GALACTOSAEMIA

BY

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The terms ' galactosaemia ', ' galactose diabetes ', ' chronic galactaemia ' and ' chronic galactosuria ' have been used more or less synonymously to denote a clinical syndrome characterized by retarded development, hepatomegaly, albuminuria and galactosuria. Cataracts have been observed in some of the cases.

Since the original description by von Reuss in 1908, 14 proven cases of galactosaemia have been placed on record, and 11 other probable but not substantiated examples have been reported. The satisfactory response which may follow treatment before irreparable changes become established makes early and accurate diagnosis a matter of great practical importance. Diagnosis may not be easy on account of the difficulty in identifying the urinary reducing substance, which may be present in only small amounts at any particular time. The purpose of this paper, in addition to recording three further cases, is to demonstrate the diagnostic help given by chromatography.

Case Reports

Case 1. Richard T., aged 12 months, was referred by Dr. George of Haverfordwest for investigation of liver enlargement, defective vision and retarded development. He was admitted to Llandough Hospital in June, 1951.

The only child of young healthy parents, he was born at full term by a normal labour after a normal pregnancy. There was no parental consanguinity and no hereditary or familial disease. The birth weight was 6 lb. 14 oz. During the first month of life he was ill with vomiting and mild but persistent jaundice, and his weight fell to 5 lb. Liver enlargement was noticed at this time. Subsequently he slowly improved, though physical and mental development were tardy. At the age of 2 months a cataract was seen in the right eye. His doctor also found albumin in the urine from time to time, and the presence of some reducing substance was noted.

On admission the baby was under weight (16½ lb.) with bilateral cataracts, the left not so easily seen as the right (Fig. 1). Vision was obviously impaired, but its exact extent was difficult to determine, though a response was obtained to a bright light. Some frontal and parietal bossing was noted, but the anterior fontanelle was normal and no clinical evidence of rickets was found.

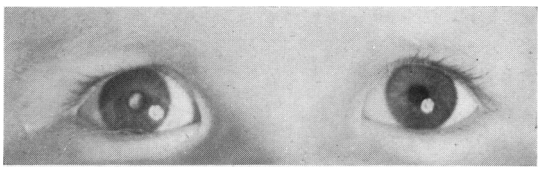

FIG. 1.—Case 1, R.T., aged 12 months, showing cataract in right eye.

He was unable to sit up without support, and took little notice of his surroundings. The lower central incisors were erupted. The lower ribs were somewhat splayed, and dilated veins were seen on the upper part of the anterior abdominal wall. The liver was enlarged, hard and smooth; the lower edge reached to the level of the umbilicus in the nipple line. The spleen could not be felt. During his seven weeks' stay in hospital he ran an irregular, low grade fever occasionally reaching 101° F. rectally.

Haemoglobin was 60% (8·8 g.); leucocytes 10,200 c.mm. (30% neutrophils, 65% lymphocytes).

Urine analysis showed albuminuria, and Benedict's solution was reduced equivalent to 0·5% glucose. The deposit showed a number of granular casts. Tests for urinary ketones were negative. The reducing substance was not fermented by yeast. Tauber's test for pentoses was negative, but Tollen's phloroglucinol test with spectroscopic examination suggested the presence of galactose. Confirmation was obtained by chromatography (see Appendix) of the urine.

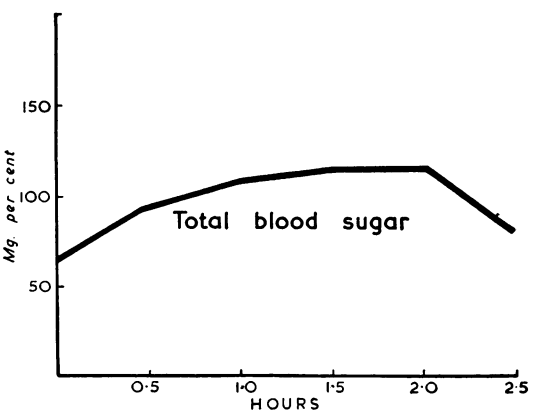

FIG. 2.—Case 1. Glucose tolerance test (1·75 g. per kg.).

An oral glucose tolerance test (Fig. 2) showed that fasting blood glucose was 65 mg.%, and after oral administration of 14 g. glucose, a maximum rise to 110 mg.% occurred at one and a half hours, falling to 80 mg.% at two and a half hours.

A galactose tolerance test (Fig. 3) gave a result typical

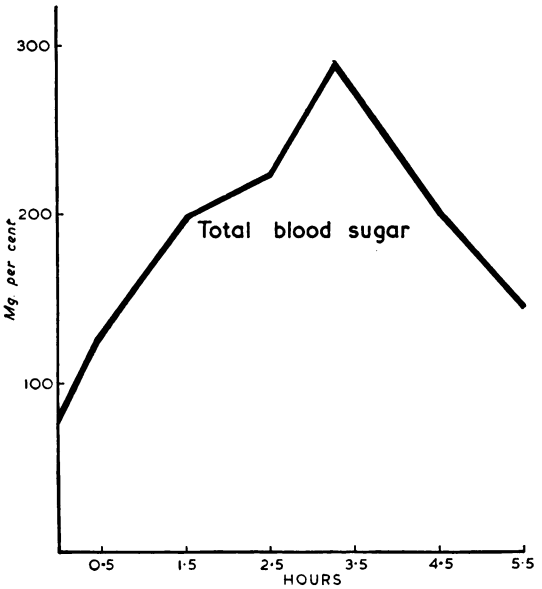

FIG. 3.—Case 1. Galactose tolerance test (1·75 g. per kg.)

of galactosaemia, the total blood sugar rising to a maximum of 265 mg. % at three and a half hours, and even at five and a half hours a value of 170 mg. % was found.

