The sum of the colour values for all the spots exceeded 80 in 6 of the 9 specimens. A plasma chromatogram of one of our patients is shown in Fig.11. An increase in the plasma level of various aminoacids was thus indicated, but owing to the semi-quantitative nature of chromatography, not proved.

In order to obtain more precise quantitative results urine and plasma specimens of some of our patients were sent to Prof. Krebs, Sheffield, and Dr. Schreier, Heidelberg, to whom I am greatly indebted for their willingness to carry out glutamine estimations by the glutaminase method (Krebs 1948) and microbiological assay (Schreier and Plückthun 1950). Compared with normal values, the urine of three patients (Table 8) showed an up to twentyfold increase of valine, leucine, methionine and tryptophane. The excretion of tyrosine, phenylalanine, and isoleucine showed an up to tenfold increase, threonine, lysine, cystine and arginine up to fivefold. The histidine excretion was normal or slightly raised. Only these 12 aminoacids were assayed.

The results of microbiological assay on deproteinised plasma of four patients are given in Table 9. Compared with the normal values our patients' plasma showed an increase of up to 100 per cent in

Table 8.

AMINOACID EXCRETION IN URINE IN LIGNAC-FANCONI DISEASE EXPRESSED AS MG. PER 24 HRS.

(Microbiological assay by Dr. Schreier)

	Normal children*	Cases 1.K.C.		2.P.R	8.M.R.	
Arginine	8-15	57	65	69	35	72
Cystine	app. 50	app. 270	app. 190	app. 380	app. 150	app. 220
Histidine	up to app. 120	138	170	164	140	115
Iso-leucine	up to 5	52	18	37	18	32
Leucine	up to 5	108	149	99	68	70
Lysine	up to 20	92	115	57	46	81
Methionine	app. 1	16	36	18	8	19
Phenyl-alanine	up to 8	72	78	37	36	29
Threonine	app. 10	53	64	45	28	29
Tryptophane	up to 5	95	83	80	52	61
Tyrosine	up to 5	68	37	33	18	61
Valine	up to 5	140	46	108	85	31

* Schreier 1950 and personal communication.

Table 9.

AMINOACID PLASMA LEVELS IN LIGNAC-FANCONI DISEASE
EXPRESSED AS mg. per 100 ml.

(Microbiological assay by Dr. Schreier)

	Normal average*	Cases 1. K.C.		2. P.R.		5.J.N.	8.M.R.
Arginine	2.4	2.9	3.6	3.1			
Cystine	2.0	3.1	3.5	4.0	2.1	2.7	2.7
Histidine	1.7	1.7	3.5	1.7		1.5	2.2
Iso-leucine	1.5-1.6	2.1	3.0	2.3	2.4	3.2	3.0
Leucine	2.1	3.5	5.4	3.8	4.1	6.8	4.0
Lysine	3.0	4.3	5.6	4.1	3.9	4.4	4.1
Methionine	0.3-0.4	0.6	0.6	0.5	0.65	1.2	0.8
Phenyl-alanine	1.6	2.6	3.3	3.0			
Threonine	2.2	3.7	2.8	3.6	3.4		
Tryptophane	1.1	1.9	2.4	2.4	2.1	2.3	1.9
Tyrosine	1.6		1.1		3.8	3.2	5.0
Valine	3.0	4.2		5.0			

* Schreier 1950 and personal communication.

tryptophane, tyrosine, phenylalanine, leucine, isoleucine, cystine and methionine. The level of threonine, valine, lysine and arginine was raised by 50 per cent; the level of histidine was normal in 3 of 5 estimations.

The results of glutamine and glutamic acid estimations in plasma and urine of three patients are given in Table 10. Compared with normal figures our patients showed an increase up to tenfold of glutamine and glutamic acid in the urine. Three estimations in the plasma of our patients showed an increase of up to 100 per cent in the plasma level. Four further estimations were carried out on specimens taken during periods when the patients were under alkali therapy with sodium citrate and showed no aminoaciduria in urine chromatograms; they all showed normal plasma levels.

Some further observations made during my chromatographic study may be summarised as follows;

(a) In each individual case the pattern of the aminoaciduria showed little variability, but the concentration of the aminoaciduria as a whole varied from day to day and was occasionally completely absent.

(b) There was a rough relationship between the concentration of aminoacids in the urine and the severity of the disease. As a rule aminoaciduria was strong in the acute, infantile form of Lignac-Fanconi disease, but weak and easily overlooked in the chronic form of later childhood. The pattern of the aminoaciduria varied only slightly from case to case, as may be seen from Table 6.

Table 10.

GLUTAMINE + GLUTAMIC ACID ESTIMATIONS IN PLASMA AND URINE OF 3 PATIENTS WITH LIGNAC-FANCONI DISEASE.

Case	Date	Glutamine + Glutamic Acid	
		Plasma mg%	Urine mg./24 hrs.
Normal		7 - 10(1)	23 - 106(2) aver. 66
1	1.11.50		443
	26. 2.51		940
	15. 4.51	8.3(3)	
	29. 5.51	13.3	556
2	15.11.50		388
	15. 4.51	8.4(3)	
	18. 5.51	17.5	210
	24. 5.51	22.3	266
8	15.11.50		245
	26. 2.51		49(3)
	15. 4.51	8.3(3)	
	23. 5.51	8.1(3)	(12.3mg.%)(3)

(1) Krebs, H.A., Eggleton, L.V., Hems, D., Biochem. J. 1949, 44, 159

(2) Unpublished findings of Krebs, H.A. in 10 children with various unrelated diseases. (personal communication).

(3) Under alkali therapy, with normal plasma CO_2 combining power and normal urine chromatogram.

(c) The concentration of aminoacids in the urine runs closely parallel to that in the plasma. This was seen in one of our patients in 21 plasma and urine chromatograms over a period of 8 months.

(d) Prolonged alkalinisation with a sodium citrate-citric acid water solution normalised the CO_2-combining power of the plasma and led to cessation of the aminoaciduria in all seven patients thus treated.

Discussion.

The results obtained by three different methods confirm earlier chromatographic findings of a substantial and generalised aminoaciduria in Lignac-Fanconi disease (Fanconi and Bickel 1949, d'Avignon and Vahlquist 1949). Up to 20 aminoacids, essential and unessential, are involved, but in contrast to various other aminoacidurias, histidine and taurine are of normal concentration.

There has been a discussion as to whether this aminoaciduria is due primarily to disturbed reabsorption of the aminoacids in the tubules or to an overflow from an increased blood level into the urine. The latter explanation now appears to be the more probable, as the plasma level of the aminoacids excreted in excess in the urine is 50-100 per cent above normal. If the tubular defect were the primary factor in this disorder, we have no satisfactory explanation of either the raised aminoacid plasma level or the cystine storage, which was a constant finding in all our patients and has repeatedly been observed in the early stages of the disease during the first and second year of life (Hottinger 1947 and the author's observations). It remains to be seen by future clearance work if the rise in the aminoacid blood level is high enough to account by itself for the strong aminoaciduria or if a disturbance

of the tubular reabsorption is a contributing factor, perhaps as a result of the advancing kidney destruction in the course of this disease, or due to a generalised enzyme deficiency affecting the kidney as well as other organs.

It is unlikely that the actual aminoacid loss in the urine is an important factor in the disease. If compared with the daily intake, the aminoacid loss seems negligible. For example, in one of our acutely ill patients, aged 2 years, the strongest aminoacids excreted were leucine (149 mg. per day) and valine (140 mg. per day). This patient consumed in his daily milk alone about 4g. leucine and 3g. valine. Rose's figures (1949) of the minimum daily requirement for man of these two aminoacids are 1.1 and 0.8 g. respectively. After having subtracted the aminoacid loss in the urine from the intake, a sufficient amount of leucine and valine should, therefore, be available to satisfy the metabolic requirements of this patient, and the same is true of the other essential aminoacids.

The cystine storage is perhaps the most striking feature of the disease. It is limited to certain parts of the reticuloendothelial system and is nearly always found intracellularly (Baar 1951). My hypothesis is that the cystine storage is the visible manifestation of a far more extensive disturbance of the aminoacid metabolism in which cystine, being poorly soluble, becomes deposited in the form of crystals, while the other aminoacids remain invisible in solution. Thus, whereas in the reticuloendothelial system only the excess of cystine is apparent, in the plasma the level of many aminoacids is raised, as shown by chemical and microbiological estimations, while in

the urine the full extent of the metabolic disorder is mirrored in the general aminoaciduria. The metabolic disorder does not seem to concern desamination, as urea and ammonia formation are usually undisturbed. Further research into the protein synthesis might throw more light on the site of the defect and for this purpose tracer work may be useful.

VI. AMINOACIDURIA IN PHENYLPYRUVIC OLIGOPHRENIA.

General considerations.

This disease, first described by Følling in 1934, is apparently not as rare as was originally believed. Jervis (1939), in a survey of 20,300 inmates of American mental institutions, discovered 200 cases and estimated the incidence of phenylpyruvic oligophrenia to be from 0.5 to 3 per cent of mental defectives. Munro (1947) found 30 such cases among 2457 idiots and imbeciles in Britain, and concluded that the incidence of phenylpyruvic oligophrenia in the general population would be approximately 4 per 100,000; there are, therefore, probably about 1600 cases in Britain.

The degree of mental deficiency varies; Penrose (1946) has estimated that 60 per cent of the patients are idiots, 30 per cent imbeciles and 10 per cent higher grades. Other clinical features are fair hair, frequent eczema, a musty, mouse-like smell of the skin, hyperhidrosis, some microcephalia, brisk reflexes, hypertonicity of the limbs, epileptic fits, and hyperkinetic movements, often with stereotype digital mannerisms. The patients are usually symmetrically and well built.

The biochemical error that accompanies and probably causes the disease is an inborn disturbance of the metabolism of phenylalanine. While this aminoacid is normally converted to tyrosine, Jervis, Block and their associates (1940, 1947) provided good evidence that in phenylpyruvic oligophrenia there is a block in this reaction; as a result phenylalanine accumulates in the blood stream and

overflows into the urine, where daily excretions of 200-600 mg. have been observed (Dann, Marples and Levine, 1943, Woolf and Vulliamy, 1951), compared with a normal excretion of up to 20 mg. It is assumed that the kidney, besides excreting phenylalanine, tries to compensate the prerenal metabolic block by desaminating phenylalanine to phenylpyruvic and phenylacetic acid (Woolf and Vulliamy, 1951). Phenylpyruvic acid especially is excreted in considerable amounts, i.e. from 0.5 gram to 2 grams daily.

The excessive excretion of phenylpyruvic acid is probably of little importance in the production of the mental damage, for no phenylpyruvic acid can be detected in the blood stream (Jervis, Block and associates, 1940). Tyrosine deficiency has also been suggested as a possible cause of the mental retardation, but this hypothesis still lacks adequate confirmation, as the tyrosine plasma level in the few estimations recorded was normal or only slightly reduced (Jervis 1947, and author's observation). Moreover, Mautner and Quinn (1949) gave two patients large amounts of tyrosine for a period of two months and Cowie (1951) treated three further patients for over four months in the same way without any improvement in their mental condition. It may be suggested that the incorporation of phenylalanine into body proteins is disturbed in phenylpyruvic oligophrenia, but this is unlikely, as Block, Jervis, Bolling and Webb (1940) found no difference between the phenylalanine and tyrosine content of the brain and other tissues in these patients and normals. Moreover, my chromatographic analysis of the phenylalanine and tyrosine content of the plasma protein in one phenylketonuric patient showed no variation from

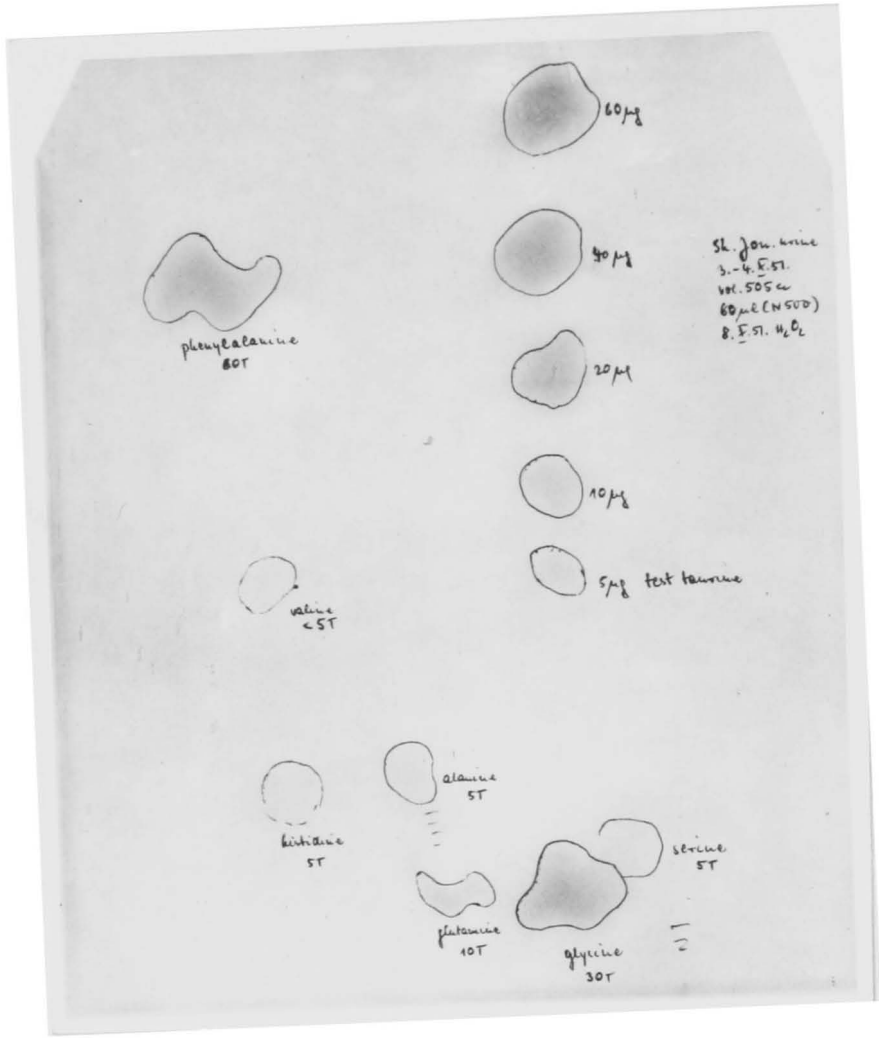


Fig. 12. Urine chromatogram of a patient with phenylpyruvic oligophrenia (Sh.J.). Note the strong phenylalanine spot. The five spots in the right upper quadrant are the taurine test spots. Total colour sum 130T.

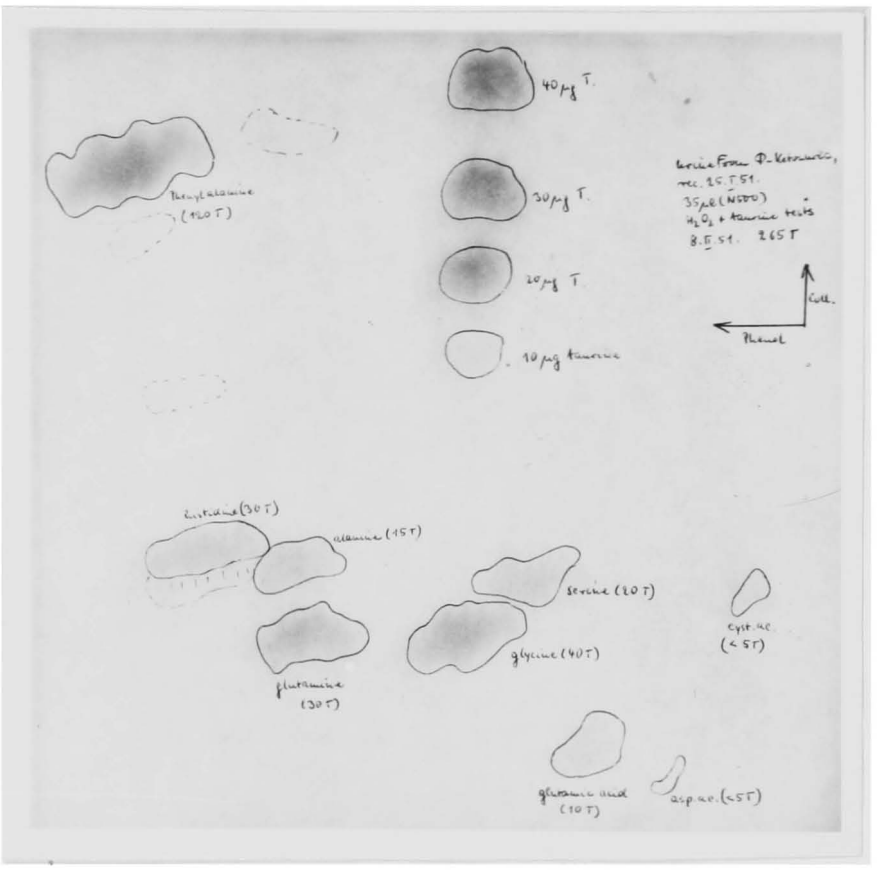


Fig. 13. Urine chromatogram of another patient with phenylpyruvic oligophrenia. Intense phenylalanine spot.
Colour sum of other aminoacids slightly raised (150T, normal up to 80T).

