A CLINICAL AND BIOCHEMICAL STUDY OF GALACTOSAEMIA

A POSSIBLE EXPLANATION OF THE NATURE OF THE BIOCHEMICAL LESION

BY

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(RECEIVED FOR PUBLICATION JANUARY 27, 1956)

The clinical syndrome of galactosaemia is now well known and more than 40 cases have been carefully described in the literature (Bell, Lindsay and Watson, 1950; Bray, Isaac and Watkins, 1952; Donnell and Lann, 1951; Darling and Mortensen, 1954; Goldbloom and Brickman, 1946; Goldstein and Ennis, 1948; Göppert, 1917; Greenman and Rathbun, 1948; Hudson, Ireland, Ockenden and White-Jones, 1954; Johns, 1953; Maris and Valcke, 1953; Mason and Turner, 1935; Mellinkoff, Roth and MacLaggan, 1945; Mortensen and Søndergaard, 1954; Norman and Fashena, 1943; Reiter and Lasky, 1952; Townsend, Mason and Strong, 1951; Unshelm, 1934).

It is generally agreed that the disorder is the result of an inborn error of metabolism and it is thought that the condition is transmitted as a homozygous recessive gene (Holzel and Komrower, 1955). There is no definite knowledge of the aetiology of the condition but writers have suggested that there is an interference with the normal metabolic process:

$$\text{galactose} + \text{ATP} \xrightarrow{\text{galactokinase}} \text{galactose-1-phosphate} + \text{ADP}$$

uridyl transferase and

$$\text{galactose-1-phosphate} \xleftarrow{\text{galactowaldenase}} \text{glucose-1-phosphate}$$

and that the transformation of galactose-1-phosphate to glucose-1-phosphate is the site of the disturbance, as there is a deficiency of one of the two enzymes, uridyl transferase or galactowaldenase, which catalyse this reaction (Kalckar, Braganca and Munch-Petersen, 1953; Trucco, Caputto, Leloir and Mittelman, 1948). Galactose has always been implicated as the toxic agent, and the development of hepatomegaly, jaundice, cataract, mental retardtion, proteinuria, and, more recently, aminoaciduria, have been attributed to the damaging effect of this sugar on each of the organs concerned.

The objects of this communication are, first, to present two further cases of the condition with the results of routine investigations; next, to report the observations made on these children during milk or galactose feeding and, finally, to relate to these findings the biochemical studies of erythrocyte metabolism that have been made in the disease. In this way we shall endeavour to confirm the site of the interference and to indicate the nature of the metabolic upset.

Case Reports

Case 1. E.F., a girl, was born on June 7, 1951, of a normal delivery (birth weight 8 lb. 15 oz., breast fed). This was the sixth pregnancy, three had terminated with a miscarriage, one infant died aged 14 days, jaundiced, vomiting and with hepatomegaly: the coroner's necropsy report was 'fatty changes in the liver and liver failure'. The other child is alive and well but has an abnormal galactose tolerance and is in much better health when milk is omitted from his diet.

On the fifth day of life vomiting began; a marked loss of weight (1 lb.) was observed, also jaundice which increased in intensity during the next five days. On the tenth day the liver was palpable two fingers below the right costal margin. A radiograph of the chest was normal as was a barium swallow. The child was noticed to be drowsy and inactive although she took her feeds well. The urine on examination was found to contain protein and a reducing substance.

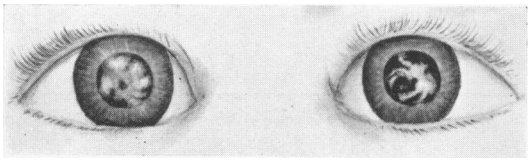
On the tenth day of life glucose saline feeds were started and given for three days; vomiting ceased and the child appeared less lethargic. On June 20 when breast feeding was restarted, the vomiting began again and the lethargy became more apparent. On June 21 the blood sugar (total) was noted to be 225 mg. per 100 ml. The reducing substance in the urine was found to be non-fermentable sugar. By this time the weight loss was 20 oz. and the liver had enlarged yet further to three fingers below the costal margin. The child was once again put on glucose saline feeds with 'casilan'. On June 23, when the child was 16 days old, the diagnosis of galactosaemia was made. On examination a small

superficial infection of the abdominal wall was noted where subcutaneous saline had been given; this required aureomycin therapy.

On June 27 the infant was transferred to the sick infants' ward of the hospital for further observation: she was still on the glucose saline and 'casilan' feeds and was gaining weight. On June 30 the blood sugar (total) was 108 mg. per 100 ml. On July 4 the milk feeds were restarted for the purposes of investigation. The following day the blood sugar (total) was noted to have increased to 202 mg. and following this the stools became loose, the vomiting recurred and an examination of the urine by chromatography confirmed the galactosuria and revealed a gross aminoaciduria.