Liver function tests gave the following results:

June 26, 1951	Serum bilirubin	0·1	mg. %
	Alkaline phosphatase	37	KA units
	Thymol turbidity	8	units
August 9, 1951	Serum bilirubin	0·2	mg. %
	Alkaline phosphatase	18	KA units
	Thymol turbidity	1	unit
	Serum proteins	6·3	g. %
	albumin	4·4	g. %
	globulin	1·9	g. %

A liver biopsy (Figs. 4, 5) was taken. The surgeon described the liver as considerably enlarged and firm, with a finely granular surface.

Microscopical examination was reported as follows:

> 'There is a diffuse fibrosis involving portal areas, and bands of cellular fibrous tissue forming a continuous network throughout the liver. The portal veins appear to be involved and there is a slight fibroblastic proliferation around some of the centrilobular veins. The fibrous tissue shows slight diffuse infiltration by lymphocytes and there is an occasional focal collection of these cells. The liver parenchymal cells are swollen and their cytoplasm shows a finely vacuolated appearance, while some are distended by a clear vacuole. The latter do not show a definite zonal distribution, but are scattered in single cells or groups in a haphazard manner throughout the lobules.
>
> 'With fat stains the liver cells show fine sudanophil droplets and some show large globules. Glycogen is also demonstrated.'

An x-ray examination of the skull, long bones and abdomen showed no abnormality. Translucent areas were noted in the necks of the fourth and fifth right ribs and in the third and fourth left ribs.

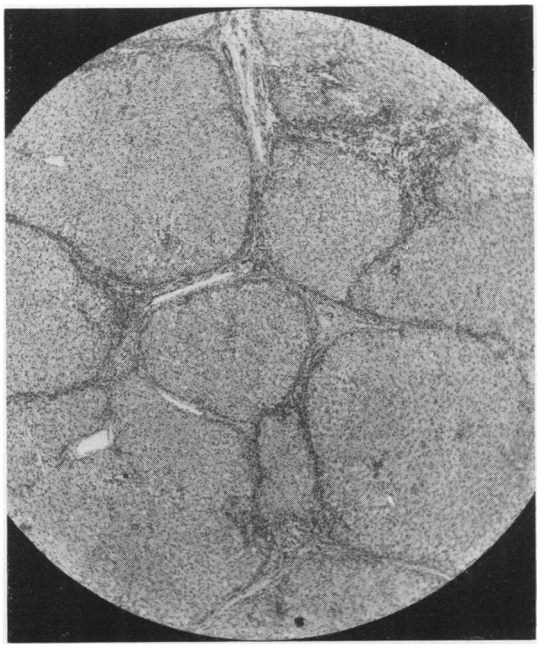

FIG. 4.—Liver biopsy, Case 1: low power view.

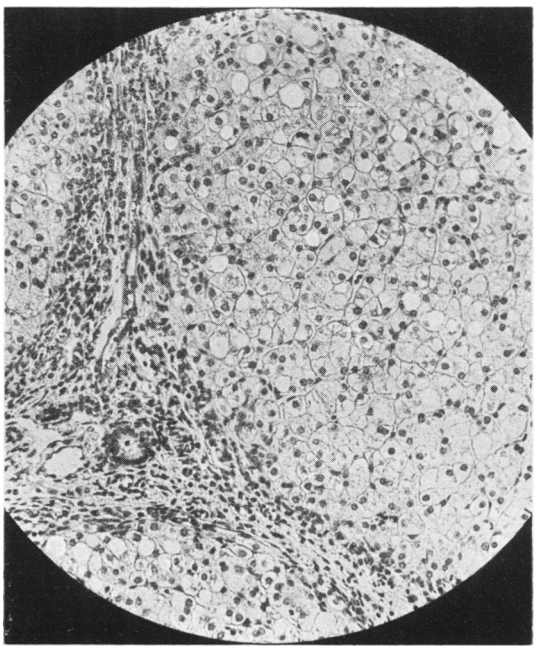

FIG. 5.—Liver biopsy, Case 1: high power view.

The blood cholesterol level was 55 mg. %. The Wassermann and Kahn tests were negative. The bone marrow was normal, with no cystinosis.

A lactose-free diet was constructed, using a soya bean preparation, a protein hydrolysate, and arachis oil, together with lactose-free solids. Powdered glucose was later added.

Considerable difficulty was experienced in getting the child to take much of this diet, although his mother gave him most of his feeds, and his weight fell by 11 oz. There was troublesome vomiting at times, but when seen one month after discharge from hospital his mother had been more successful in persuading him to take the diet and he had gained 2 lb. 1 oz. His general condition had improved, and he seemed altogether more lively, was able to sit up and had cut three further teeth. He seemed able to follow objects more easily, although it was difficult to detect any difference in the appearance of the cataracts. The liver clinically was unchanged, but a new finding was that the tip of the spleen was now palpable.