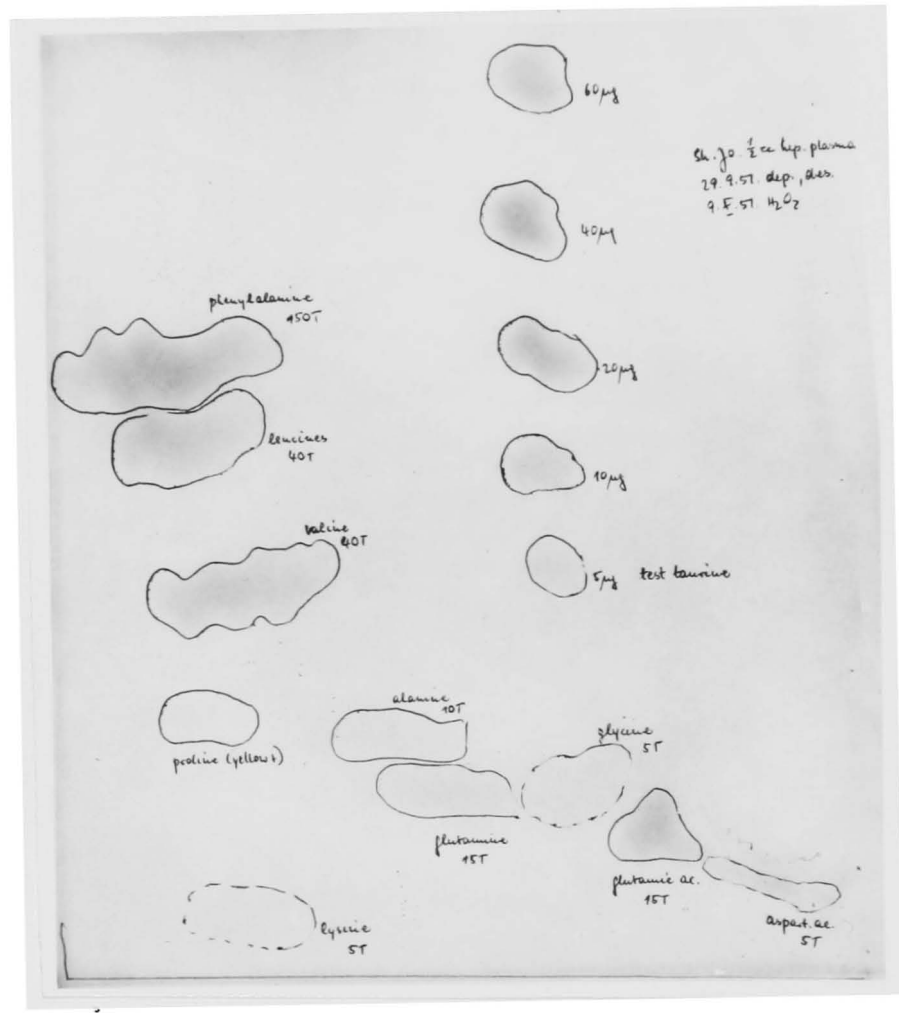


Fig. 14. Plasma chromatogram of Sh.J. Note the strong phenylalanine spot.
Total colour sum 285T.

the normal. It may, therefore, be that the high phenylalanine level in the blood is itself the toxic factor which damages the higher centres and that an attempt to lower the level by restricting the phenylalanine intake might be beneficial. Unfortunately phenylalanine is an essential aminoacid and its intake can, therefore, only be restricted to the minimum necessary for life. Furthermore, the preparation of such a diet presents great practical difficulties, as phenylalanine is a constituent of every food protein.

A description of the chromatographic findings in phenylpyruvic oligophrenia and of an attempt to feed one of our patients on a phenylalanine-free diet follows.

Results of chromatographic studies.

The urine of five and the plasma of two patients with phenylpyruvic oligophrenia were studied. The results were so similar that they can be summarised as follows:

(a) All urine chromatograms showed a strong increase of phenylalanine, as can be seen in Fig.12.

(b) In the urine of all patients, besides phenylalanine, the same aminoacids were found as in normal urine. In three patients their concentration was normal; in the two patients with the strongest phenylalanine excretion, the concentration of these aminoacids (alanine, glutamine, histidine, glycine, serine) was slightly increased or on the upper limit of normal (Fig.13).

(c) Plasma chromatograms in two patients showed a greatly increased phenylalanine spot, which exceeded in colour strength the leucine and valine spots (Fig.14). In normal plasma chromatograms phenylalanine was not seen at all or only in traces.

(d) The other aminoacids in the plasma were of normal concentration. The tyrosine level was not raised.

Attempt at feeding a phenylalanine-free diet.

It was Dr. L. I. Woolf, (Hospital for Sick Children, Great Ormond Street, London) who drew my attention to a method of removing phenylalanine from casein hydrolysate; he also generously sent me a gift of phenylpyruvic and α- acetylaminocinnamic acid for preparation of the standards. Dr. E. M. Hickmans carried out the phenylpyruvic acid estimations and helped in the preparation of the food. The trial was carried out on our youngest patient (Sh.J., 2 years 1 month) and consisted of four periods with different diets, namely:-

Period 1 Normal ward diet (7 days)
Period 2a Diet free of phenylalanine and tyrosine (5 days)
Period 2b Diet free of phenylalanine only (38 days)
Period 3 Phenylalanine-poor diet, in hospital (52 days)
Period 4 Phenylalanine-poor diet, at home (began 6 days ago)

During the periods 1-3 the plasma content of phenylalanine and tyrosine and the urine content of phenylalanine, phenylpyruvic acid and tyrosine were repeatedly tested, while the mental development and behaviour of the patient were followed as closely as possible.

Methods.

(a) Preparation of the phenylalanine-free food.

While the necessary salts, vitamins, fats and carbohydrates were provided in the pure state, the only nitrogen source was an acid hydrolysed casein (A & H) treated to remove phenylalanine and tyrosine by adsorption on activated charcoal which

had been further treated with 5% acetic acid (Block and Bolling, 1951). The hydrolysed casein is free of tryptophane. 1.5 g. tyrosine and 0.25 g. tryptophane per day were then added to the hydrolysate, except for the tyrosine-free period (2a), where only tryptophane was added. The hydrolysate prepared in this way was tested for phenylalanine by microbiological assay. The amount of casein hydrolysate ingested daily was equivalent to approximately 10 g. protein, the total daily intake provided about 1100 calories. The protein intake was limited because hydrolysed casein is expensive and its further preparation is difficult.

(b) Phenylalanine-poor food.

It was obvious from the beginning of the test that on a regime free of the essential aminoacid phenylalanine the patient would soon show a negative nitrogen balance and lose weight. Thus, after the second period, in which the child lost 3 lbs. 10 oz., she was given a diet containing just enough phenylalanine to maintain her weight. As Rose (1949) gives a tentative value of 1.1 g. for the minimum requirement of phenylalanine by adults, 0.7-0.8 g. phenylalanine in the form of about 400 ml. full cream milk was added daily for the next 52 days (period 3), providing altogether 20 to 25 g. protein. She has been sent home on a similar though somewhat less restricted diet, including an addition of 1 oz. of bread and Weetabix, 1 oz. butter, and those fruits and vegetables which contain practically no protein. These additions give about 2.3 g. protein and about 0.15 g. of phenylalanine.

(c) Other methods.

Phenylalanine and tyrosine were determined by microbiological assay on deproteinised plasma.

As the girl was incontinent, she was kept on a metabolic bed and a special apparatus for collecting 24-hour urine specimens was prepared according to the description by Black (1951). The urine was collected under toluol, transferred every twelve hours to a refrigerator and kept at-10°C. Phenylpyruvic acid estimations were carried out within the next two days using the dinitrophenylhydrazine method of Penrose and Quastel (1937). A semiquantitative estimation of phenylalanine was made by paper chromatography. Phenylalanine test spots of 20-40-60-80µg. were run with a constant fraction of the 24-hour-urine and two chromatograms were prepared from each specimen. The range of error of this method is believed to be less than 30%. Microbiological assay of phenylalanine in the urine after separation from its desamination products by ether extraction is in progress but is not yet completed. Tyrosine in the urine was tested by microbiological assay. Other changes in the aminoacid pattern of the urine were demonstrated by paper chromatography.

Results as recorded in Fig.15.

(a) Period 1. In the control period the patient's phenylalanine plasma level was65 mg. per 100 ml. (normal approx. 1.6 mg. per 100 ml.). The tyrosine plasma level at 0.8 mg. per 100 ml. was slightly below the normal range. The phenylpyruvic acid excretion in urine ranged from 350 to 550 mg. per day (none in normal urine), the phenylalanine excretion was about 500 mg. per day (normal up to 8 mg. per day). The tyrosine excretion was within the normal range.

(b) Period 2a and b: During the first ten days of the phenylalanine-free period the phenylalanine content of plasma and urine gradually fell to normal

levels and the excretion of phenylpyruvic acid dropped to 38 mg. per day. The tyrosine content of the plasma during the first five days of tyrosine-free food dropped to such low levels that it was no longer measurable, but it rose to normal levels when tyrosine was added to the diet. During the following weeks the level of phenylalanine in the plasma fell to subnormal levels and in one specimen was too low to be measured. The urine chromatograms showed none, or only traces of phenylalanine, with the exception of the urine chromatogram of the twenty-eighth day of period 2b, which showed a phenylalanine excretion of about 100 mg. per day. At the same time the phenylpyruvic acid excretion rose, but in the other estimations of this period it was below 50 mg. per day, a tenth of its former value. The ferric chloride test persistently gave an atypical white or white-brown colour reaction.

During the third week of the period, and particularly towards the end, general aminoaciduria developed with involvement of some nine aminoacids, namely, serine, glycine, alanine, glutamine, glutamic acid, lysine, histidine, valine and the leucines. The patient slowly but steadily lost weight, without otherwise being unduly upset. Unfortunately nitrogen balances could not be carried out as the child was very constipated and also vomited from time to time. When towards the end of the period vomiting became more frequent and the child seemed upset, it was decided to change to the phenylalanine-poor diet of period 3 described above.

(c) Period 3. Twenty-four hour urine collections were restricted for the sake of the child to two days at the beginning and two towards the end of the period. The phenylalanine and phenylpyruvic acid content of these specimens and the level of phenylalanine in the plasma under the new diet rose

considerably above the level of period 2 but were only about a third of the values in period 1. The child recovered quickly and kept her weight constant, gaining slightly towards the end of the period.

(d) Period 4. The child appears to be doing well at home, but it is too early to make a final judgment.

Mental development.

The assessment of any changes in the mental capacity and behaviour of the patient presented great difficulties. The child was too young and of too low an intelligence grade to be given any intelligence test. She behaved rather like a nine months old baby. There certainly was no sudden improvement under phenylalanine-free food, apart from the fact that she soon became quieter and more contented and ceased banging her head against the pillow. This, however, might have been because she was now accustomed to her new environment. Livelier interest in her surroundings, a more intelligent expression in her eyes, attempts to stand, etc., were recorded by the nursing staff but are too vague to be indicative of any real improvement. The general impression of the child's mother and her neighbours is that there is a definite though not dramatic improvement. The mother reports as follows:

> "Since Sheila returned home from hospital, her eyes seem brighter and livelier than before. She plays with more toys, crawls more and tried to pull herself up. She makes noises as if she wants to talk. She begins to notice when her name is called, whereas before she seemed deaf. She is interested in all food, crawls to pick up a biscuit from the floor and puts it into her mouth. This is the first time she has done this. She has nearly stopped rolling her head from side to side. She has now begun to quarrel with another baby about toys".

Discussion.

The aminoaciduria in phenylpyruvic oligophrenia, with its isolated phenylalanine increase, shows a typical chromatographic pattern, but as the urine also gives a strong ferric chloride reaction, chromatography is only an additional, not an essential diagnostic aid. The slight increase of certain other aminoacids, seen in the chromatogram of two patients, is probably of secondary importance, as there was no such increase in the urines of the other children. The two patients were those who excreted most phenylalanine and it is possible that the reabsorption of the excess of phenylalanine partly blocked the reabsorption of the other aminoacids. A common reabsorption mechanism for various aminoacids has been suggested by the experimental work of Pitts (1944). The excessive loss of phenylalanine and its desamination products in the urine is due to an overflow from an increased phenylalanine blood level, which is clearly seen in the plasma chromatograms.

By depriving a phenylpyruvic patient at first totally and then partially of phenylalanine the blood and urine levels of this aminoacid were considerably lowered. Simultaneously the excretion of phenylpyruvic acid dropped. There was, however, still some phenylpyruvic acid in the urine, even when the phenylalanine excretion had ceased. This might have been derived from phenylalanine liberated from increasing breakdown of body proteins. Another explanation might be provided by Berry and Woolf's finding (1952) that the dinitrophenylhydrazine method produces too high values due to a still unidentified phenol.

In this test it took nearly ten days for the high phenylalanine content of blood and urine to fall to normal levels. This was perhaps due to the excessive amount of phenylalanine accumulated in the body fluids,

which could only gradually be excreted through the kidney. Had nitrogen balances been possible it would have been interesting to see how soon they became negative. During the first days of the test, when the phenylalanine blood level was still raised and there was no tyrosine in the diet, a negative nitrogen balance would have provided support for the assumption that tyrosine is in this disease an essential aminoacid which must be supplied in the food, as it cannot be formed by the conversion of phenylalanine. The correctness of this assumption is indicated by the prompt loss of weight, despite sufficient caloric intake, as soon as the tyrosine-free food was started, and by the slight gain in weight when tyrosine was again added to the diet. The further loss of weight after this gain may be taken as evidence of a negative nitrogen balance due to the phenylalanine deficiency.

In the second half of the phenylalan ine-free period the general aminoaciduria and the temporary phenylalanine increase in the urine was probably due to increased breakdown of body proteins, which is also suggested by the loss of weight despite sufficient caloric intake. This is supported by the finding of Penrose and Quastel (1937) that partial protein starvation of their patient with phenylketonuria reduced the excretion of phenylpyruvic acid only temporarily and that the production of the acid soon rose again.

Any mental improvement on a phenylalanine-poor diet will depend on whether or not the cerebral lesion is reversible. If the lesion is not reversible, a phenylalanine-poor diet would only be of use if initiated in infancy. Its success would depend on the possibility of keeping the phenylalanine plasma level low enough to avoid toxic damage to the brain

while still giving enough of this essential amino-acid to permit normal development of the growing child. It is possible that these two aims are incompatible and that some compromise must be found between them.

It is, of course, still not certain that the high phenylalanine level in the blood is the toxic factor in this disease. The cerebral damage may result from a slight decrease in the tyrosine plasma level. Jervis (1947) alone has recorded tyrosine plasma figures in phenylpyruvic patients; his levels are slightly below normal, as was a single tyrosine plasma value in the control period of our patient. Peters and van Slyke (1946) remarked on the extra-ordinary economy with which tyrosine is handled by the body. They suggested that this may arise from the fact that tyrosine serves a multitude of special functions. Until the influence of a tyrosine deficiency in this disease is clarified it will be advisable to give a diet poor in phenylalanine with a plentiful additon of tyrosine.

VII. AMINOACIDURIA IN CERTAIN LIVER DISORDERS.

General considerations.

Earlier animal experiments suggested that though the liver is indispensable for the desamination of aminoacids and the formation of urea, demonstrable impairment of these liver functions did not occur until 90 per cent of the organ had been removed (McMaster and Drury 1929). Peters and van Slyke (1946) in a review of the subject claimed that this was why the concentration of aminoacids in the blood was little disturbed in diseases of the liver, and why normal values had been reported in hepatitis, cirrhosis, infectious and toxic jaundice. A considerable rise in the aminoacid blood level was recorded only in terminal stages of acute yellow atrophy (Stadie and van Slyke, 1920, and others). The same authors and also Greene (1941) mentioned that normal amino-nitrogen levels were found even in the blood of patients dying of hepatic insufficiency due to liver cirrhosis.

More recent reports, however, present a different picture. This is due to the fact that later workers have been able to analyse not merely the total amino-nitrogen but also the individual aminoacids by microbiological assay (Eckhardt, Cooper, Faloon and Davidson, 1948, and Dunn, Akawaie, Yeh and Martin, 1950) and by paper chromatography (Dent and Walshe, 1951, Walshe, 1951). Thus considerable variations in the aminoacid pattern of blood and urine in liver disorders have been demonstrated where there was no significant change in the total amino-nitrogen level.

Paper chromatography reveals even small deviations from the normal aminoacid pattern in the urine of patients with liver disease and has been applied in the course of my investigations to a variety of disorders with proved or suspected liver damage. This chapter, however, will deal with only a limited number of cases for two reasons:

(a) Well-defined liver disorders, such as cirrhosis and acute yellow atrophy, are rare in childhood.

(b) In a multitude of toxic and infectious diseases chromatographic changes were seen which were probably due to liver damage. In only six of these cases was the evidence of liver damage well established and it seemed probable that the underlying diseases had no influence on the aminoaciduria.

The following account has, therefore, been confined to children suffering from liver cirrhosis (10 cases, 9 were confirmed at necropsy), infectious hepatitis (9 cases, none died) and toxic jaundice with liver damage (6 cases, 5 confirmed at necropsy).

In view of the complexity of the problem the number of cases studied is very small, while other diseases, such as acute yellow atrophy, tumour, abscess and syphilis of the liver have not been encountered at all. This should, therefore, be regarded as a preliminary and necessarily incomplete account of some examples of aminoaciduria due to liver damage.

Other diseases with aminoaciduria, in which the rôle played by the diseased liver is not yet clearly established, will be discussed in other chapters, and comprise galactosaemia, hepatolenticular degeneration and steatorrhoea.

Table 11.

CHROMATOGRAPHIC FINDINGS IN THE URINE OF PATIENTS WITH LIVER DISEASES, EXPRESSED IN UNITS OF AN ARBITRARY COLOUR SCALE 1 FOR THE WEAKEST, ≫10 FOR THE STRONGEST COLOUR.