On December 8 we received the following report from Dr. George:

'Richard T. is now 17½ months old. He recently had a cold but seems to have got over that very well. He takes an intelligent interest in his surroundings and his manner is bright. He utters monosyllables, and has little accomplishments in which he seems to take a pride, e.g. "Clap hands till Daddy comes home", and others. He is unable to sit up without help, but when placed in a sitting position, maintains it and plays happily. He is able to stand by catching hold of furniture if first placed in the standing position. His appetite is good and he is taking a mixed diet. I understand his motions have been rather loose since coming from Llandough Hospital, but they have now become more formed and normal in appearance.

On examination, both fontanelles were closed. A large cataract is still present in the right eye but it is possibly less opaque than formerly. There is, however, room for two opinions about this. The child is able to see with at least one eye and picks up articles without hesitation. He has four incisor teeth in both the upper and the lower jaws.

His abdomen is considerably less protuberant than formerly. The liver can be felt one and a half to two fingerbreadths below the right costal margin. It is not as firm as formerly. He has a hydrococoele of the right spermatic cord.

There is no albumin, reducing substance or acetone in the urine.

On September 1, 1951, he weighed 17 lb. 14 oz. and today 23 lb.

This child has made a considerable advance since his condition was recognized last July. While still backward, he is no longer the apathetic child that he was then. It is difficult to be sure about the state of the cataract, but it is my impression that, on the periphery, at least, it is not so opaque as formerly. The liver has diminished in size very considerably. It formerly extended some four fingerbreadths below the right costal margin.'

Case 2. Raymond R., 12 days old, was admitted to the Port Talbot Hospital on account of jaundice of three days' duration. He was the firstborn of healthy parents. Delivery at term was normal and the pregnancy had been uncomplicated. The birth weight was 7 lb. and the feeds were of breast milk. There was no family history of hereditary or metabolic disorders.

He was a somewhat inert infant with normal tissue tone. There had been no vomiting or diarrhoea. The skin and sclera were slightly icteric. No abnormal dryness of the skin was observed and there were no cutaneous haemangiomata. Both breasts showed 'mastitis neonatorum'. He weighed 6 lb. 7 oz. The temperature and respiratory rate were normal. The abdomen was slightly distended but no prominent superficial veins were seen. The liver, the edge of which was palpable 6 cm. below the costal margin, was smooth in outline and firm (Fig. 6). The spleen was 2 cm. below the costal margin. There was no evidence of ascites and no other abnormal organs or masses were palpable. No abnormal physical signs were detected in the heart, lungs or central nervous system.

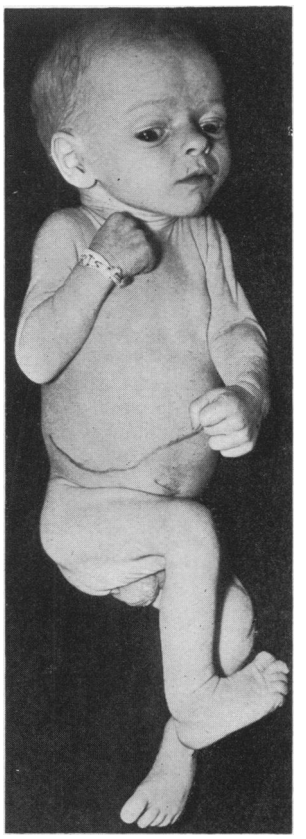

FIG. 6.

In both eyes the lenses appeared hazy (Fig. 7).

Movements of the eyes were normal in all directions, and they were attracted by lights from all quadrants. The tension of each eye was normal to finger pressure. Pupil reflexes were normal to light and well sustained. The lenses showed general clouding with increased opacity through the posterior cortex of each. The appearance suggested 'intumescence' of the lenses. There were no discrete opacities. Fundi were not clearly seen owing to lens changes, but appeared normal.

The urine was opalescent and of a colour reminiscent of lemon cheese. It gave a strong reduction of Benedict's solution and this, and the physical signs, suggested the diagnosis of galactosaemia.

During the subsequent days when investigations were proceeding, breast feeding was continued, but weight loss was progressive and there was an increase in the jaundice associated with greater apathy and inertness. On the seventh day after admission the

laboratory reported that the reducing substance in the urine was galactose. Breast feeds were therefore discontinued and a formula of 1½ oz. of calcium caseinate, 2 oz. of glucose and ¾ oz. of arachis oil was used. Later a supply of 'nutramigen' was obtained.

The infant's weight was now 5 lb. 12 oz. and he was wasted.

Hartmann solution, 100 ml., followed by 90 ml. of whole blood were given by scalp vein transfusion with improvement in general condition and increased vigour in feeding.

Urine specimens consistently showed a negative Benedict's test (Fig. 8) on the formula feed. On breast

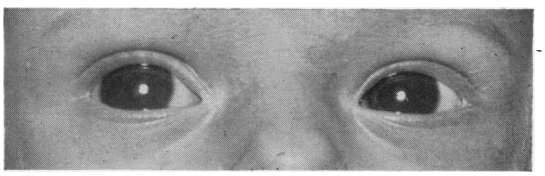

FIG. 7.—Case 2, R.R., aged 3 weeks, showing haziness of lenses.

feeding urine analysis showed that albumin was present and the *p*H 5·5. Benedict's test was strongly positive. Rothera's and Gerhardt's tests were negative. Tests for bile salts and bile pigments were respectively negative and positive. There was no increase in urobilinogen. Total sugar was 0·8 g. %. Rubner's and Bial's tests were negative. Barfoed's test for monosaccharides was positive as was the mucic acid test.