	Liver cirrhosis cases											Infectious hepatitis cases										Toxic jaundice cases.							Total Aver.	Aver. normals
	1	2	3	4	5	6	7	8	9	10	Av.	11	12	13	14	15	16	17	18	19	Av.	20	21	22	23	24	25	Av.		
Glycine	≫10	10	>10	10	8	>10	10	8	9	9	11	>10	≫10	7	8	10	>10	10	10	>10	12	>10	10	8	10	>10	10	11	11	6
Alanine	6	9	>10	9	6	10	8	4	8	4	8	>10	4	7	4	3	6		8		5	9	10	9	10	8		8	7	2.7
Glutamine	6		>10			9	6		9	2	5	>10	4	3	4	10	4		3	4	5	8	8		5	4		4	5	1.8
Cystine	2	8	4	9	8	8	7	10		8	6		3	9	3	3	3	2	3		3	6	10	7	5	3	4	6	5	0.5
Taurine	4	>10		10	8		8	10		3	6	>10	3	4				9			3	9	10	8	4	4	4	7	5	1.1
Serine	≫10	10	10	8	6	9	9	2			7	7		4			3	5			2.1	9	10		5	5	4	6	5	0.5
Glutamic acid	6	10	3	10	8	5	3				5			4	3	4					1.2	7	9	5	5	2	1	5	4	3
Histidine	4		6	9	8	6			4	4	4		4	5	4	4			3	7	3	8						1.3	2.8	2.2
β-amino-iso-butyric acid		10					6	3			1.9	>10			>10						3		2	4		2		1.3	2.1	0.5
Methionine-sulphone	4		2	1							0.7	3		3		2	3	3			1.5			9				1.5	1.2	0
Tyrosine	7	7	2	1	4				1		2.2	4	2								0.7			3				0.5	1.1	0.2
γ-amino-butyric acid	5	3	4			2					1.4			2		4		3			1.0	2		10				2.0	1.1	0
Aspartic acid	6	4		6			4				2										0	6						1.0	1.0	0
Methyl-histidine	8				6		4				1.8		6		3						1.0							0	0.9	0
Ethanolamine						5					0.5	4									0.4	7						1.2	0.7	0
Threonine	3	3	6	2	2						1.6										0							0	0.5	0
Valine		3			3						0.6	4	1	1							0.7	2						0.3	0.5	0.2
The leucines					7						0.7		2	1							0.3	1						0.2	0.4	0
Ethanolamine phosphate ?		9									0.9										0							0	0.3	0
Lysine		2									0.2						4				0.4							0	0.2	0.05
Phenylalanine			2								0.2		2								0.2							0	0.1	0
α-amino-n-butyric acid		1									0.1										0							0	0	0
Total	101	101	80	75	74	67	65	37	37	30	66.8	97	51	50	44	44	34	32	27	26	43.5	89	69	63	44	44	23	56.3	54.9	19

Note. Cases 3, acute; 1,5, subacute; 2,4,6-10, chronic cirrhosis; 8,9, without jaundice. Cases 11-19 were jaundiced; specimens collected at height of disease. Cases 20,21,22,24,25, severe fatty liver degeneration at autopsy.
For the total sum > 10 is calculated as 15, ≫ 10 as 20.

Results of chromatographic studies.

(a) Liver Cirrhosis.

Ten cases were investigated, in nine of which the diagnosis "liver cirrhosis" was confirmed at autopsy (Table 11, Cases 1-9). Case 10 is still alive and is suffering from familial liver cirrhosis. She is the sister of Case 6, and at two months old is the youngest of these patients, has a hard enlarged liver palpable two fingerbreadths below the costal margin, slight jaundice, dilated veins on the abdominal wall, moderate urobilinogenuria and a plasma phosphatase of 30 units. Case 3 exhibited the severest course of the disease; she developed cirrhosis rapidly following an infectious hepatitis and died six weeks after the onset of symptoms. Cases 1 and 5 had subacute cirrhosis, which in Case 1 was complicated by subacute glomerulonephritis. This probably had no influence on the aminoaciduria, as the pattern of the urine chromatogram was very similar to that of the other cases without kidney diseases; moreover, nine cases of glomerulonephritis without liver disorder showed no aminoaciduria. Cases 2, 4 and 6-10 suffered from chronic cirrhosis of the liver, which in Case 9 was found at autopsy to be a complication of congenital cystic liver and polycystic kidneys.

Table 11 summarises the findings of a representative urine chromatogram from each of the ten cases. None of the specimens were taken in the terminal stage of the disease, the time of collection being three weeks (Case 3) or longer before death. All specimens were early morning samples. Seven of the ten patients showed a definite aminoaciduria with a total of 65 to 101 colour units (normal range 3-55). Of the aminoacids which are usually found in

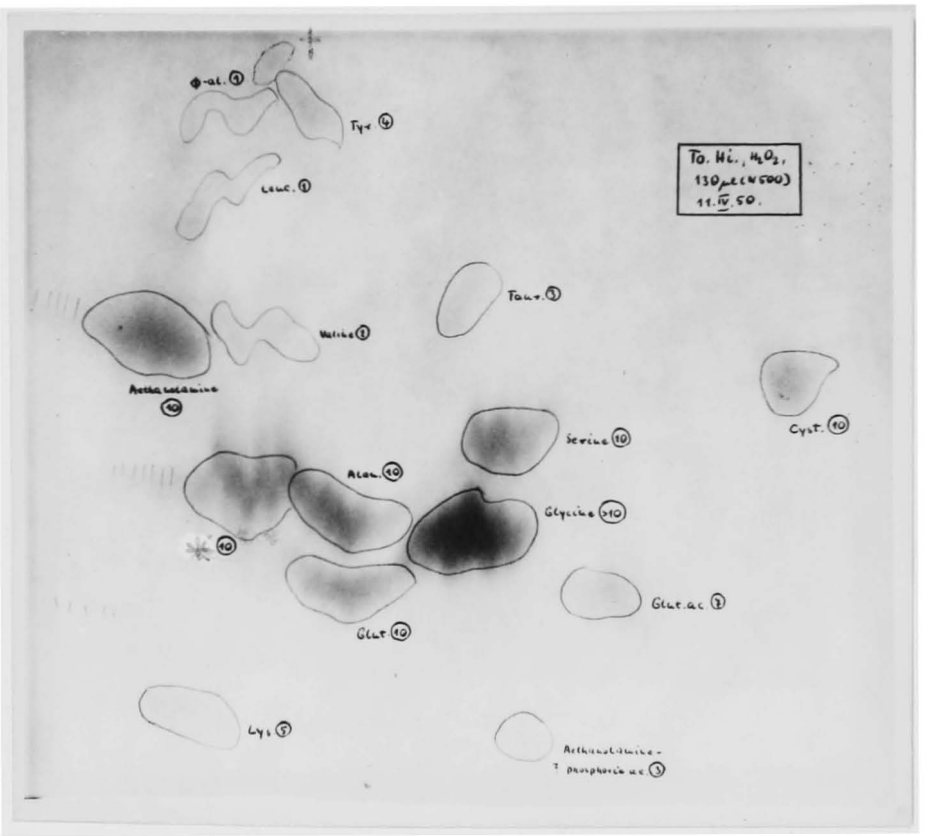


Fig. 16. Urine chromatogram of a patient with liver cirrhosis (Case 6). Note the increase of ethanolamine, β-amino-isobutyric acid (*) and cystine. Traces of tryptophane at the top of the print (†).
Total colour sum 101.

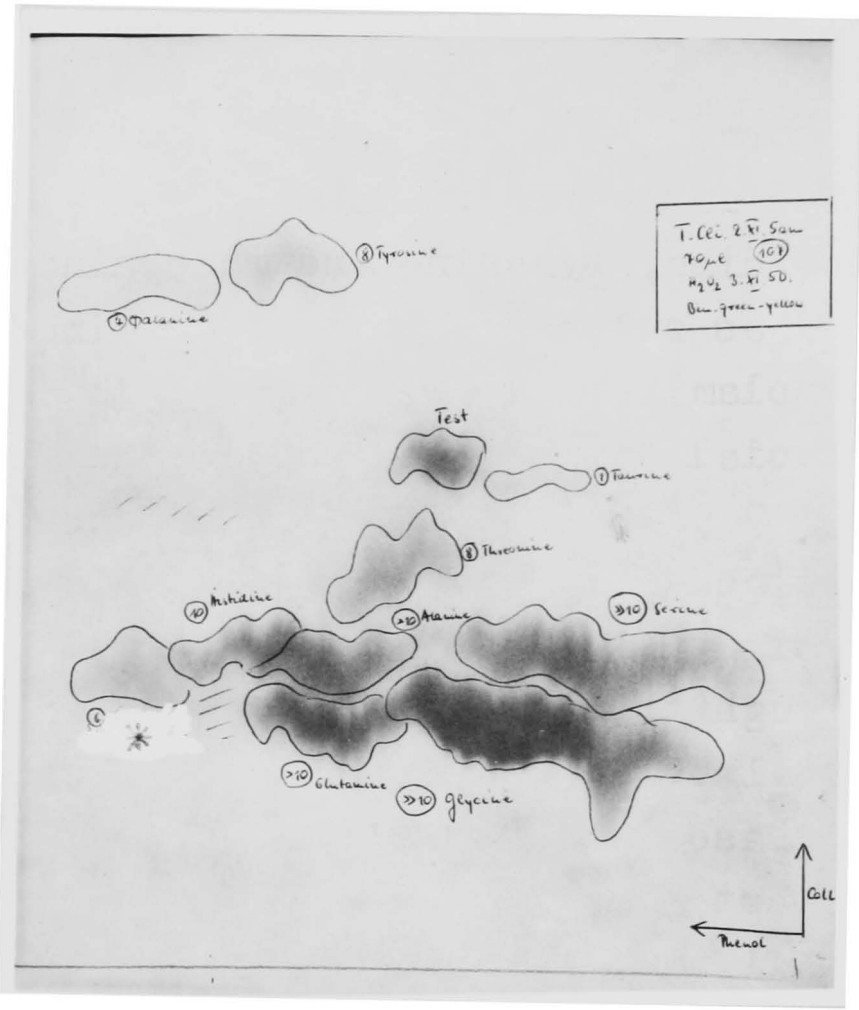


Fig. 17. Urine chromatogram of another patient with liver cirrhosis. Note the increase of serine, threonine, γ-amino-butyric acid (*) and tyrosine.
Total colour sum 107. (>10 calculated as 15, >>10 as 20.).

normal urine, the increase of cystine, taurine and serine was especially marked, but the glycine and alanine concentration was also raised. These aminoacids were present in most cases while others were only seen occasionally and, for no obvious reason, were absent in other cases. Threonine was above the normal level in five instances; γ-amino-butyric acid and aspartic acid in four instances; methyl-histidine, tyrosine and threonine in three; methionine (as sulphone) in two; β-amino-isobutyric acid, ethanolamine and the leucines in one instance.

Of special interest are the urine chromatograms of Cases 8, 9 and 10, none of which showed an increase in the total colour sum. The aminoacid pattern in the urine of Case 9 was the only normal one of this series, though the pattern of Cases 8 and 10 was also nearly normal but for the increase of cystine and taurine in Case 8 and cystine in Case 10. Cases 8 and 9 were not jaundiced, and Case 10 only slightly so, while all the other cases had marked jaundice.

Examples of urine chromatograms from Cases 1 and 6 and a plasma chromatogram from Case 1 are reproduced in Figures 16, 17 and 18. In Fig. 16, the increase of ethanolamine is remarkable; it has previously been observed in liver diseases by Dent and Walshe (1951). Some difficulty was encountered in diagnosing spot "6" in Fig.17 and spot "10" in Fig. 18 as they lie in an area where the aminoacids γ-amino-butyric acid and methionine sulphoxide occupy practically identical positions. Spots "6" and "10" were diagnosed as γ-amino-butyric acid, as they were stable to H_2O_2 in the presence of ammonium molybdate (Dent 1948).

The plasma chromatogram of Case 1 (Fig.18) showed an increase of the total colour sum to 119

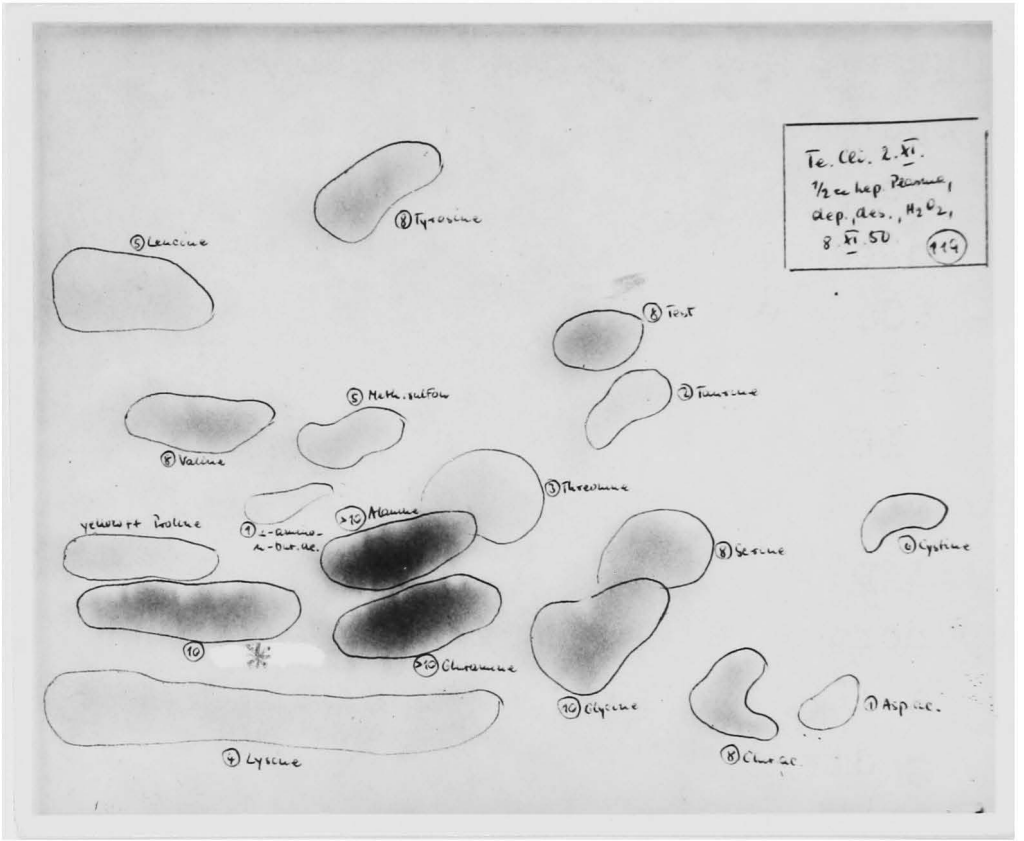


Fig. 18. Plasma chromatogram of the same patient. Note the similarity to the aminoacid pattern of the urine, especially the increase of γ-amino-butyric acid (*) and of tyrosine. Raised plasma level of methionine (as sulphone). Hyperamino-acidaemia with a total colour sum 109 (normal up to 78).

(normal average 52) and an abnormal pattern with an increase of methionine as sulphone, of γ-amino-butyric acid and of tyrosine, while cystine was at the upper limit of normal. In this patient the chromatographic findings in urine and plasma were followed up during two months of increasing liver coma until death. The pattern remained similar, though cystine, methionine sulphone and tryptophane appeared in a few urine chromatograms. During the last days of life the taurine spot grew stronger in plasma and urine. There was, however, no significant increase of the total colour sum of the chromatograms. In five further patients (Cases 2, 4, 5, 6, 9) plasma chromatograms were prepared. Their total colour sum was, in order of cases, 165 - 144 - 81 - 68 - 46, and was thus definitely increased in Cases 2, 4 and 5, whereas the normal plasma chromatogram of Case 9 agreed well with his normal urine chromatogram. The pattern of the plasma chromatograms resembled that of the urine in showing disproportionately strong increases of tyrosine, cystine, taurine, aspartic acid, threonine and γ-amino-butyric acid in Case 2; cystine, taurine, tyrosine, aspartic acid and threonine in Case 4; cystine, taurine and tyrosine in Case 5; cystine and ethanolamine in Case 6.

(b) Infectious hepatitis.

In all nine cases the diagnosis was based on clinical and biochemical evidence and all the patients recovered from their disease. In five cases (Table 11, Nos. 12, 14, 15, 17, 19) the source of infection was believed to be known. Case 13 was suffering from a very severe hepatitis with grossly abnormal liver function tests and a liver extending to the umbilicus,

but made a complete recovery after four months. Case 16 was, at nine weeks old, the youngest of the series. Cases 18 and 19 suffered from a mild form of the disease, showed no pathological liver function tests and made a rapid recovery.

The chromatographic pattern in the urine of these patients resembled those of the patients with liver cirrhosis but the total colour strength of the chromatograms was above normal only in Case 11, a patient with an acute onset and high fever. Thus, the abnormality of the urine chromatograms lay with one exception, not in a general increase of many aminoacids, but in an abnormal concentration of a few aminoacids, namely of cystine, taurine, methionine as sulphone, β-amino-isobutyric acid, methyl-histidine, γ-amino-butyric acid, tyrosine, valine and ethanolamine. Of these, only cystine, taurine and methionine were seen in four or more of the nine patients, whereas the other aminoacids were encountered only in one or two instances. In only two patients was the aminoacid pattern of the urine completely normal.

(c) Toxic jaundice.

Of the six patients studied (Table 11, Cases 20-25) five succumbed to their illness (Nos. 20, 21, 22, 24, 25). At autopsy Cases 20 and 21 showed miliary tuberculosis with severe fatty degeneration of the liver and the beginning of cirrhotic changes. The urine specimens were collected five and eight days before death. Case 22 showed gangrene of the small intestine in addition to severe fatty degeneration of the liver. The urine specimen was collected two days before death. Cases 23 and 25 suffered from septicaemia with toxic jaundice; Case 25, whose urine

specimen was collected two weeks before death, showed at autopsy severe congestion and fatty degeneration of the liver. Case 24 suffered from gastroenteritis with toxic jaundice, and his urine was collected one week before death; autopsy revealed enlargement and severe fatty degeneration of the liver. All the patients were jaundiced and various liver function tests were grossly abnormal.

The urine chromatograms of these patients are listed in Table 11, Nos. 20-25. The total colour sum of the chromatograms was on the upper limit or above the normal range in five of the six cases. The pattern of the chromatograms again showed some features common to those of the two previous groups of cases, namely an increase of cystine and taurine and occasionally of methionine, γ-amino-butyric acid, ethanolamine and aspartic acid. In three of the six cases (Cases 23, 24, 25), however, the changes in cystine and taurine excretion were too mild to be clearly distinguished from normal urine.

Summary and discussion.

The chromatographic studies in the cases described above may be summarised as follows:

(a) A moderate but definite general amino-aciduria was found in 11 of the 25 cases, particularly in the patients with cirrhosis.

(b) The pattern of the urine chromatogram differed from the normal in all but six cases, and in all but one of the patients with cirrhosis.

(c) Plasma chromatograms of four out of six cases of liver cirrhosis showed a general aminoacidaemia with a pattern similar to that of the urine. The same changes were indicated in the fifth but absent in the sixth patient, who was the only cirrhotic patient without aminoaciduria.

These findings suggest that alterations of the aminoacid pattern in blood and urine are common in liver diseases, not only in acute and final stages but also in early cirrhosis (Case 10) as well as in transitory and relatively mild hepatitis (Case 11). The same conclusion was reached by Dent and Walshe (1951), who in chromatographic studies of ten cases of cirrhosis and eleven cases of infectious hepatitis found some changes of the urine aminoacid pattern in all of them. The moderate increase of the total colour sum in less than half of our patients may account for the failure of less specific quantitative methods, such as formol titration and Folin's method, to detect this form of aminoaciduria.

The pattern of the aminoaciduria in liver disease is not so characteristic as, for instance, in cystine-lysinuria or in phenylpyruvic oligophrenia. There appears to be no typical pattern with an excess of certain special aminoacids common to all patients. There are, however, some chromatographic changes which were frequently or occasionally found in our patients and which may be summarised as follows:

Of the aminoacids which may be found in normal urine:

(a) Cystine was found in 88% of the patients (normal 24%) its colour intensity averaged 5 (normally 0.5).

(b) Taurine was found in 68% (normally in 33%); its colour intensity averaged 5 (normally 1.1).

(c) Serine was found in 68% (normally in 17%); its colour intensity averaged 5 (normally 0.5).

(d) Tyrosine was found in 36% (normally in 11%); its colour intensity averaged 1.1 (normally 0.2).

(e) β-amino-isobutyric acid was found in 32% (normal 11%); its colour intensity averaged 2.1 (normally 0.5).

(f) Valine was found in 24% (normally in 11%); its colour intensity averaged 0.5 (normally 0.2).

Of the aminoacids not usually found in normal urine:

(a) Methionine as sulphone was found in 36%; its colour intensity averaged 1.2.

(b) γ-amino-butyric acid was found in 36%; its colour intensity averaged 1.1.

(c) Methyl-histidine, aspartic acid and threonine were found in 20%; their colour intensity averaged 0.9, 1.0 and 0.5.

(d) The leucines were found in 16%; their colour intensity averaged 0.4.

(e) Ethanolamine was found in 12%; its colour intensity averaged 0.7.

The aminoacids in the second group are of special diagnostic importance, as their occurrence in urine is definitely pathological. Their frequency and concentration is probably still higher than in the above estimations, as methionine, methyl-histidine, threonine, and tyrosine have a low ninhydrin sensitivity. Moreover, methionine is excreted only in traces in normal urine, namely about 1 mg. per day (in contrast to a histidine excretion of 50 to 100 mg. per day); considerable increases of the methionine output can thus be easily overlooked by paper chromatography. To a lesser degree the same is true of tyrosine, tryptophane and phenylalanine, which not only have a low ninhydrin sensitivity but are excreted in quantities of 5 mg. per day or less.

Dent and Walshe (1951) in their chromatographic study distinguished six different types of response to liver damage:

Response A showed many aminoacids in great excess in the urine.
Response B many aminoacids in smaller excess than in A.
Response C cystine alone in excess.
Response D cystine and β-amino-isobutyric acid in excess.
Response E cystine, β-amino-isobutyric acid and methyl-histidine in excess.
Response F ethanolamine alone in excess.

Responses A and B were found in acute yellow atrophy and fatal liver coma, responses B, C, D and E in infectious hepatitis and non-terminal liver cirrhosis, response F in a patient suffering from a primary hepatoma. Two further responses were briefly mentioned in the same paper, one involving the aromatic acids in addition to the E response, the other involving methionine in addition to response C. The plasma was tested in 6 cases with response C and showed a greatly increased concentration of cystine. My results confirm their findings, in so far as the aminoacids they observed to be excessive were also found in our patients.

Dunn, Akawaie, Yeh and Martin (1950) approached the problem with the aid of microbiological assay. They tested 3 to 15 aminoacids in 25 patients suffering from various liver diseases, mainly subacute and chronic cirrhosis. 38% of the total aminoacid values were abnormal, 20% of them increased, 18% below normal. Most of the high values were found in cirrhotic patients with jaundice. 50% or more of the values for methionine, tyrosine and valine were high, as were 38% of the cystine values, 28% of threonine, 25% of phenylalanine, 18% of tryptophane, 14% of aspartic acid and 12.5% of the histidine values. Almost half the lysine and histidine values were low. Many of the aminoacids in which an increase was observed by Dent and Walshe and by me were not tested, e.g. taurine, β-amino-isobutyric acid, γ-amino-butyric acid, methyl-histidine and ethanolamine. The results of Dunn and associates support my chromatographic findings and supplement them by showing that in liver diseases the urinary aminoacids may be decreased as well as increased.

The finer mechanism of the aminoaciduria in liver disease is still unknown. Dunn and associates point out that the high dietary protein intake given to most of their patients, and also to ours, does not lead to aminoaciduria in normals (Kisner, Sheffner and Palmer 1949).

This factor was probably not of great importance in our cases, as the urine was always taken from an early morning collection before breakfast. In four of our patients an aminoacidaemia with a pattern similar to that of the urine suggests an overflow mechanism for the aminoaciduria. The increase of the aminoacids which act as protein building blocks (e.g. serine, tyrosine, valine etc.) is probably due to increased tissue breakdown and decreased desamination and transamination in the liver. It is more difficult to explain the high concentration of aminoacids not belonging to the protein building blocks, such as β-amino-isobutyric acid and methyl-histidine. For these Dent and Walshe (1951) suggest a liberation from intracellular peptides into the blood stream. β-amino-isobutyric acid, methyl-histidine and cystine, being only slowly metabolised, build up appreciable concentrations in the blood and overflow into the urine.

Certainly the fact that the rise of the aminoacid level in blood and urine does not affect all aminoacids to the same extent suggests a more complicated dysfunction of the desamination. For the metabolism of the various aminoacids the liver may perform a multitude of separate functions and provide a variety of enzyme systems which can be affected independently of each other. The oxidation of the sulphur-containing aminoacids methionine and cystine seems to be affected particularly early in the disease, and also the oxidation of tyrosine. This aminoacid has long been known to be excreted in excess in the urine of certain patients with advanced liver disease and has even been found in crystalline form in their urine.

A chromatographic study of the aminoaciduria in liver diseases demonstrates the value of methods capable of assessing even small deviations from the normal

aminoacid pattern in biological fluids. The diagnostic importance of such findings is obvious, though future experience must show how far they are specific for liver dysfunction only. If the specificity should prove to be high (as I suspect), then chromatography might become an important liver function test.

There are other aspects of the research into the aminoacid pattern in liver diseases. The influence of the altered aminoacid composition of biological fluids upon the clinical course of the disease has to be studied. Animal experiments provide evidence of the toxic effect of disturbing the aminoacid balance by feeding certain aminoacids in unphysiological amounts. Walshe (1951) recently reviewed this question and suggested that a similar toxic effect of the unbalanced aminoacid pattern in liver disease might be the cause of hepatic coma. Moreover, a better understanding of the structural changes of the aminoacid pattern in liver diseases may bring about a finer adjustment of therapeutic measures, such as the addition or omission of certain aminoacids to the diet, thus giving the liver a better chance of recovering from its lesion.

VIII. AMINOACIDURIA IN IDIOPATHIC STEATORRHOEA AND COELIAC DISEASE.

General considerations.

During the study of aminoaciduria in Lignac-Fanconi disease I encountered an 18-months old patient who exhibited various characteristic clinical features, such as dwarfism, anorexia, vomiting, irregular temperature, constipation, photophobia, fair hair, pronounced osteoporosis, hypophosphataemia and aminoaciduria. But the pattern of the aminoaciduria was different from that seen in Lignac-Fanconi disease and there was no glycosuria, nor could cystine storage be detected in eyes or bone marrow. A fat balance on a diet containing 50g. of fat showed a sub-normal fat absorption of 88 per cent, and a barium meal X-ray examination revealed dilated jejunal loops. A chylomicrograph gave a subnormal and prolonged rise. On a wheat-free, low fat diet the child has gained four pounds in two months and has gone home in much improved health.

Soon afterwards, urine specimens of a suspected case of Lignac-Fanconi disease were sent to me for chromatographic investigation. The patient was nineteen years old, and suffering from severe dwarfing, anorexia, bilateral genu valgum and other bone deformities. X-ray examination revealed considerable osteoporosis. In over 30 urine specimens tested during the following months the aminoacid concentration was considerably increased (Fig.19) but again the pattern was not that of the aminoaciduria in Lignac-Fanconi disease. Further clinical investigation of the patient by

Professor Thomson revealed a disturbed fat absorption and other features typical of idiopathic steatorrhoea. The onset could be traced back to the age of fifteen months, when the patient developed a large abdomen and frequent pale motions.

The two cases suggested that some patients with coeliac disease and idiopathic steatorrhoea excreted various aminoacids in excess in their urine. There appeared to be no previous report of aminoaciduria in the literature of steatorrhoea. To learn more about this form of aminoaciduria and its frequency, I investigated 22 other cases of coeliac disease and 52 further adult patients with idiopathic steatorrhoea. I am greatly indebted to Professor Thomson and Dr. Trevor Cooke for providing me with numerous urine samples and clinical notes of the adult patients. Whereas aminoaciduria was found in only three patients with coeliac disease, twelve of the adult patients with steatorrhoea repeatedly showed a moderate or marked increase of aminoacids in the urine.

Results of chromatographic and microbiological studies.

The findings in the adult patients are included in this study so that the number of cases might be sufficient on which to base conclusions. This was thought to be justifiable as the pattern of the aminoaciduria was very much the same in adults and children and the majority of the adult patients had actually suffered from coeliac disease in childhood.

Table 12 summarises representative urine chromatograms from each of the cases. All adult patients exhibited a fat absorption of 80% or less on a 50g. fat intake, low glucose tolerance curves and anaemia, generally of the macrocytic type. Cases 1, 2, 3 and 9 showed osteoporosis, Cases 6 and 8

osteomalacia with Looser's zones. Cases 1, 2, 3, 5, 6, 8, 9 and 11 had had the disease since childhood. Case 3 died in a hypokalaemic crisis and was found at autopsy to have severe fatty infiltration of the liver. This was the only case who showed some evidence of liver damage in the usual liver function tests (thymol turbidity, flocculation tests, albumin-globulin ratio, cholesterol and phosphatase), though in some of the other cases too few tests were carried out to assess the liver function.

It may be of some significance that the first three cases who showed the strongest aminoaciduria had had chronic severe steatorrhoea since childhood with a fat absorption of about 70%, marked anaemia, bone changes, etc. There was, however, no strict relation between the severity of the disease and of the amino-aciduria, and occasionally patients with chronic, severe steatorrhoea showed normal urine chromatograms.

Of the three coeliac patients with aminoaciduria, two (Cases 13 and 14 in Table 12), aged 18 months and 16 months, were acutely and rather severely affected. They were both wasted, miserable-looking children, hypotonic and pale. More details about Case 13 are given in the first paragraph of this chapter. Case 14 with a fat intake of 20g. fat excreted 11.9g. and on another day 17.3g. faecal fat, estimated as fatty acid. The chylomicrograph showed a fairly good rise, although to subnormal levels. The glucose tolerance curve was flat and there was osteoporosis present. On a high protein, low fat diet containing no wheat flour the child's general condition improved steadily and he gained weight satisfactorily. Case 15 had suffered from coeliac disease between the ages of five and six years but had been put on a low fat diet for one year and later on a normal diet, and

Table 12. AMINOACIDURIA IN STEATORRHOEA AND IN COELIAC DISEASE, EXPRESSED IN COLOUR UNITS, 1 FOR THE WEAKEST, > 10 FOR THE STRONGEST COLOUR.

	Steatorrhoea in adults.												Coeliac disease			Patients.		Normal children	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	frequency	Average colour strength	frequency	Average colour strength
Glycine	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	10	>10	8	>10	100%	14	97%	6
Alanine	10	9	10	10	10	6	10	8	8	9	8	7	10	8	8	100%	9	74%	2.7
Glutamine	10	9	10	8	6	3	9		5	4	9	7	9	4	8	93%	7	42%	1.8
Histidine	5	10	4	3	10	8	5	4	3	10	10	3	4	3	3	100%	6	54%	2.2
Serine	10	7	8	5	6	8	8	8	4		3	8	9	3	4	93%	6	17%	0.5
Glutamic acid	7	8	4		2	9			3	3	5	2	7	8	3	80%	4	68%	3
Cystine	8	5	2	2	2	5	2	3	2	2	2	4	4	4	3	100%	3	24%	0.5
Tyrosine	9	5	2	3	2	3		2	3	3	2		1	4	1	87%	3	11%	0.2
Threonine	9	4	2	8	3	5	4	4			2					60%	3	-	-
Taurine	10	8	5		6	3	2	8						5		53%	3	33%	1.1
Lysine	10	4	4	5		3			3				7	5		53%	3	3%	0.05
β-amino-iso-butyric acid			6	7			5	5	7	5		6		3		53%	3	11%	0.5
Methyl-histidine	4		4						3			4	5	3	7	47%	2.0	-	-
Aspartic acid	6	5			2								7	6		33%	1.7	-	-
Valine	2		1					2	1	8			3		1	47%	1.2	11%	0.25
Phenylalanine	3		3		2				1		2		1		1	47%	0.9	4%	0.04
The leucines	2		4		1			1	1				2		1	47%	0.7	7%	0.1
Methionine sulphone	2			3	1											20%	0.4	-	-
γ-amino-butyric acid		2	1													13%	0.2	-	-
Methionine sulphoxide				3												7%	0.2	-	-
Total	122	91	85	72	68	68	60	60	59	59	58	51	84	64	55		71.3		19

Note. For the total sum >10 is calculated as 15.

was symptom-free. At eleven years old the child had a short attack of diarrhoea and it was at this time that the urine for chromatography was collected. She was soon sent home again without any specific treatment and apparently in good health.

In the ten other cases of coeliac disease studied by chromatography there was no aminoaciduria, though some of them were as severely affected as Cases 13 and 14. There was, therefore, no obvious relationship between the severity of the coeliac disease and of the aminoaciduria.

In Table 12 only chromatograms with a total colour sum of above 50 (normal range 0-55) are listed. Values of over 80 were reached in three adults with steatorrhoea and in one coeliac patient. More striking than the total colour increase of the chromatograms was their deviation from the normal aminoacid pattern. This was also obvious in various other patients with steatorrhoea, whose urine chromatograms did not show an increase of the total colour strength.

Of the aminoacids which are commonly found in normal urine, there was an increase of serine, cystine, and to a lesser degree of alanine, glutamine, histidine and glycine in the urine of practically every patient. Taurine was present in the urine of more than half of the patients. Of the aminoacids rarely or never encountered in normal urine tyrosine, threonine, lysine and β-amino-isobutyric acid were found in eight or more of the fifteen cases; methylhistidine, valine, the leucines and phenylalanine in seven; aspartic acid in five; methionine sulphone in three and γ-amino-butyric acid in two cases.

The pattern thus produced is shown in the urine chromatograms of Case 1 (Fig.19) and Case 2 (Fig.20). They resemble the findings in liver disease (see later),

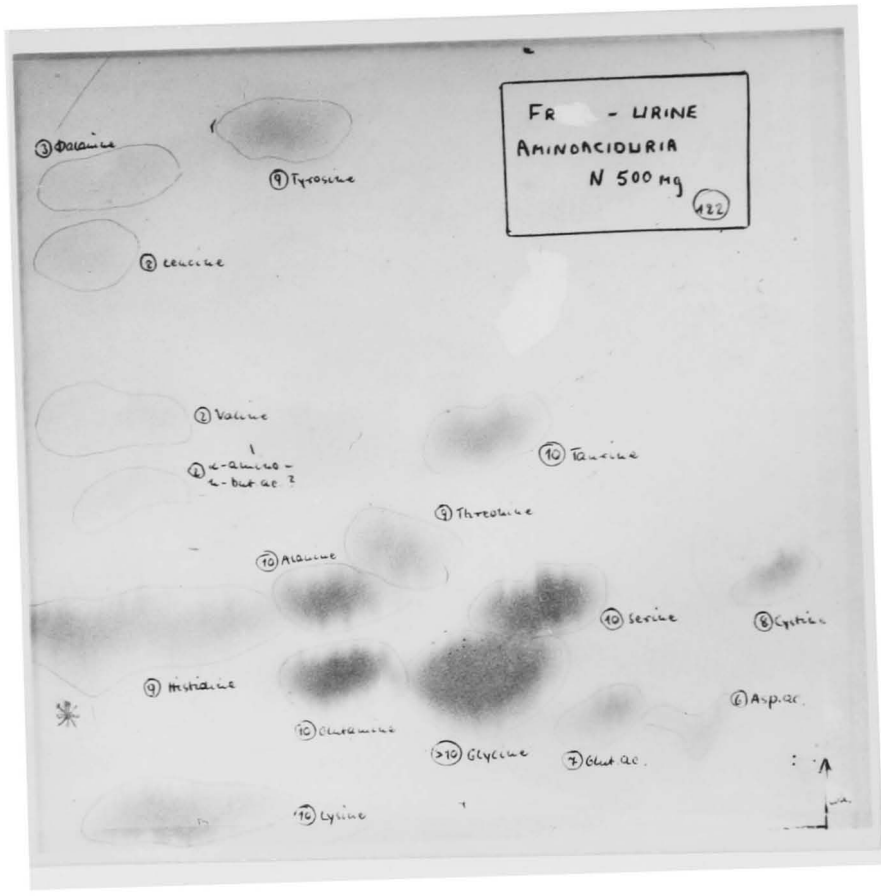


Fig. 19. Urine chromatogram of a patient with steatorrhoea (Case 1). Note the increase of cystine, serine, taurine, threonine, lysine, histidine + methyl-histidine (*) and tyrosine.
Total colour sum 122.