A centrifugalized deposit showed epithelial cells and granular casts, but no bacterial growth after 18 hours' incubation.

Phenylosazone crystals were identical in appearance and melting point with those prepared from a sample of galactose.

Serum protein analysis gave total proteins 7 g. % (albumin 4 g. %, globulin 3 g. %) A : G ratio 1·3 : 1.

Serum alkaline phosphatase was 30 units. A cephalin flocculation test was positive. A serum colloidal gold test gave 00000 and thymol turbidity 3 units. There were 3,400,000 red blood cells per c.mm. Red cells showed some macrocytosis with accompanying anisocytosis. The blood group was AII rr. Haemoglobin was 80% (Haldane), and the colour index 1·18. Leucocytes were 5,600 (polymorphs 50%, lymphocytes 45%, eosinophils 5%). Fasting blood sugar was 190 mg. %. The direct Coombs test was negative. The direct van den Bergh test formed a deep wine colour immediately. The indirect reaction gave a serum bilirubin level of 3·6 mg. %.

The Wassermann and Kahn reactions were negative. A galactose tolerance test (1·75 g. body weight), showed the abnormal values recorded in Fig. 9.

Radiographs of the skull, ribs and bones of the extremities did not show any abnormality.

Paper chromatography (see Appendix) showed a considerable excretion of galactose as demonstrated in 100 ml. of the urine.

Examination of the parents showed no change in their blood or urine and both had normal galactose tolerance

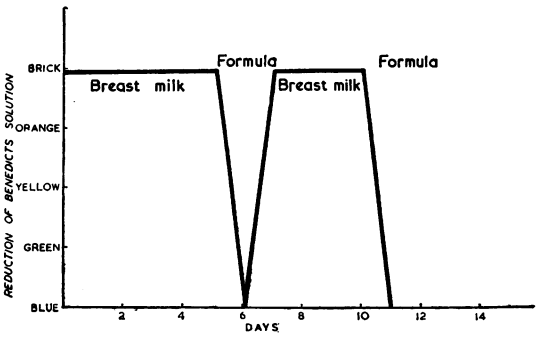

FIG. 8.—Case 2. Effect of diet on urinary reducing substance.

curves. It was noted that the parents of this case were natives of the same township as Case 1, but careful enquiry showed no known relationship.

Case 3. Hilda J., aged 7 years, was referred from a residential school for blind children for investigation of abdominal enlargement. Full details of her previous medical and family history were not available, but the following facts are known.

She was one of twins; her sister died soon after birth from an undetermined cause. Bilateral cataracts were found at an early age, and in spite of several operations vision remained poor. Physical growth

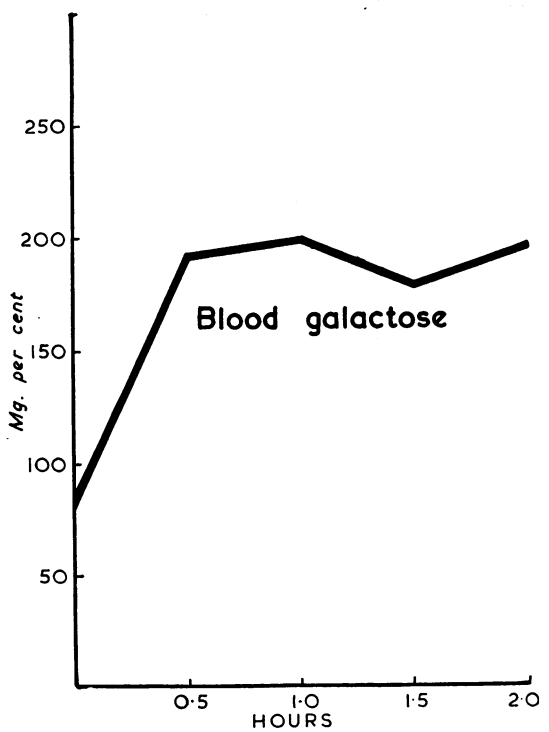

FIG. 9.—Case 2. Galactose tolerance test (1·75 g. per kg.).

and mental development were also retarded. The liver was found to be moderately enlarged and firm, the lower edge reaching midway to the umbilicus. No splenomegaly or ascites was found. On an ordinary diet containing a pint of cow's milk daily no reducing substance was found in the urine. A galactose tolerance test, however, showed hypergalactosaemia and galactosuria indicating impaired liver metabolism of galactose. Glucose tolerance was normal. Other liver function tests showed no abnormality.

The brief summary of this case is included in this report because, although it is incomplete, it suggests that a review of children in residential schools for the blind might reveal further cases.