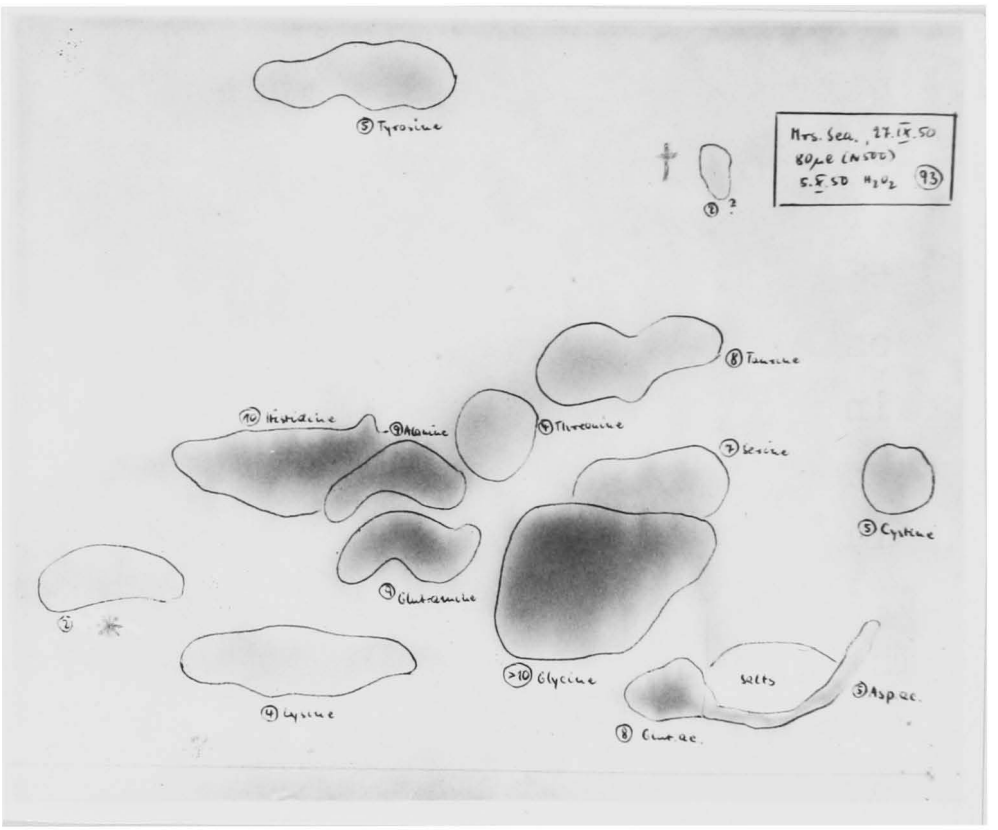


Fig. 20. Urine chromatogram of another patient with steatorrhoea (Case 2). Note the similarity of the aminoacid pattern to Case 1.
*? fast-arginine (Dent 1948, Dent and Rose 1951). † unidentified substance.
Total colour sum 93.

except for the increase of histidine and lysine, It is possible that histidine in some cases has overlapped with β-amino-isobutyric acid and vice versa and that in such cases the weaker component has escaped detection.

Plasma chromatograms were carried out in Cases 1, 2 and 13. The total colour sums of the chromatograms were 68, 76 and 74 and were thus on the upper limit of the normal range (28-78) but not definitely raised. The semiquantitative nature of such estimations has been stressed before. The plasma aminoacid pattern was similar to the urine pattern of these patients, especially the relatively strong spots of cystine, serine, tyrosine, threonine and lysine, which indicates that the aminoaciduria is pre-renal in origin.

In order to obtain more quantitative values for the aminoacid plasma level, microbiological assay was carried out on fasting plasma specimens of Cases 5, 7 and 8. The results (expressed in mg/100 ml) are shown, below together with Schreier's (1951) normal range and our controls.

	Threonine.	Phenylalanine	Valine	Arginine	Tryptophane.
Schreier's normal range	1.6-2.2	1.2-1.8	2.5-3.0	2.0-2.5	1.2-1.5
Controls of the same assay	1.5-2.3	1.7	2.6-3.0	1.3-2.0	1.0
Case 5	2.8	2.1	2.4		1.0
Case 7	2.2	1.7	2.4	1.5	1.0
Case 8	2.6		3.5	2.0	

Case 5 showed a slight rise in the plasma level of threonine and phenylalanine, and Case 8 of threonine and valine, whereas the plasma levels in Case 7 were normal. A chromatogram of a fasting urine specimen, collected from the patients at the same time, showed no aminoacid

increase in Case 7; there was a slight increase of various aminoacids including threonine in Case 5, and of threonine and valine in Case 8, Phenylalanine, arginine and tryptophane were not visible in the chromatograms.

Discussion.

The aminoaciduria in steatorrhoea and coeliac disease is usually mild and this is probably the reason why it seems to have been overlooked up to the present time. In only 23 per cent of the adult patients and 14 per cent of the children was there any increase of the total ninhydrin intensity of the chromatograms above the normal range, and only 5 per cent of all the patients studied showed a considerable aminoaciduria. In the other 32 per cent with aminoaciduria the amino-acid increase was easily overlooked and sometimes only detected after several urine specimens had been tested. The pattern of the aminoaciduria resembles in many respects that in liver diseases. A comparison follows of the relative frequency with which certain amino-acids occur in the urine of these patients.

	steatorrhoea	liver disease	normals
histidine	100%	56%	54%
cystine	100%	88%	24%
serine	93%	68%	17%
tyrosine	87%	36%	11%
threonine	60%	20%	-
taurine	53%	68%	33%
lysine	53%	8%	3%
β-amino-isobutyric acid	53%	32%	11%
methyl-histidine	47%	20%	-
valine	47%	24%	11%
methionine sulphone	20%	36%	-
γ-amino-butyric acid	13%	36%	-

that the increase in the frequency of cystine, serine, taurine, β-amino-isobutyric acid, methyl-histidine, valine and methionine (as sulphone) was similar in both diseases. Lysine, threonine and tyrosine were more frequently encountered in steatorrhoea, γ-amino-butyric acid more frequently in liver disease. Ethanolamine was only observed in liver disease, an increase of histidine only in steatorrhoea. This comparison indicates that, despite many common features in the urine chromatogram of both diseases, some differences do exist. A larger series of cases of both conditions is necessary to decide whether these differences are significant enough to distinguish the two patterns.

The question whether the aminoaciduria is renal or pre-renal in origin may be answered by estimating the plasma level of various aminoacids. Unfortunately I have not yet had the opportunity of testing the plasma levels of patients with really strong aminoaciduria, such as Cases 1, 2, 3 and 13, by microbiological assay. The few aminoacid assays in Cases 5 and 8 showing normal or slightly raised values do not provide sufficient evidence for the assumption that there is an overflow from high blood levels into the urine.

It is unlikely that the aminoaciduria in steatorrhoea is due to any extensive liver destruction. Clinical and biochemical evidence of severe liver damage, such as in liver cirrhosis and infectious hepatitis, was not encountered in our cases. Nor is the high protein diet which is usually given to patients with steatorrhoea likely to be a major factor. Cases 1, 13, 14 and 15 were on a normal diet when the aminoaciduria was detected. In cases 13 and 14, after a high protein diet had been given and the children had improved, the aminoaciduria disappeared.

I am more inclined to connect the aminoaciduria with the fundamental disturbance of the fat metabolism in this disease. The interrelationship between the fat and protein metabolism in steatorrhoea has recently been demonstrated in the intestinal absorption of these substances. In patients with idiopathic steatorrhoea Bassett et al. (1939) found an increased faecal nitrogen up to 4.4g. per day, which tended to vary with the quantity of lipids in the stools. Animal experiments provide another interesting insight into the metabolic connection between fat and protein. Pearson and Panzer (1949) examined by microbiological assay the excretion of phenylalanine, valine, lysine and methionine in the stool and urine of rats fed on a normal and a fat-free diet. The aminoacid loss in the stool of the rats receiving the fat-free diet was significantly higher than in that of the rats fed on the normal diet, and the same was true of the excretion of valine and methionine in the urine. This animal experiment suggests that a sufficient intake and absorption of fat is essential to the proper utilisation of aminoacids.

The aminoaciduria in steatorrhoea may be explained on similar lines. The poor fat absorption and the resulting deprivation of the fuel value of fat may lead to a disturbance in the building up of proteins, and an increased output of aminoacids in the urine may be the result. Desamination may also be affected and conditions may become comparable with those in liver disease. The fact that not all the protein building aminoacids are affected to the same extent, resulting in an aminoacid pattern resembling that in liver disease, suggests certain similarities of the disturbance in both conditions.

IX. AMINOACIDURIA IN GALACTOSAEMIA.

General considerations.

Galactosaemia is an inborn error of metabolism characterised by the inability of the body to utilise ingested galactose. This leads to feeding difficulties and malnutrition even in the neonatal period, to hepatomegaly, galactosuria, mental deficiency and not infrequently to nuclear cataracts.

Galactosaemia is regarded as a rare metabolic anomaly and so far only 21 cases have been reported. With the introduction into clinical medicine of more sensitive and specific sugar tests, such as sugar chromatography, the diagnosis will probably be made more frequently. The disease was first described in 1908 by Reuss in a child showing malnutrition, hepatomegaly and mellituria, which disappeared with the elimination of milk from the diet. At autopsy the liver was found to be cirrhotic. Bruck and Rapoport (1945) first observed in their 7 weeks-old patient nuclear cataracts as a salient feature of the condition. Since then cataracts have been observed in eight of the twenty-one cases.

Until recently little has been known about the nature of the liver lesion. Von Reuss reported cirrhosis of the liver in his patient, and Bell et al. (1950) found a fatty liver with early fibrotic changes in a biopsy specimen of a proved case, and an enlarged liver with severe, diffuse fatty infiltration in the sibling, who was also a suspected though not proved case of galactosaemia. Donnell and Lann (1951) observed at the autopsy of one of their patients a fatty cirrhotic liver and Townsend, Mason and Strong

(1951) reported true Laennec's cirrhosis in their Cases 1 and 6. They concluded that the fatty infiltration found by Bell might well be an earlier stage of the degeneration of the liver which may proceed to a cirrhotic state under the toxic influence of unusually high levels of galactose.

Mental retardation is another important feature of the disease. Townsend, Mason and Strong have suggested that it is due to the direct toxic effect of the high galactose blood level upon the brain similar to that upon the liver. They found the severest retardation in the patient who had ingested galactose over the longest period of time. As evidence for their hypothesis they quoted the animal experiments of Dam (1944) who produced convulsions in chicks by giving them galactose in excess. Another view is that hypoglucosaemia, which accompanies galactosaemia, is the damaging factor in this disease (Mason and Turner, 1935).

The early diagnosis of galactosaemia is of great clinical importance, as it is possible to effect a considerable improvement by omitting galactose from the diet. This therapy may save the child's life even in advanced liver dysfunction, as it has been repeatedly observed that after a galactose-free period the enlargement of the liver disappears and the liver function tests become normal. The prognosis for the mental recovery, however, seems to be less favourable if the disease has persisted over any length of time.

By the kindness of Professor Watkins, Cardiff, Dr. Thursby-Pelham, Stoke-on-Trent, and Dr. Black, London, I have seen five patients suffering from

galactosaemia and in four of them I have studied the urine by paper chromatography. Urine of another case was sent to me by Dr. Snyder, New Orleans.

Results of chromatographic studies.

Of the five patients whose urine was studied, three were still at the height of their disease and subjected to no dietetic measures. Case 1, aged twelve months, showed the fully developed clinical picture with mental retardation, enlarged liver and cataracts. Case 2, six weeks old, was a premature baby, did not thrive, vomited and had an enlarged liver. Her urine showed the presence of albumin and a reducing substance which was identified by paper chromatography as galactose (see later). The infant succumbed to her illness, but the autopsy findings have not yet been received. Case 3 had suffered from jaundice, hepatomegaly and bilateral cataracts since birth. Since the collection of the urine specimens, the child has been put on a galactose-free diet and is slowly improving. Case 4 had already been on a galactose-free diet for about four weeks and had considerably improved when, at two months of age, urine was collected for chromatography. Before the diet, the infant had an enlarged liver, albuminuria and did not thrive; galactosuria had been demonstrated by chemical methods. Case 5 was an infant of two months of age whose brother had died of the disease and in whom the diagnosis had been established soon after birth by the chemical identification of galactose in the urine and by galactose tolerance tests. A galactose-free diet was introduced and her condition soon improved. The urine for chromatography was collected after she had been treated for some weeks.

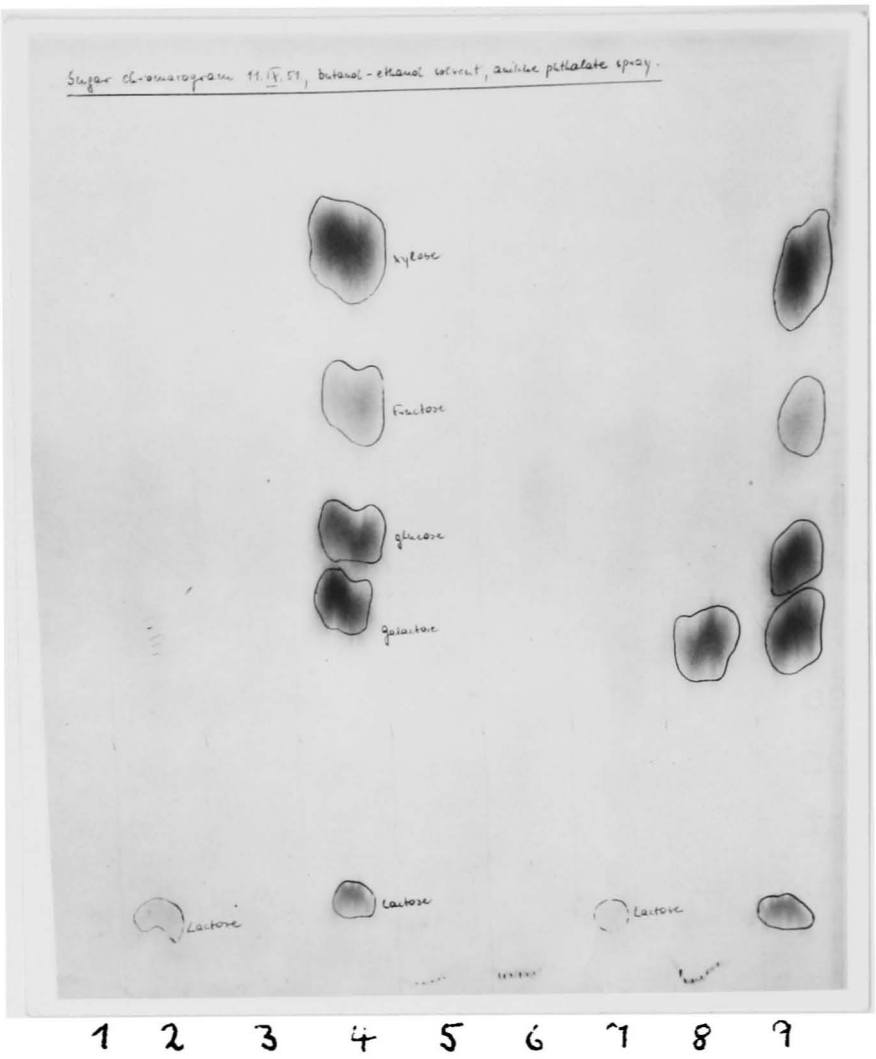


Fig. 21. One-dimensional sugar chromatogram showing galactosuria (column 8) in a patient with galactosaemia (Case 1). Column 1, normal urine; columns 4 and 9 pure lactose, galactose, glucose, fructose and xylose; columns 2, 5 and 7 urine of normal child under additional lactose ingestion.

The nature of the reducing substance in the urine of Cases 1, 2 and 3 was established by sugar chromatography. Subsequently, aminoacid chromatography was used in order to demonstrate the aminoacid pattern in the urine of galactosaemia.

(a) Sugar chromatography.

The technique employed was that described by Partridge and Westall (1948) and by Horrocks and Manning (1949). The chromatograms were one-dimensional butanol-ethanol-ammonia runs, the developer being aniline phthalate. The urine volume pipetted at the base of each column was chosen according to the colour reaction of the Benedict test, namely 100µl. in the case of a blue reaction, 50µl. in case of a green and 25µl. or less in case of a green-yellow reaction. Pure lactose, galactose, glucose, fructose and xylose were run simultaneously in two or three columns on every chromatogram and the position of these test sugars assisted the diagnosis of the unknown sugar. Finally 100-200µl. of normal urine was run in another column of the chromatogram.

Normal urine does not give any colour reaction with aniline phthalate, even when volumes of 200µl. are used. This point has been established by a chromatographic study of the urine of 100 school children and 30 infants from the age of one month onward. Fig. 21 shows the sugar chromatogram of the urine of Case 1 (column 8). The urine contains galactose, which takes up the same position as the pure galactose in columns 4 and 9. Column 1 shows a normal urine which gives no colour reaction at all. Columns 2, 5 and 7 show the urine of a healthy child under additional lactose ingestion; only traces of lactose but no galactose are excreted.

Table 13. CHROMATOGRAPHIC FINDINGS IN THE URINE OF PATIENTS WITH GALACTOSAEMIA, EXPRESSED IN TAURINE UNITS, 1 T FOR THE WEAKEST, ≫60 T FOR THE STRONGEST COLOUR.

	Cases				Normal range.
	1	2	3	4	
Glycine	≫ 60T	> 60T	≫ 60T	80T	0- >60T
Serine	60T	20T	40T	15T	0- 5 T
Glutamine	40T	20T		20T	0- 20T
Alanine	10T	20T	15T	30T	0- 15T
Lysine	3T	20T	20T	30T	0- 15T
Threonine	20T	5T	5T	5T	-
Cystine	2T	10T	3T	15T	0- 15T
Valine	5T	20T		5T	0- 5T
Histidine	5T	5T		10T	0- 25T
Glutamic acid	3T	8T		5T	0- 20T
Tyrosine	3T	5T	3T	3T	0- 2T
The leucines	5T	3T		5T	-
Taurine	2T		10T		0-10T
Methionine sulphone			5T		-
Proline			yellow +		-
Hydroxy-proline		yellow +			-
β-amino-iso-butyric acid			3T		0-20T
Phenylalanine		3T			-
Argihine	2T				-
Total	290T	229T	224T	223T	0-80T

Note. For the total sum >60T is calculated as 90T, ≫60T as 120T

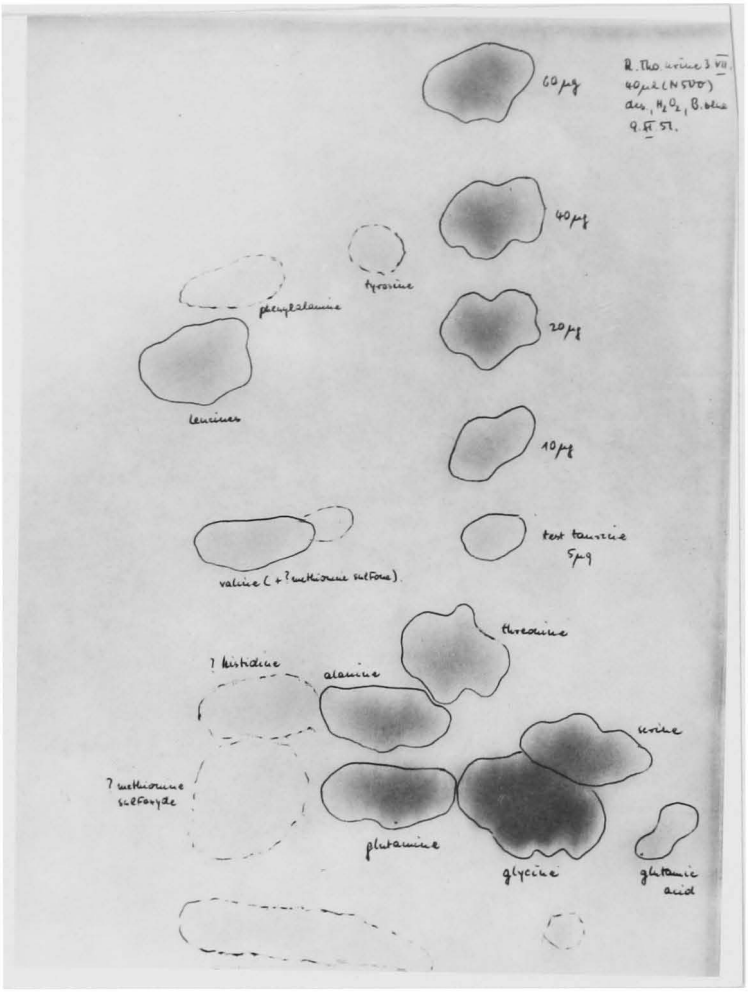


Fig. 22. Aminoacid chromatogram of urine from a patient with galactosaemia (Case 1). Note the increase of serine, threonine, the leucines and valine.