Review of the Literature

Von Reuss (1908) published the first recorded case of galactosaemia in a marasmic infant with melituria; an enlarged cirrhotic liver was also found. Göppert (1917) reported a case in a child aged 4 years; three siblings had a suggestive history, but the diagnosis of galactosaemia was never established. Fanconi (1933) described a case of galactose intolerance in a 9-year-old boy, but hepatomegaly and cataracts were not present. In the case reported by Unshelm (1934) galactosaemia and liver enlargement returned to normal after galactose was withdrawn from the diet. Mason and Turner (1935) studied the case of a male negro infant with galactosuria, malnutrition, hepatosplenomegaly and evidence of impaired hepatic and renal function. Cataracts developed later. A follow-up of this patient to the age of 18 years is quoted in the paper by Townsend, Mason and Strong (1951). As the child grew older the galactose tolerance increased somewhat and no evidence of permanent liver disease was found, but he remained educationally subnormal (I.Q. 64).

The case described by Norman and Fashena (1943) resembled Unshelm's in that a galactose-free diet caused galactosaemia to disappear and liver function returned to normal. A similar case was recorded by Mellinkoff, Roth and MacLaggan (1945).

Detailed studies of carbohydrate metabolism in a case of galactosaemia are recorded by Bruck and Rapoport (1945). These authors emphasized the reciprocal relationship of galactose and glucose. Greenman and Rathbun (1948) also noted this, and found that the tolerance to galactose could be improved by adding glucose to the diet.

Goldbloom and Brickman (1946) reported two typical cases. In one of their cases, and in the patient described by Goldstein and Ennis (1948), lamellar cataracts showed signs of clearing after persisting for months on a galactose-free regime.

Liver biopsy in a case of galactosaemia was first described by Bell, Davidson and Scarborough (1950). The histological features were early fibrotic changes, focal cellular necrosis, and many liver cells distended by a single large lipid vacuole.

Donnell and Lann (1951) present four additional cases; three occurred in one family, though in two of these the diagnosis was not suspected before death, and galactose was not certainly identified. In one case portal cirrhosis and fatty infiltration of the liver was found at necropsy.

DuShane and Hartman (1951) report another case diagnosed at $4\frac{1}{2}$ months, and treated by eliminating milk from the diet and substituting a soya bean preparation. At the age of 24 months the infant appeared normal.

Townsend *et al.* (1951) present five additional cases and a follow-up of Mason's original patient. Liver biopsy in one patient showed a typical picture of Laennec's cirrhosis. These authors emphasize mental retardation as a salient complication of galactosaemia and the potential reversibility of the liver cirrhosis.

Gorter (1951) described three cases in a sibship of four, and quotes de Haas as having observed a family of 10 children of whom four had galactosuria. Gorter considers the disease to be an inborn error of metabolism of galactose, and states that symptoms are due to the toxic effect of galactose on the liver and kidney.

Discussion

Galactose, a dextro-rotatory stereo-isomer of glucose, occurs in nature as a constituent of lactose, and in certain complex lipids and proteins. Lactose, a β-galactoside in composition, occurs in the milk of mammals and is synthesized in the mammary gland. After ingestion lactose is hydrolyzed in the intestinal tract to glucose and galactose. The absorbed galactose is converted to glycogen by the liver. According to Bell, Davidson and Scarborough (1950) and Bridge and Mulholland (1951) the galactose is first converted to glucose by way of phosphorylated derivatives, and then to glycogen. Mason and Andersen (1941), discussing one form of glycogen storage disease, state that the liver in that condition is unable to convert glycogen to glucose or glucose to glycogen, but that a slow accumulation of hepatic glycogen results from the conversion of dietary galactose.

Where liver function is impaired, galactose is imperfectly metabolized, and after ingestion hypergalactosaemia and galactosuria occur. These findings are utilized in the galactose tolerance test for liver disease (Maclagan, 1940). Alimentary galactosuria is said to occur in normal infants after excessive ingestion of lactose (Rapoport, 1950). (See Appendix.)

The condition termed 'galactosaemia', on the other hand, is considered to be an inherent defect of carbohydrate metabolism in which the liver is unable to metabolize galactose. It has been placed among the 'inborn errors of metabolism' (Taggart and Mason, 1950), and Donnell and Lann (1951) refer to the likelihood of a specific gene, necessary for the metabolism of galactose, being absent or altered in this disease. A familial incidence has been reported on several occasions (Bell, Blair, Lindsay and Watson, 1950; Donnell and Lann, 1951; Gorter, 1951).

In most of the reported cases the neonatal course of the infant has been a stormy one, characterized by feeding difficulties, jaundice and failure to gain weight. Albuminuria and melituria are present. The reducing substance may vary in amount from day to day, depending on the diet, and identification may be very difficult. Galactose reduces Benedict's solution, but is not ordinarily fermented by yeast, though many samples of brewer's yeast will ferment it. Barfoed's test will show the reducing substance to be a monosaccharide, and Bial's and Tauber's tests will eliminate pentose. A positive mucic acid test will show that the sugar is either lactose or galactose, and the Rubner test can be used to eliminate the former. Tollen's phloroglucinol reaction and the preparation of galactose osazone confirm the occurrence of galactosuria. In Case 2, a breast-fed infant, identification of galactosuria was definite using the above tests. A breast-fed baby taking 600 g. milk, receives about 22·5 g. of galactose per day. In Case 1, the relative proportion of dietary galactose was much less, and the galactosuria less constant and profuse. In both these cases the diagnostic help afforded by chromatography was great. In Case 3 no spontaneous galactosuria was observed, but oral administration of galactose produced hypergalactosaemia and galactosuria. This patient resembles that of Fanconi (1933), and Mason and Turner's patient when seen at 7 years old, who could tolerate 200 ml. milk at each meal without melituria.