Sugar chromatograms of the urine of Cases 2 and 3 gave similar results whereas the urine of Cases 4 and 5, who were on a galactose-free diet, did not show any sugar spots. The high sensitivity and specificity of sugar chromatography renders it superior to the Benedict test; in Cases 1, 2 and 3 galactose was demonstrated in the urine even when Benedict's reduction test gave an indefinite blue-green or green colour reaction. Thus, chromatography will reveal a galactosuria which might escape diagnosis if the Benedict test only is used.

(b) Aminoacid chromatography.

In four of the five patients (Cases 1, 2, 3 and 4) the urine showed a definite aminoaciduria. The findings in representative chromatograms are summarised in Table 13. The total colour intensity of these chromatograms ranged from 223 to 290 taurine units (normal 0-80 taurine units). The aminoacid pattern of the urines was quite constant in each individual case, but differed somewhat from one case to another. For instance, proline and methionine sulphone were seen only in Case 3, hydroxy-proline and an increase of valine only in Case 2. The leucines were encountered in the urine of Cases 1, 2 and 4. On the other hand, some pathological findings were common to all four cases, especially the increase of serine, threonine, lysine and tyrosine, while the excretion of glycine and alanine was also on the upper limit of normal or moderately raised in all four cases. The urine chromatogram of Case 1 is reproduced in Fig. 22, hich shows some of the changes reported above. The increase of serine, threonine, valine and the leucines, and also of glycine, alanine and glutamine is apparent. The increase of tyrosine and lysine is only indicated in this chromatogram.

Discussion.

When my chromatographic findings of aminoaciduria in Cases 1 and 3 were included as an addendum to the publication of Bray, Jacobs and Watkins in December 1951 this condition had not yet been described in galactosaemia. A month later, however, Holzel, Komrower and Wilson (1952) published a short report of two infants suffering from galactosaemia and also exhibiting aminoaciduria, which was discovered by paper chromatography. There was a gross aminoaciduria in both patients with disproportionately large quantities of serine, threonine, methylhistidine, lysine and tyrosine. One interesting point observed by these authors was that the aminoaciduria disappeared on a milk-free diet, but reappeared when galactose was added to the food. Dent commented on the aminoaciduria in these two infants: "It could be that this pattern is specific for the disease".

My observations agree in many but not in all respects with those of Holzel, Komrower and Wilson. A gross aminoaciduria was present in all patients who received normal galactose-containing food (Cases 1, 2, 3), but also in one infant who was fed on a galactose-free diet and had ceased to excrete galactose in the urine (Case 4). Another infant also receiving a galactose-free diet exhibited no aminoaciduria (Case 5). Thus, whereas the absence of an aminoaciduria in Case 5 on a galactose-free diet confirms Holzel's observations, the persistence of the aminoaciduria in Case 4 does not. An explanation of this contradictory behaviour may be the presence of a more advanced and not easily reversible lesion of the liver or kidneys causing the aminoaciduria.

As regards the aminoacid pattern of the aminoaciduria, large quantities of serine and threonine as described by Holzel were also found in all our cases; an increase of tyrosine was also observed, though this was only moderate. The lysine excretion was abnormal in only three cases and methyl-histidine was not observed at all. The presence of other rare aminoacids in one or two of our cases has been mentioned above. In view of this and of the small number of cases investigated it would be premature to define a specific aminoacid pattern for the urine of patients with galactosaemia.

So far no studies of the aminoacid blood level in this disease have been reported nor did I have the opportunity to carry out such investigations. Considerations as to the origin and mechanism of the aminoaciduria are, therefore, still purely speculative. It is possible that the liver damage in galactosaemic patients is the cause of the aminoaciduria. However, the pattern of the aminoaciduria observed so far in such patients does not bear much resemblance to that in liver disease. The absence of an increase of cystine and taurine and the frequent and strong excretion of threonine and lysine are the main points of difference. Furthermore, the albuminuria in galactosaemia points to a kidney lesion in this disease. Possibly the excess of galactose damages not only the liver and brain, but also the renal tubules, and thus interferes with the proper reabsorption of the aminoacids.

X. AMINOACIDURIA IN HEPATOLENTICULAR DEGENERATION.

General considerations.

Hepatolenticular degeneration is a familial disease, often affecting several siblings in one generation without having been found in previous generations. It was first described by Kinnier Wilson in 1912 and has since aroused much interest because of the unusual association between cirrhosis of the liver and specific cerebral degeneration most conspicuous in the basal ganglia and, in particular, the putamen.

A salient feature of the disease is the characteristic, dark brown pigmentation of the cornea (Kayser-Fleischer ring) which has been observed in all but a few cases where death rapidly ensued. The detection of this ring provides definite proof of the disease, as it has not been found in any other condition.

André (1946) in a review of the literature quoted 145 cases of hepatolenticular degeneration verified by the presence of the Kayser-Fleischer ring or by proven disease of the siblings. The youngest patient was four years old, although the disease usually starts in adolescence or later. Cirrhosis of the liver nearly always develops before the nervous symptoms and the patient may die of the consequences of a progressive Laennec cirrhosis before neurological symptoms are apparent. Occasionally the reverse is true, as in one of the patients of Uzman and Denny-Brown (1948), who showed definite neurological symptoms without cirrhosis of the liver, which was

excluded by punch biopsy.

Clinical evidence of the liver lesion is often minimal up to the late stages of the disease. There may be recurrent episodes of digestive disorder, perhaps with mild jaundice. A hard enlarged liver may be palpable but often the liver cannot be felt. The spleen is frequently enlarged and may suggest the diagnosis of Banti's disease. Biochemical tests of the liver function are of little diagnostic value as they usually only become positive when the liver damage is already obvious by clinical examination. The most sensitive tests seem to be the serum colloidal gold reaction, the cephalin flocculation test and the demonstration of urobilin and urobilinogen in the urine.

Clinically the liver lesion is less striking than the nervous symptoms. These may begin with unsteadiness on the legs, clumsy movements, indistinct speech and deterioration of the handwriting. Anti-social behaviour may be another early feature; one of our patients began to steal at school. The patients become emotionally unbalanced, and pass from an euphoric friendly mood to uncontrolled fits of weeping or bad temper. Action tremor, especially of the upper limbs and tongue, an empty, fatuous smile, increased salivation, adiadochokinesis, extrapyramidal rigidity and finally severe immobilisation and distortion of posture are further features. Progressive mental incapacity may develop , and the outcome of the disease is always fatal, its duration varying from a few months in young children (Howard and Royce, 1919) to over 40 years in the chronic pseudo-sclerotic type of Westphal (1883) and Strumpell (1898), which was shown by Hall (1921) to be identical with the disease described by Kinnier Wilson.

The cause of the disease is still obscure, though

within the last few years interesting new facts have been discovered which may throw light on its pathogenesis. In the search for some toxic agent which might damage both liver and brain, two biochemical abnormalities have been discovered, which so far, reveal no clear connection with each other.

(a) Excess of copper in tissues and urine.

Haurowitz (1930), Glazebrook (1945), Cumings (1948) and Spillane, Keyser and Parker (1952) estimated the copper content of liver and brain in hepatolenticular degeneration and found an up to tenfold increase of this substance as compared with normal liver and brain. Mandelbrote, Stainier, Thompson and Thruston (1948), Porter (1949), Cumings (1951), Denny-Brown and Porter (1951), and Spillane, Keyser and Parker (1952) have reported increased urinary excretion of copper in patients with hepatolenticular degeneration. All these workers found a further increase of the copper excretion after BAL-injections, which were given to eliminate the excessive copper from the liver and brain. A simultaneous clinical improvement was evident in most patients, though it is still too early to assess the therapeutic possibilities of BAL over a longer period. Although the urinary copper excretion could be raised some 2-7 times by BAL, Denny-Brown and Porter (1951) estimated that only about 5% of the copper in the liver in hepatolenticular degeneration was removed during one BAL-course. There seems to be no explanation of the origin of the copper excess in the body nor is it clear why no increased morbidity from hepatolenticular degeneration is found among industrial workers dealing with copper dust. It is also remarkable that in various patients with hepatolenticular degeneration the copper blood level was normal despite

increased copper excretion in the urine (Cumings 1951, Spillane et al. (1952).

In two of our patients, an eleven year old boy and a fifteen year old girl, Dr. E. M. Hickmans found a raised copper excretion in the urine, namely 0.5-1.0 mg. per day in Case 1, and 0.4-0.6 mg. per day in Case 2 (normal up to 0.15 mg. per day). Both patients are now under treatment with BAL, which has led to a daily copper excretion of up to 1.8 mg. in both cases. It is interesting to note that a younger sister of Case 1 shows as yet very indefinite neurological symptoms of hepatolenticular degeneration, but already has a raised urinary copper excretion of 0.29 and 0.36 mg. daily. The parents' copper excretion is normal. In this case there is no obvious source of the copper excess, but the source of the copper in Case 2 probably lies in the father's occupation. He works in a factory producing electrical contacts and often brings copper dust home in the turn-ups of his trousers, etc.

(b) Aminoaciduria.

Uzman and Denny-Brown (1948) and Eckhardt, Cooper, Faloon and Davidson (1948) were the first to describe aminoaciduria in hepatolenticular degeneration, using chemical, chromatographic and microbiological methods. Their results will be discussed in more detail later. Porter (1949), Cooper, Eckhardt, Faloon and Davidson (1950), Dent (1950), Cumings (1951) and Spillane, Keyser and Parker (1952) confirmed this finding, though Case 3 of Cumings was an exception in that he showed a normal aminoacid excretion in the urine.

Thanks to the kind cooperation of Dr. Davison, Newcastle-upon-Tyne, and Dr. Gilbert Hall, Birmingham, I was able to study in detail the clinical picture of two patients with hepatolenticular degeneration (Cases 1 and 2) and to investigate their aminoacid content

or urine and blood by chromatographic and microbiological means. Of three other patients of Professor Cloake, Birmingham, Dr. Davison, Newcastle-upon-Tyne, and Professor Berg, Hamburg, I received numerous urine specimens for chromatographic investigation.

Results of chromatographic and microbiological studies.

Urine and plasma of five patients with hepatolenticular degeneration was examined. In all of them the diagnosis was proved by the presence of typical Keyser-Fleischer rings in the cornea. Cases 1, 2 and 3 were children, Cases 4 and 5 adults. Case 1, now eleven years old, has suffered from the age of five from slowly progressing symptoms of liver cirrhosis, and two years ago neurological symptoms appeared. At the present time signs of liver damage are slight, namely, oedema of the lower extremities, a slight direct positive van den Berg, raised phosphatase and occasional urobilinogenuria. The liver is no longer palpable but was enlarged two years ago. The spleen is two fingers below the costal margin. The neurological and mental changes are typical and advanced, but the boy is still able to walk about. His speech is nearly incomprehensible. In Case 2 the mental and neurological changes dominated the clinical picture and there were no obvious signs of liver disease, except a brief transient attack of jaundice at the age of eight. Four years ago, at the age of eleven, the patient developed attacks of drowsiness, and soon other typical central symptoms appeared. At the present time there are pronounced extrapyramidal changes, marked rigidity of the limbs, adiadochokinesis, almost incomprehensible speech, tremor of tongue and upper limbs and uncoordinated movements. The mental affection is similar to that in Case 1, but more advanced. The liver is not palpable but the spleen is one fingerbreadth

Table 14.

CHROMATOGRAPHIC FINDINGS IN THE URINE OF PATIENTS WITH HEPATO-LENTICULAR DEGENERATION. COLOUR INTENSITY EXPRESSED IN TAURINE UNITS, 1T FOR THE WEAKEST, ≫60T FOR THE STRONGEST COLOUR.

	Children			Adults		Normal range.
	Case 1	Case 2	Case 3	Case 4	Case 5	
Glycine	> 60T	80T	80T	> 60T	≫ 60T	40T	0-≯60T
Histidine	> 60T	20T	40T	15T	> 60T	5T	0- 25T
Glutamine	> 60T	40T	60T	20T	40T	10T	0- 20T
Serine	> 60T	40T	30T	30T	30T	5T	0- 5T
Alanine	> 60T	20T	30T	10T	40T	10T	0- 15T
Threonine	> 60T	10T	20T	15T	10T		-
Lysine	60T	2T			20T		0- 15T
Cystine	10T	7T			40T	2T	0- 15T
Tyrosine	20T	8T	10T	10T	10T		0- < 5T
γ-amino-butyric acid		10T	20T	5T	30T		-
Glutamic acid				5T	20T	3T	0- 20T
Phenylalanine	5T	5T	5T	10T	5T		-
Methyl-histidine	20T						0- 3T
Valine	5T	2T			10T		0- 5T
The leucines	5T	1T			10T		-
Methionine as sulphone			10T				-
Tryptophane	< 5T						-
Taurine	< 5T						0- 20T
Total	670T	245T	305T	210T	475T	75T	0- 80T

Note. For the total sum > 60T is calculated as 90T, ≫60T as 120T

under the costal margin. Liver function tests are normal but for a raised serum alkaline phosphatase and prothrombin level. Case 3, whose elder sister is a proved case of hepatolenticular degeneration, is still in an early stage of the disease. A year ago he was admitted to hospital because of recent oedema of the legs, ascites and anorexia. The urine was found to contain albumin, R.B.C., W.B.C, and casts. There were scarcely any neurological or mental changes present, except that the boy tended to stumble when running, had a coarse tremor of the tongue, was emotionally rather labile and his right plantar response was extensor. No further deterioration has occurred since then. His liver and spleen have always been just palpable and the ascites has disappeared. The albuminuria has always been present. Case 4, a middle-aged woman, was quite well up to six years ago, when tremor of hands and head developed. Four years ago, without clinical signs of liver disease, she was noticed to have occasional urobilinogenuria and a thymol turbidity of 4 units. Other liver function tests were normal. The liver was not enlarged, but the spleen was palpable two inches below the costal margin. Gross ascites and oedema of the legs developed, the tremor increased and the patient became unable to walk, though there were few other neurological or mental changes. The patient died some months after the urine collections were made. Of Case 5 the only information available is that she is a woman with proved hepatolenticular degeneration, exhibiting a Kayser-Fleischer ring.

(a) Chromatographic findings.

The results in the urine of these patients are summarised in Table 14. All patients but No.4 had a

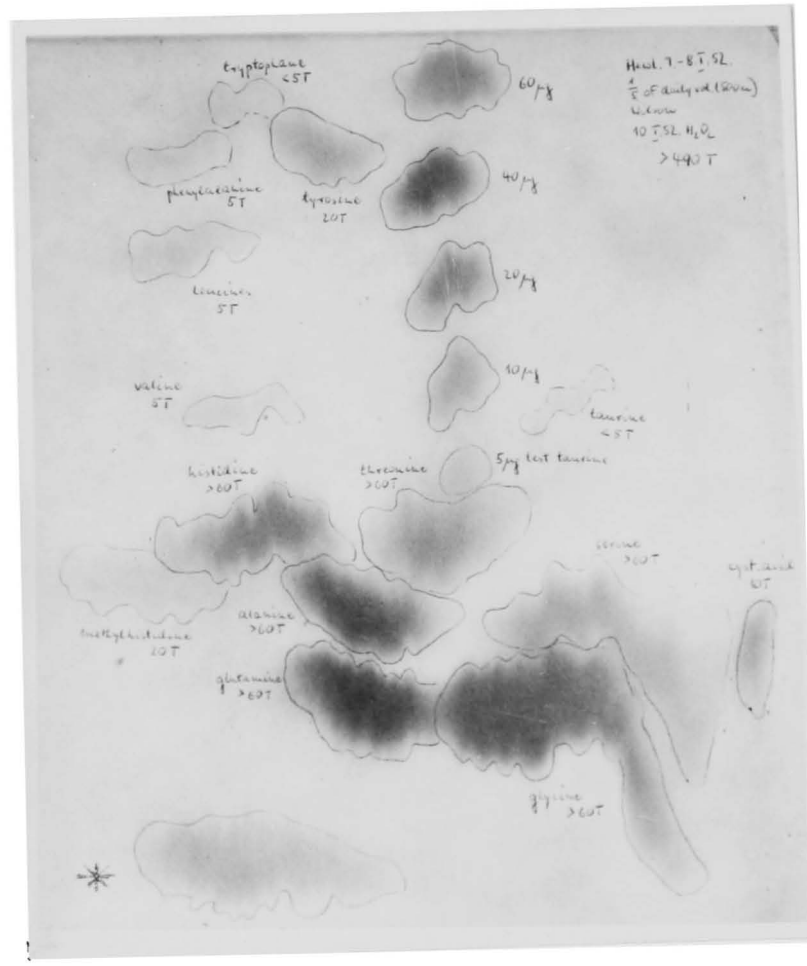


Fig. 23. Urine chromatogram of a patient with hepatolenticular degeneration (Case 1). Note the increase of serine, threonine, lysine (*), histidine, methyl-histidine, tyrosine and, to a lesser degree, of cystine, valine, the leucines, phenylalanine and tyrosine.
Total colour sum >490T
> [illegible] calculated as 90T, >60T as 120T.

pathological aminoaciduria, which was strongest in Cases 1 and 4. The normal aminoacid excretion in the urine of Case 5 has been confirmed in repeated specimens during the last year.