Much interest has been shown in the liver enlargement constantly present in this disease. Abnormal liver function tests are an unusual finding in the reported cases. Cases 1 and 2 showed a high phosphatase level, and in Case 2 hyperbilirubinaemia was also present.

Biopsy studies are recorded by Bell *et al.* (1950) and by Townsend, Bell, *et al.* (1951). In the former histological examination showed many hepatic cells distended by a single large lipid vacuole, focal cellular necrosis and early fibrotic changes. The authors considered infiltration with fat to be the basic cause of the liver enlargement. In Townsend's case liver biopsy showed typical Laennec's cirrhosis. There was no vacuolation within the hepatic cells. Cirrhosis of the liver was found at necropsy in four other cases of galactosaemia.

Liver biopsy in Case 1 (Figs. 2, 3), the third to be reported, shows features of both the previously described cases. Diffuse hepatic fibrosis is present, the appearance closely resembling that of Townsend's case, and fatty changes are also prominent.

The cause of the hepatic cirrhosis is uncertain. Mason and Turner (1935) thought it might be due to hypoglucosaemia; the more likely explanation is a direct toxic action of galactose.

A curious feature of all the proved cases of galactosaemia is the absence of ketosis, which occurs commonly in glycogen storage disease and in conditions associated with fatty cirrhotic livers. Possible explanations are the normal liver glycogen, or the toxic action of galactose preventing the formation of ketone bodies.

Cataracts have been reported in nine cases of galactosaemia. In three cases they disappeared after the child had been on a galactose-free diet, though taking several months to do so. In two other cases the opacities became less, and no improvement occurred in the remainder.

Mitchell and Dodge (1935) and Yudkin and Arnold (1935) described cataracts as occurring fairly constantly in young rats fed on a diet in which the main carbohydrate was lactose or galactose. The cause of the cataracts is still obscure. Bellows and Rosner (1938) noted a decrease in the permeability of the lens capsule preceding the formation of cataracts, and Bellows and Chinn (1941) produced cataracts in young animals within a few minutes of an intravenous injection of hypertonic galactose solution. These authors suggest an upset in osmotic balance in the lens, together with alterations in the permeability of the lens capsule, as responsible for the formation of galactose cataracts. Weekers (1943) stated that hypocalcaemia was necessary for galactose cataracts to develop. An interesting observation recorded by Bannon, Higginbottom, McConnell and Kaan (1945) was that galactose cataracts produced in embryos affected only the nucleus of the lens, the actively growing regions remaining unaffected. Well-developed cataracts were present in Cases 1 and 3; in Case 2 the lenses showed a diffuse haziness, described as 'intumescence'. The earliest reported cataract was in Bruck and Rapoport's patient aged 7 weeks.

The nomenclature of the disease may, with advantage, be revised; that at present in use is open to various objections. The term commonly

used, ' galactosaemia ', like ' uraemia ' and ' hyperglycaemia ' connotes a particular finding in the blood which may occur in a number of diseases. ' Galactose diabetes ' on the other hand suggests too close an analogy with diabetes mellitus, unsupported by the pathogenesis of the disease. If it is accepted that the condition is an inborn error of metabolism, then to bring it into line with the other conditions in that group, the term ' essential galactosuria ' or ' congenital galactosuria ' would be preferable. Further, if it were agreed that damage to the eyes, liver and kidney resulted from toxic action of the galactose, then an analogy with the de Toni-Fanconi syndrome becomes apparent, and the most suitable name for the condition would be either ' galactose disease ' or ' galactosis '.

Summary and Conclusions

Two cases of galactosaemia in infants are presented, and a third case of probable galactosaemia in a 7-year-old girl with cataracts, liver enlargement and galactose intolerance.

The importance of early diagnosis is emphasized by the improvement shown in Case 2 when the infant was placed on a lactose-free diet.

The diagnostic help given by chromatography is demonstrated.

The results of liver biopsy are described, the findings being intermediate between the only two previously reported biopsy studies.

It is suggested that the name of the disease be ' galactosis '.

We gratefully acknowledge the help we have received from Dr. H. Bickel, of the Biochemical Department of the Children's Hospital, Birmingham, both in providing us with the report on chromatography and in giving his diagnostic help.

To Mr. Foster who performed the liver biopsy, to Dr. Richards who reported on the sections and to Mr. Rupert Parry and Mr. Hibberd who gave us reports on the eyes, we express gratitude for their valuable assistance. We offer thanks to Dr. George for his interesting comment on Richard T., also Mr. Salter and Mr. Bennett for technical assistance. Finally, our thanks are due to Mr. Napper and Mr. Griffiths for supplying the photographs.

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Addendum

When last seen on April 27, 1952, Richard T. was considerably improved and was gaining weight, talking and walking. The cataracts showed little, if any, change.

On July 10, 1952, Raymond R. was described by his mother as a normal child, and was making excellent progress. Both children have been kept on a lactose-free diet.

APPENDIX

PAPER CHROMATOGRAPHIC INVESTIGATIONS ON THE URINE OF PATIENTS R.T. AND R.R.

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Technique

Sugar Chromatography. The urine specimens were preserved with thymol and arrived in good condition. 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 aniline phthalate. The volume pipetted at the base of each column was 50 µl. for Richard T., 10 µl. for R.R., 50 or 100 µl. for the normal controls.