There was a close resemblance of the aminoacid pattern of the urine in all four cases with aminoaciduria; they showed a well-marked increase of serine, threonine, tyrosine and phenylalanine. Histidine, alanine, glutamine, the leucines, and γ-amino-butyric acid were increased in three, lysine in two, cystine, methionine, valine, tryptophane and methyl-histidine in one case. A second chromatogram is given for Case 2, which was typical for a period of about one month, when methionine as sulphone was found in nearly all urine specimens. Later the methionine excretion ceased and the chromatograms once more resembled the first example shown of this case. There was no obvious clinical change during this period. A chromatogram with urine of Case 1 is reproduced in Fig.23.

Plasma chromatograms in Cases 2 and 5 showed a normal aminoacid pattern and normal total colour intensity. No plasma chromatogram could be prepared in Case 2 during the period when methionine was excreted in the urine. The normal plasma chromatogram of Case 2, who at the same time showed a strong aminoaciduria, indicates a renal mechanism for the aminoaciduria.

(b) Microbiological assay.

To obtain further evidence the plasma level of two aminoacids was determined on three different days by microbiological assay. The results, expressed as mg. per 100 ml. were:

		tyrosine	threonine
Normal range (Schreier)		1.0-1.6	1.8-2.2
Normal control of the same assay		1.0	2.1
Case 1	specimen 1	0.9	2.0
	specimen 2	1.0	2.3
	specimen 3	1.0	2.0
Case 2	specimen 1	1.4	1.8
	specimen 2	1.6	2.3
	specimen 3	1.6	2.1

Thus microbiological assay gave a normal plasma level for tyrosine and threonine, though in urine chromatograms of the same days these aminoacids were shown to be greatly increased.

Further evidence of the normal aminoacid plasma level was provided by the kind co-operation of Professor H. A. Krebs, Sheffield, who estimated the glutamine + glutamic acid content of a plasma and 24 hour-urine specimen of Case 2 by the glutaminase method (Krebs 1948). The result in the plasma was 8.5 mg. in 100 ml. (normal 7-10 mg. in 100 ml.) and in the urine 335 mg. per day (normal 23-106 mg. per day). The figures confirm the chromatographic and microbiological findings of a normal aminoacid plasma level despite aminoaciduria.

Discussion.

Paper chromatography revealed a marked aminoaciduria in four of our five patients. The total colour sum of the chromatograms was twice to eight times as high as normal. In Case 2 the increase was about threefold and this agreed well with Dr. Hickmans' finding of an amino-nitrogen coefficient of 5% (normal 1-2%) and a total amino-nitrogen excretion of between 600-800 mg. per day (normal 100-200 mg), and with Professor Krebs'

finding of a threefold increase of the glutamine + glutamic acid output. The amino-nitrogen was estimated by formol titration. It has been stressed by Uzman and Denny-Brown (1948) that the severity of the aminoaciduria does not seem to be related to the extent of the liver damage, and this is borne out by our Case 5, who showed no aminoaciduria although her liver lesion was more advanced than that of Cases 1-3, the state of Case 4 being unknown. In our cases there was some parallelism between the severity of the aminoaciduria and of the neurological symptoms, which were most advanced in Case 1, but only mild in Cases 3 and 5, who showed little or no aminoaciduria. The number of cases is, however, much too small to draw any conclusion from this observation.

Uzman and Denny-Brown employed one-dimensional paper chromatography for the study of this aminoaciduria but the urine contains far too many aminoacids to be separated clearly by this method. Their conclusion that none of the aminoacids was present in excess over the others has been disproved by Cooper et al. (1950) who employed microbiological assay on the urine of patients with hepatolenticular degeneration. These workers assayed ten essential aminoacids and found a variation of the excretion from twice the normal amount of isoleucine to more than 12 times the normal amount of threonine. Leucine was increased 10 times, valine 9 times, phenylalanine 8 times, lysine 5 times, methionine, arginine and tryptophane 4 times and histidine 2½ times above normal.

Two-dimensional chromatography, despite the fact that it is only semiquantitative and subject to the variable ninhydrin sensitivity of different aminoacids, reveals an excessive excretion of certain other aminoacids, namely serine, tyrosine, alanine, glutamine, γ-amino-butyric acid, cystine and methyl-histidine. Dent and Harris (1951) also recorded small amounts of citrulline and aspartic acid

in the urine chromatogram of their patient.

A larger number of cases must be investigated before deciding whether the aminoacid pattern in the urine is sufficiently specific to be of great diagnostic value or to allow of far-reaching pathogenetic conclusions. The completely normal pattern in Case 5 and the temporary strong methionine excretion in Case 2 demonstrate its variability. On the other hand Cases 1-4 show a close resemblance in their urinary aminoacid pattern, which in turn resembles that of the case reported by Dent and Harris. The increase of serine, threonine, tyrosine and phenylalanine was striking in each of the many urine chromatograms completed in Cases 1-4 and is possibly characteristic of this form of aminoaciduria. An increase of methionine, methyl-histidine, and γ-amino-butyric acid has been seen less constantly and may depend upon the state of the patient's liver function.

The pattern of the aminoaciduria is distinctly different from that of cystine-lysinuria, phenylketonuria and Lignac-Fanconi disease, where relatively stronger concentrations of valine, the leucines and proline are observed, while histidine is not increased. It differs from the urine pattern in liver disease because of the inconsistent increase of cystine and the normal taurine concentration. The pattern is more like that in galactosaemia and steatorrhoea, though in galactosaemia the tyrosine and threonine increase was less striking and there was no histidine increase. In steatorrhoea cystine, taurine and methyl-histidine are found in higher concentration than in hepatolenticular degeneration.

In order to learn more about the mechanism of this aminoaciduria the plasma amino-nitrogen has been estimated by various authors. Uzman and Denny-Brown (1948) as well as Cooper et al. (1950) and Cumings (1951)

found in their patients a normal or slightly raised level. Uzman argued that a normal aminoacid plasma level did not necessarily exclude a pre-renal origin of the aminoaciduria, as even healthy adults with a normal blood amino-nitrogen level excrete as much as 100-200 mg. alpha-amino-nitrogen daily in their urine so that an increase of the aminoacid output might well take place without necessarily being reflected in the aminoacid blood level. This explanation served Uzman and Denny-Brown to justify their hypothesis that there was a prerenal upset of the aminoacid metabolism in this disease. Cooper and his colleagues, however, found no demonstrable defect of the intermediary amino-acid metabolism but renal glycosuria in three of their patients, and they concluded a lowered renal threshold to be the explanation of the aminoaciduria. Cumings (1951) and Dent and Harris (1951) were in agreement with this opinion.

So far no quantitative estimations of the plasma level of individual aminoacids in this disease have been reported. Microbiological assay with plasma of two of our patients showed normal levels for tyrosine and threonine though two of the values were on the upper limit of normal. This finding and the normal pattern of the plasma chromatograms in Case 2 support the hypothesis of a renal mechanism for this aminoaciduria. The constant albuminuria in Case 3 and a moderate hypostenuria in Case 2 revealed in a water concentration test, also point to the existence of an anomaly of renal function in hepatolenticular degeneration.

The absence of an aminoacid increase in the urine of Case 5, who was severely ill, suggests that amino-aciduria is not so essential a feature of this disease as, for instance, of phenylketonuria. The normal aminoacid excretion in Case 5 does not support the hypothesis of Uzman and Denny-Brown that hepatolenticular

degeneration presents a basic error in the metabolism of aminoacids. These workers suggested that enzymatic dysfunction results in disturbed utilisation and consequently excessive loss of aminoacids through the kidney, thus producing an aminoacid deficiency and damage to the brain and liver. Cooper et al. (1950) calculated that an average loss of 400 mg. alpha-amino-nitrogen per day in their patients was equivalent to about 3.2 grams of protein, a small and probably negligible portion of the daily protein intake.

Cumings (1951) and Dent and Harris (1951) reported other instances of patients with hepatolenticular degeneration who had no aminoaciduria. Furthermore, a young sister of our Case 1, with very early neurological signs of the disease and an increased copper excretion in the urine, has not yet exhibited any aminoaciduria. I concluded from these observations that aminoaciduria in hepatolenticular degeneration is not the result of a primary metabolic disturbance but a complication occurring in the course of the disease. It may be due to damage of the renal tubules produced by the same agent that causes the liver and brain lesion, possibly by copper. The pattern of this aminoaciduria may later be influenced to some extent by the progressive liver damage. This may explain the slightly raised alpha-amino-nitrogen plasma levels recorded in the literature.

XI. AMINOACIDURIA IN AN OBSCURE SYNDROME.

In the course of a series of urine investigations by paper chromatography for Dr. Thursby-Pelham, he permitted me to study urine, plasma and, at a later date, the clinical picture and biochemistry of one of his patients, a boy aged four years, five months, who had received medical attention because of his retarded development, and had shown a strong aminoaciduria in a preliminary chromatographic test. This case was of special interest, as the child exhibited various symptoms of two conditions previously discussed, namely Lignac-Fanconi disease and galactosaemia. There were, however, sufficient distinguishing features to differentiate his condition from these disorders, and it appears to present a new syndrome of still obscure aetiology and pathogenesis.

Case record.

Case history. The boy is the second child of an otherwise healthy family. The pregnancy was undisturbed, but the child was delivered by forceps after having been overdue for two weeks. Birthweight 9 lbs. At 4 months old bilateral cataracts were noticed, which reduced the vision of the child almost to the point of blindness. From the second year onward static and mental underdevelopment became apparent and a striking motility was noticed. The joints could be bent "like a real acrobat", the child moved his arms, legs and head continuously in a senseless manner and often grimaced. He has not yet learnt to stand but can feed himself and knows some

nursery rhymes. Appetite poor, often constipated, but no vomiting. At the Royal Infirmary, Stoke-on-Trent, active rickets and osteoporosis were noticed and in the urine some albumin, pus cells and granular casts were found, but no sugar. On a low calcium intake the calcium excretion in the urine was found to be above normal. There was no excess of fat in the faeces. The child was transferred to the Children's Hospital, Birmingham, for further investigation.

Findings on admission. A boy of four years, five months, dwarfed (34½", normal 39"), and underweight (27 lbs., normal for age 35 lbs.,) who refused to stand, was very hypotonic, hyper-flexible, with a lax puffy skin and acrobatic motility. His motility was peculiar, somewhat choreiform, with frequent mannerisms. His vision was so poor that it was difficult to judge of his intelligence, which was later assessed by a psychologist to be below that of a normal child of two. The facial expression was empty but characterised by a considerable enophthalmus due to lack of periocular fat and a coarse nystagmus of both eyes. The abdomen was protuberant due to muscular hypotonia, there was a deviation of the recti and an umbilical hernia, but no enlargement of liver or spleen. There was frontal bossing, a slight rosary and enlarged wrists and knock-knees. The reflexes were weak, due to the muscular hypotony. No pathological reflexes.

X-ray examination showed active rickets with pronounced lack of calcium and the suggestion of a pseudofracture of the left femur. Carpal ossification equivalent to that of a boy of two years. No calcification nor

structural abnormality of kidneys at subcutaneous urography.

Ophthalmological examination showed bilateral capsulo-lenticular cataracts. No cystine crystals at slit-lamp investigation.

Haematology. Normal values for R.B.C., Hb., W.B.C., differential count, prothrombin time, platelets.

Bone marrow: Differential count gave low cellularity with a drift to the left. No cystine crystals.

Blood chemistry: Constant moderate acidosis with a CO_2-combining power of 43-49-44-43 vol. per cent. Hypophosphataemia of 2.4-2.8-2.6-2.1 mg. phosphorus per 100 ml. Increase of the alkaline phosphatase to 40.3-46.1-40.0 with a drop under vitamin D therapy to 18.6 Kay-units. Serum calcium normal apart from one low value of 9.1 mg. per 100 ml. and, after vitamin D, a slight increase to 11.6 mg. per 100 ml. N.P.N., cholesterol, fasting blood sugar, bilirubin, thymol turbidity, sodium, potassium and chloride normal except for one slightly raised sodium chloride value of 639 mg. per 100 ml.

Urinary findings. Normal 24 hr.-volume (300-500-600 ml.). Traces of albumin, Benedict's test and sugar chromatography always negative except in two specimens, where traces of glucose and galactose were detected. No cellular elements in the centrifuged deposit, but there were some tyrosine crystals. Urine pH between 7.2 and 6.6, once 5.7. Bicarbonate excretion very low, as might be expected during acidosis. Ammonia coefficient 4.4 and 10.4 (normal 2-4). Aminoacid coefficient 4.5 (normal 1-2).

Galactose tolerance test. Within four hours after

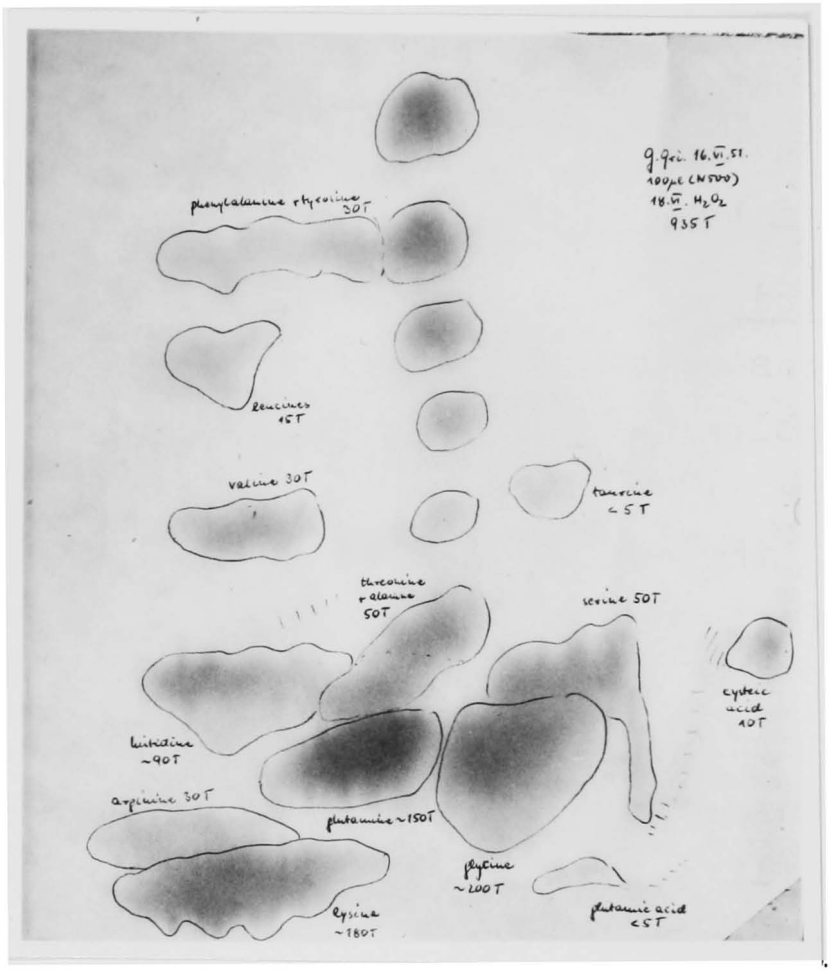


Fig. 24. Urine chromatogram of G.Gr. Generalised aminoaciduria similar to that in Lignac-Fanconi disease but with some differences (strong histidine, no proline, relatively too strong tyrosine spot etc., see text). The five spots in the middle and top of the print are taurine tests.
Total colour intensity 500T.

ingestion of 30g. galactose, only 333 mg. galactose was excreted (normal result).

Calcium-phosphorus balance. The calcium retention was only 13 per cent of the intake (normal 26%, Macy 1942), due to increased calcium loss in the faeces. The phosphorus retention was good. Of the total output a normal percentage of calcium and phosphorus was present in the urine. The balance was possibly influenced by the vitamin D treatment before admission.

Investigations of the aminoacid metabolism. Chromatograms of numerous urine specimens showed a constant and considerable aminoaciduria (Fig.24). Up to 15 amino-acids were excreted in excess, especially lysine, arginine, threonine, tyrosine, phenylalanine, valine and leucine, but also the aminoacids commonly found in urine, such as serine, histidine, alanine, glutamine, glycine, cystine and occasionally taurine. The increase of tyrosine and phenylalanine was often greater than that of valine and the leucines, and cystine was generally excreted in larger amounts than in the chromatogram reproduced. The total colour intensity of the chromatogram was at times ten times that of normal urine.

Professor H. A. Krebs kindly estimated the glutamine + glutamic acid content of the plasma and found a raised level of 13.8 and 14.0 mg. per 100 ml. (normal 7-10 mg. per 100 ml.). The daily urine excretion of these aminoacids was 535 mg. (normal up to 100 mg. per day). Chromatographic investigation of two plasma specimens gave no conclusive evidence of an increased aminoacid concentration, though the level of various aminoacids, especially of cystine, lysine, threonine, tyrosine and phenylalanine was on the upper limit of normal or slightly raised. The

pattern resembled the urine pattern. Microbiological assay was not yet available at the time of the observation.

To summarise the case record the patient exhibited bilateral cataracts, enophthalmus, rickets and dwarfing. He was backward in his mental and static development and showed peculiar motility and mannerisms. Acidaemia, hypophosphataemia and raised alkaline phosphatase characterised the blood chemistry, and in the urine moderate albuminuria and a strong general aminoaciduria were found.

Discussion.

This peculiar syndrome bore some resemblance to Lignac-Fanconi disease in the dwarfing, anorexia and constipation, albuminuria, general aminoaciduria and hypophosphataemic rickets. But this diagnosis was excluded by the absence of cystine storage, glycosuria and hypopotassaemia, and by the atypical aminoacid pattern in the urine with the relatively strong increase of histidine, tyrosine, phenylalanine and threonine. There was also no proline in any of the specimens. Furthermore the mental retardation and cataracts are not characteristic of Lignac-Fanconi disease but are more suggestive of galactosaemia. This disease was ruled out by the normal galactose tolerance test and because galactose tests of the urine were nearly always negative.

The cause of the aminoaciduria, one of the most striking features of this condition, is still unknown. The increased glutamine level of the plasma suggested an overflow from raised plasma levels and thus a metabolic origin. On the other hand, the albuminuria and the finding of pus cells and granular casts at a

previous date pointed to some kidney lesion.