Amino-acid Chromatography. The technique employed was that of Consden, Gordon and Martin (1944), of Dent (1947 and 1948), and of Hermann, Bickel and Fanconi (1949). The urine specimens were de-proteinized by passing them through a collodion sac (Greenberg and Gunther, 1930) to remove albumin, and if necessary, de-salted (Consden *et al.*, 1947). For each urine chromatogram the quantity of urine used contained 500 µg. nitrogen. All the chromatograms were two-dimensional phenol-collidine-lutidine runs and were treated with perhydrol. After developing the papers with ninhydrin the colour intensity of the spots is expressed either in figures—1 for the weakest and 10 for the strongest—or compared with test spots of pure taurine placed above the urine spot in five different positions and concentrations (5-10-20-40-60 µg. of taurine) before the run is started.

Results

Sugar Chromatography. The sugar chromatograms of the patients Richard T. and R.R. showed a definite galactosuria as demonstrated by the strongly coloured spots in the runs of their urine (Fig. 1, column 3, Fig. 2, columns 2, 3, 4, 6). The position of these spots is identical with that of pure galactose, which was run in Fig. 1 in columns 1 and 6, in Fig. 2 in columns 1 and 7 together with several other pure sugars. Normal urine (Fig. 1, column 7, Fig. 2, column 5) does not give any colour reaction with aniline phthalate, even when volumes of 200 µl. 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 1 month onward. Fig. 3 shows the sugar chromatograms with the urines of Richard T. and a normal control child during three days' lactose ingestion. The normal child excreted traces of lactose in the urine on the first and last lactose day, but no other sugars, and, in particular, no galactose. Richard T. did not excrete any sugars during the first two lactose days, but a definite galactosuria developed on the third lactose day.

Amino-acid Chromatography. Glycine and serine are the only amino-acids of sufficient concentration to give a colour reaction with the ninhydrin spray. More than 200 urines of children of all ages were tested by the authors (unpublished data) to establish the chromatographic pattern and spot intensity in healthy children. None of the normals, with the exception of newborns, exhibited an amino-aciduria comparable with that of

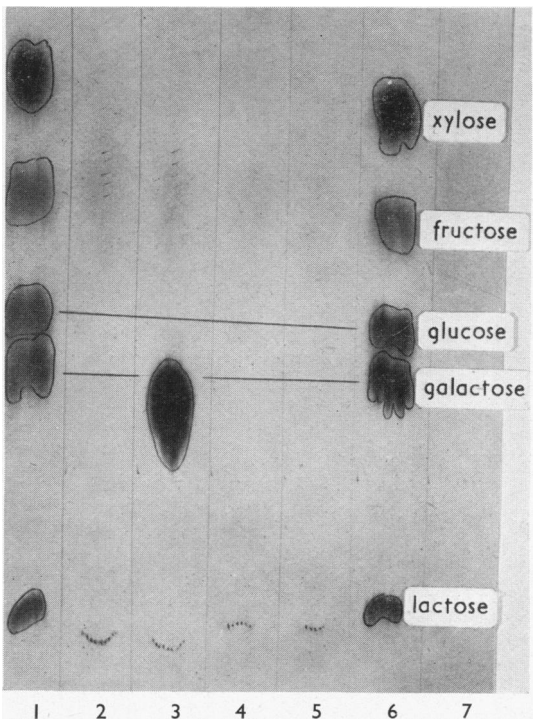

FIG. 1.—One-dimensional sugar chromatogram from 100 µg. each of pure lactose, galactose, glucose, fructose and xylose (columns 1 and 6); 50-200 µl. normal urines (columns 2, 4, 5 and 7); 50µl. urine of Richard T., of June 30, 1951 (column 3). Benedict's test green-yellow.

Richard T. and R.R. A normal urine of a volume containing 500 μg. nitrogen rarely shows more than three to six faint amino-acid spots, generally glycine, alanine, glutamine, glutamic acid, sometimes traces of histidine, cystine and, still more rarely, serine, taurine and β-amino-isobutyric acid. Richard T., on the contrary, showed a marked amino-aciduria; glycine, serine, alanine, glutamine were excreted in excess, but still more pathological was the excretion of amino-acids not occurring in normal urine, such as threonine, valine and the leucines.

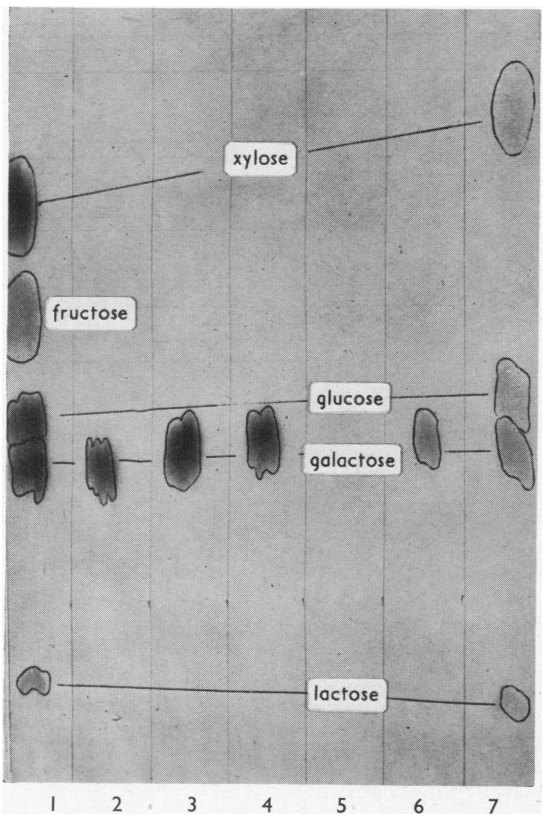

FIG. 2.—One-dimensional sugar chromatogram from pure sugars (column 1 and 7); 100 μl. of normal urine (column 5); 10 μl. urine of R.R. on different days (columns 2, 3, 4 and 6). Benedict test on urines in columns 2, 3 and 4 green-yellow; in column 6 green.