Quite recently Lowe, Terrey and MacLachlan (1952) of Boston have described in three young children a very similar syndrome, which they call "organic-aciduria, decreased renal ammonia production, hydrophthalmus and mental retardation". The photograph of one of their patients showed a striking resemblance to our patient. Clinically Lowe's patients were characterised by hyperactivity, hydrophthalmus in all three, cataracts in two children, nystagmus, hyporeflexia, flabby musculature and frontal bossing. X-ray examination showed decreased bone opacity in all and active rickets in two cases. There was acidaemia and hypophosphataemia, and the urine showed albuminuria, renal glycosuria and a relative insufficiency of the ammonia production. Aminoaciduria was shown by chemical and chromatographic means and the chromatographic pattern closely resembled that in our patient. Lowe calculated, however, that this aminoaciduria was not alone responsible for the greatly increased excess of organic acids in the urine, and that other organic acids were probably increased as well.

It is possible that the syndrome exhibited by our patient is identical with that of Lowe's patients, though glycosuria, hydrophthalmus and deficient ammonia production were not features of our case. The underlying metabolic defect of this condition remains obscure, and further work is required to extend our knowledge.

XII. SUMMARY AND CONCLUSIONS.

Paper partition chromatography has been used extensively to study the aminoacid excretion in the urine of healthy children, of newborn infants and of patients suffering from eight different metabolic disorders. The purpose of this investigation was to discover the aminoacid pattern in the urine and the extent to which it is characteristic of each condition, and to establish the differences between the various aminoacid patterns. In order to gain some insight into the mechanism of the various forms of aminoaciduria a comparison was made between the aminoacid pattern of the plasma and that of the urine. In some cases microbiological assay has been used to establish the plasma level of certain aminoacids more exactly than was possible by paper chromatography.

Highly characteristic aminoacid patterns of the urine were found in cystine-lysinuria and in phenylpyruvic oligophrenia. The plasma levels of the aminoacids involved are normal in cystine-lysinuria and raised in phenylpyruvic oligophrenia. In the latter condition a successful attempt has been made to lower the phenylalanine plasma level by restricting the phenylalanine intake, but the influence of this procedure on the disease cannot yet be assessed.

In Lignac-Fanconi disease, in steatorrhoea and in the newborn period greater variations of the aminoacid patterns in the urine have been observed, but the patterns may still be regarded as characteristic. In Lignac-Fanconi disease and in the newborn period evidence of an increased aminoacid plasma level has

been provided, whereas in steatorrhoea conclusive data are still lacking. The aminoaciduria in Lignac-Fanconi disease is of special interest for two reasons: it is of considerable diagnostic value in a disease where most of the other features show a confusing variability, and its pattern shows a striking resemblance to the aminoacid pattern of the hydrolysed plasma protein and of the protein-free plasma ultrafiltrate (Fig.26) which suggests a generalised disturbance of the protein synthesis or desamination.

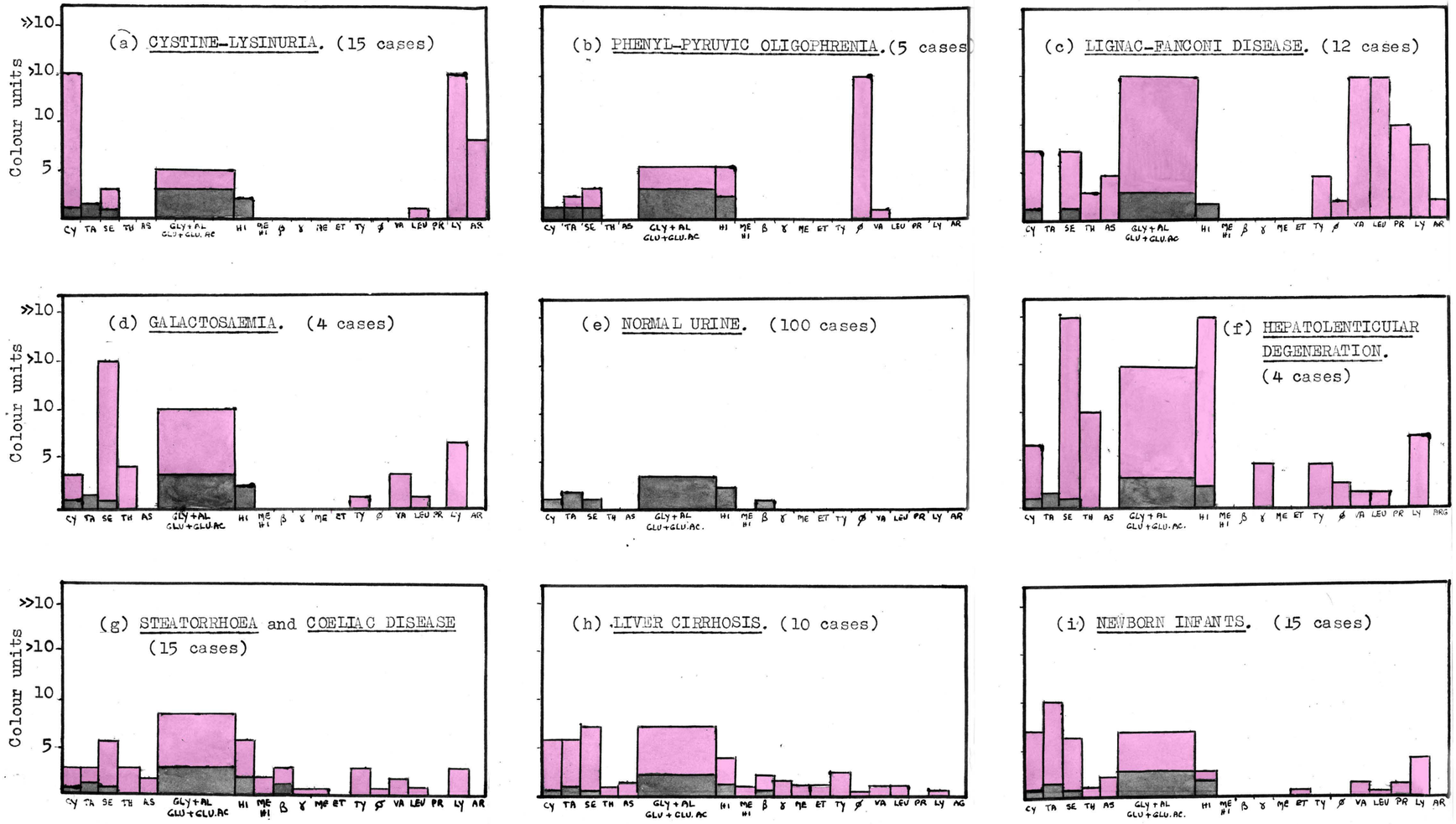
In liver disease various patterns of the amino-aciduria have been observed, which perhaps indicate a variety of lesions of different site and character. The changes are accompanied by similar changes in the plasma aminoacid pattern and are sufficiently characteristic to be of diagnostic value.

Both in galactosaemia and in hepatolenticular degeneration the number of cases studied was too small to permit of generalisation, though it seems possible that both diseases have a specific aminoacid pattern of the urine. In hepatolenticular degeneration normal aminoacid plasma levels suggest a renal origin of the aminoaciduria, which is an inconstant though frequent feature of the disease. In galactosaemia no investigations of the aminoacid plasma level have so far been carried out.

An isolated case of a still obscure syndrome is described with dwarfing, mental retardation, hyperactivity, mannerisms, muscular hypotonia, enophthalmus, nuclear cataracts, hypophosphataemia, rickets, acidaemia, mild albuminuria and a strong general aminoaciduria. It resembles a new disease recently described by Lowe and colleagues (1952).

In figure 25 an attempt is made to represent the

Fig.25. THE AMINOACID PATTERN OF URINE CHROMATOGRAMS IN EIGHT FORMS OF AMINOACIDURIA, BASED ON THE AVERAGE NINHYDRIN COLOUR INTENSITY OF THE AMINOACID SPOTS IN A LIMITED NUMBER OF CASES OF EACH CONDITION. (Strictly tentative).

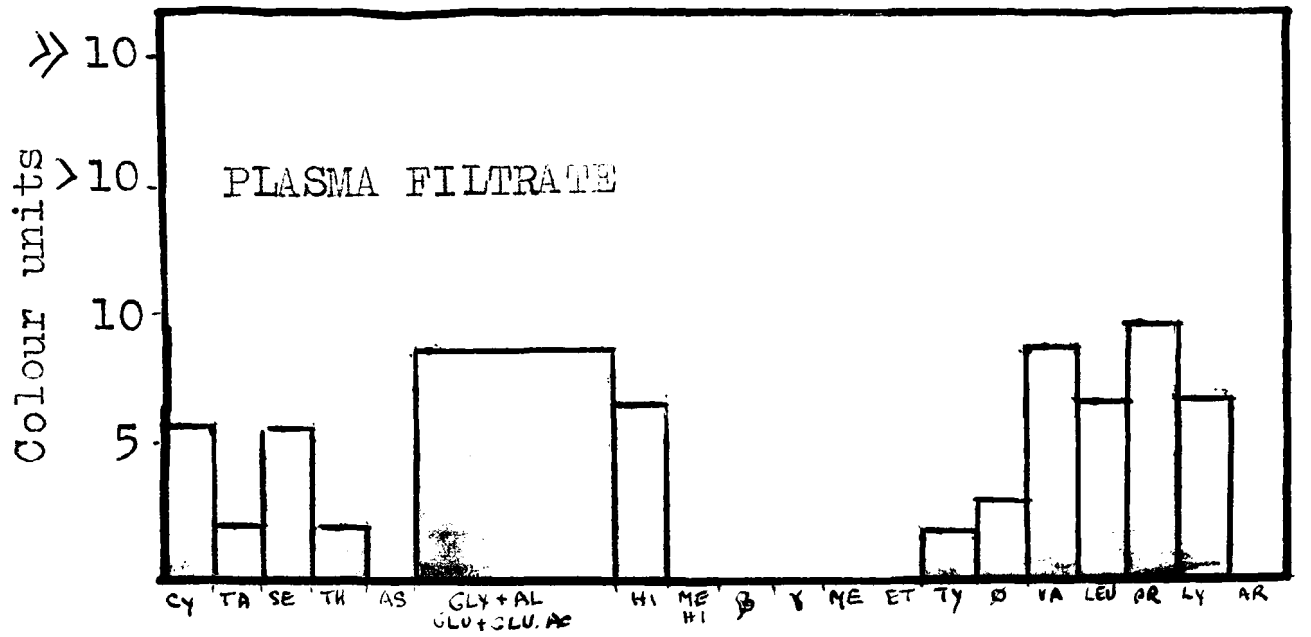


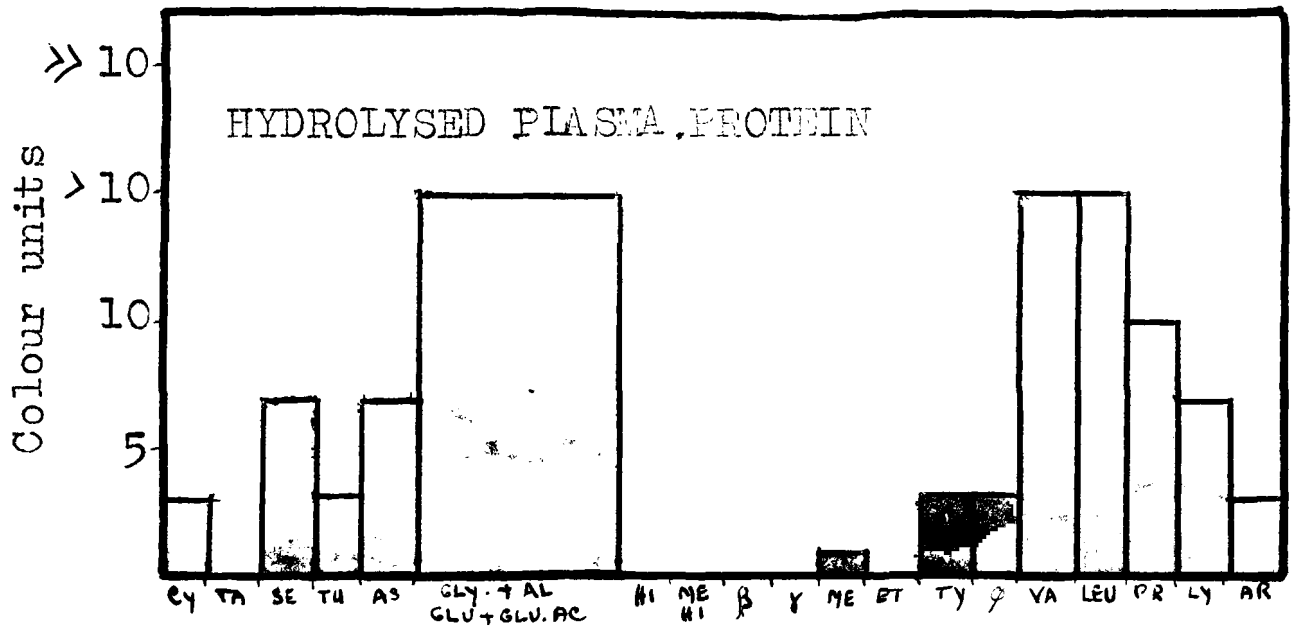
CY = cystine
TA = taurine
SE = serine
TH = threonine
AS = aspartic acid
GLY + AL / GLU + GLU.AC. = Aver. of glycine, alanine, glutamine and glutamic acid taken together
HI = histidine
ME HI = methyl histidine
β = β-amino-isobutyric acid
γ = γ-amino-butyric acid
ME = methionine
ET = ethanolamine
TY = tyrosine
Φ = phenylalanine
VA = valine
LEU = the leucines
PR = proline
LY = lysine
AR = arginine

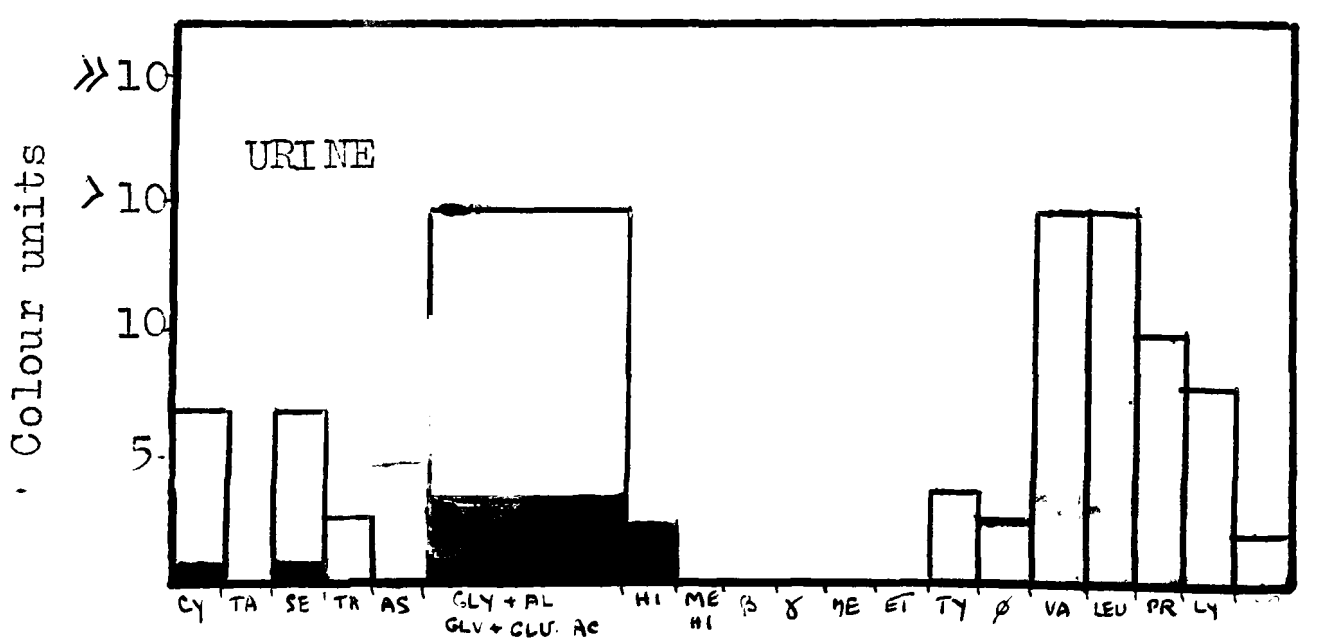
different aminoacid patterns in diagrammatic form, based on the average colour intensity of each amino-acid in the urine chromatograms of the various diseases. Fig. 25(e) shows the pattern of a normal urine chromatogram, which is also indicated in black at the basis of the other diagrams. The pathological aminoacid increase is shown in red. Diagrams 25(a) and (b) demonstrate the typical pattern of cystine-lysinuria and of phenylpyruvic oligophrenia, with a strong increase of very few aminoacids. Fig.25(c) shows the more generalised aminoaciduria in Lignac-Fanconi disease, of which the increase of valine, the leucines and proline is characteristic. In Fig.25(h) the average aminoacid findings in the urine of ten patients with liver cirrhosis have been reproduced. The first five columns show the increases of cystine, taurine, serine, threonine and aspartic acid, columns 9 to 13 those of β-amino-isobutyric acid, γ-amino-butyric acid, methionine, ethanolamine and tyrosine. The diagram of the aminoaciduria in liver disease closely resembles that of steatorrhoea (g) and, for the first five columns, that in newborn infants (i). The diagrams of the aminoaciduria in galactosaemia (d) and in hepatolenticular degeneration (f), as far as can yet be judged, are very similar to each other except for the strong increase of histidine in the latter disorder.

We still know very little about the reasons for the manifold changes of the urine aminoacid pattern in health and disease. To extend our knowledge of the function and metabolic pathway of the individual aminoacids the use of tracers and renal clearance work

Fig. 26. AMINO-ACID PATTERN OF PLASMA FILTRATE, HYDROLYSED PLASMA PROTEIN AND URINE IN LIGNAC-FANCONI DISEASE.







will be immensely valuable. Animal experiments, in producing kidney and liver lesions and in testing the effect of excessive peroral and parenteral doses of one or many aminoacids, or of their restriction in the diet, may further elucidate the significance of the various aminoacid patterns. But the history of the research into phenylpyruvic oligophrenia shows that valuable information can also be gained by careful observation of patients suffering from an inborn error of protein metabolism. In this disease the close alliance between research and treatment is demonstrated, emphasizing the ultimate aim of the student, namely, that the knowedge he is struggling to acquire may finally be employed for the treatment or cure of the patient's disease.

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