Chromatograms with other urine specimens of this patient showed in addition an increased excretion of lysine, methionine, phenylalanine, tyrosine and cystine (as cysteic acid). R.R.'s urine chromatograms also showed an increased output of various amino-acids, especially of glycine, serine, taurine, threonine, cystine, proline and methionine. The last two are rarely found together even in pathological urines.

Discussion

Paper chromatography provides an excellent and comparatively simple method of testing urine, plasma and other biological fluids for their sugar and amino-acid composition. Chromatography has the great advantage of being highly sensitive and specific; its main disadvantage is its semiquantitative nature. Sugar chromatograms on the two patients showed a definite galactosuria. Galactose excretion could be demonstrated even when Benedict's reduction test gave an indefinite blue-green or green colour reaction. Chromatography is thus superior to the Benedict test, as it is both more specific and more sensitive, and may reveal a galactosuria which could easily escape the usual Benedict test.

The demonstration of amino-aciduria in both patients is a finding which, to our knowledge, has so far not yet been mentioned in the literature on galactosaemia. Up to 15 amino-acids were excreted in excess. The pattern of the amino-acid chromatograms was similar to that seen in the urine of some patients suffering from liver diseases, such as cirrhosis and atrophy of the liver. The amino-aciduria continues even when the galactosuria has ceased as a result of a galactose-free diet. This was observed in a patient of Dr. Snyder, New Orleans, who kindly sent us a urine specimen for chromatographic investigations.

Unfortunately we have so far had no opportunity of studying the amino-acid plasma level in galactosaemia so that the mechanism of the amino-aciduria in this disease, whether over-flow or lowered renal threshold,

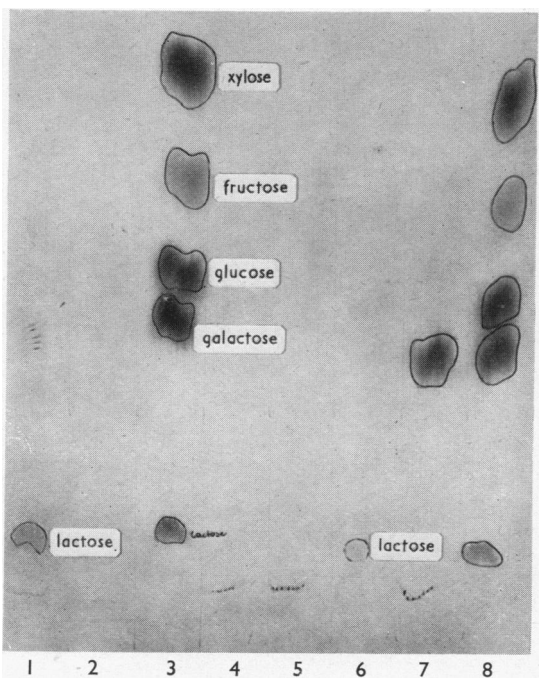

FIG. 3.—One-dimensional sugar chromatogram showing the results of lactose feeding on the urines of Richard T., first day, July 6, 1951 (column 2), second day (column 5), third day (column 7); normal control, first day, July 6, 1951 (column 1), second day (column 4), third day (column 6). Chromatograms of 100 μg. of pure sugars are shown in columns 3 and 8.

cannot be assessed. The well-known fact that the liver of galactosaemic patients is damaged (Bell, Blair, Lindsay and Watson, 1950), may lead one to suspect that this is the cause of the amino-aciduria. Parenchymal damage of the liver, however, does not always lead to amino-aciduria, even in advanced stages with considerable liver destruction. We have seen moribund patients suffering from liver cirrhosis without amino-aciduria. Furthermore, the albuminuria in galactosaemia points to a kidney lesion in this disease. Our tentative conclusion is that the amino-aciduria in galactosaemia is due either to the failure of a specific liver function essential to the amino-acid metabolism or to a disturbance of the reabsorption of certain amino-acids in the kidney in addition to the metabolic error in galactose utilization by the liver.

Summary

Paper chromatographic studies on two patients suffering from galactosaemia revealed, besides galactose, a pathological excretion of various amino-acids in the urine.

Galactosuria may be demonstrated by chromatography even where the galactose concentration is too weak for a positive Benedict test.

The amino-aciduria persists after cessation of the galactosuria following a galactose-free diet.

The pattern of the amino-aciduria is similar to that in other cases of liver damage. A kidney lesion as a possible cause of the amino-aciduria has, however, also to be taken into account. Future investigations into the plasma level of the various amino-acids may reveal the mechanism of the amino-aciduria.

References

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