During the rest of the month of July investigations of liver function, glucose and galactose tolerance tests, together with further examinations of the urine, were made. A careful examination of the fundi and media on July 23 did not reveal any abnormality.

Early in August she developed a low-grade pneumonia and a gastro-enteritis from which she recovered. She was then put on 'casilan' and dextrimaltose mixture which was thought to contain no lactose: this was subsequently found to be incorrect and explained the fact that the liver was still enlarging. On August 20 it was noted that on examination of the eyes there was an increased refractivity to light, and two days later cataracts were found and confirmed.



Bilateral cataracts in untreated case.

On August 24 a special mixture of 'farex', egg and dextrimaltose was started. There was no gain in weight for 10 days but by September 3 her general condition had improved considerably. There was no vomiting, the stools were more normal and the skin infection had improved: in addition examination of the urine did not reveal any aminoaciduria. Later in the month the strength of the feeds was adjusted and a steady improvement in general condition was noted. By the beginning of October there was a marked regression of the cataracts, the left eye being quite clear. The liver was only one and a half fingers palpable below the costal margin and the spleen tip could just be felt. It was noted that the child passed appreciable quantities of urine of low specific gravity with a maximum specific gravity 1011 which occurred 11 hours after a feed. On November 28 the glucose tolerance test was repeated while on the lactose-free diet, and was shown to be normal. Early in December liver function tests were found to be normal and the intelligence of the child, as tested by the Gesell technique, did not seem to be impaired. In addition the fundi were quite normal and there was no abnormality of the media of the eyes.

On December 8, aged 6 months, she returned home weighing 11 lb. 3 oz.

She was readmitted on February 25, 1952, for further investigation. By this time she weighed 14 lb. 3 oz., the urine contained no reducing substances and the amino-acid chromatogram was normal. In addition the glucose tolerance test showed no abnormality. The liver was just palpable, the spleen was not felt, the eyes were normal. Her general condition was excellent.

Her urine output averaged 23 oz. a day for two days with a low specific gravity of 1002. On March 3 a half-cream National dried milk feed was begun. Reducing substances were discovered in the urine after two days and protein after four days; it was noted that the aminoaciduria began after four or five days. There was, interestingly enough, a reduction in the urinary output. By the eighth day there was a gross aminoaciduria and the liver had enlarged two fingers below the costal margin.

She was discharged home on March 12 when the special diet was started again. She has since made steady progress; her weight in March, 1953, was 22½ lb., her general condition excellent and her intelligence normal. She was walking and talking, there was no hepato-splenomegaly but she was still intolerant of milk and her galactose tolerance was grossly abnormal.

Investigations made at this time showed that an aminoaciduria was produced when she was given milk feeds.

Further observations (*vide infra*) were made in October, 1954; in July, 1955 (aged 4 years), the results of a clinical examination were perfectly normal and intelligence testing suggested that her mental development was unimpaired.

Routine Investigations in Case 1

The galactose tolerance test and the glucose tolerance test are described in the section on carbohydrate studies.

LIVER FUNCTION TESTS

	* 21.6.51	* 23.7.51	4.9.51	4.12.51	* 12.3.52
Thymol-turbidity (units)	2·5	2·1	1·8	1·7	1·7
Serum alkaline phosphatase (units per 100 ml.)	17	40	23	26	50
Serum bilirubin (mg. per 100 ml.)		1·6	0·4	0·2	1·8
Serum albumin (mg. %)		3·3	3·6	4·0	3·4
Serum globulin (mg. %)		2·0	2·1	2·3	2·0

* Diet contained lactose or galactose.

A blood count on August 10, 1951, gave: red cells 3·8 m., Hb 72%, leucocytes 8,800 (polymorphs 38%. lymphocytes 62%).

Renal function is described in the section on renal investigations.

Radiographs of the hands and wrists, skull and long bones (May 27, 1952) showed that no evidence of any

deficiency disease could be detected in the skull or in the long bones: in the right hand there was a distinct suspicion of a cystic area in the neck of the third metacarpal, and possibly in the neck of the fourth.

Nothing abnormal was detected in radiographs of the hands, wrists and chest on January 24, 1953.

Case 2. A.K., a girl, was born on December 11, 1953, of a normal delivery (birth weight 7 lb. 10 oz.). She was admitted under the care of Dr. Margaret Egan on the 14th day of life because of a reluctance to feed, persistent vomiting and failure to thrive.

She was the first child, and there was no consanguinity; one distant relative had diabetes.

On examination the child was wasted and deeply jaundiced; the liver was enlarged, extending to below the umbilicus, the spleen was not palpable. There was a purulent vaginal discharge.

The immediate relevant laboratory data were as follows: serum bilirubin 14·2 mg. per 100 ml., Coombs test (direct) negative; Wassermann reaction negative. A blood film was normal without spherocytosis; haemoglobin 130%. The urine contained protein and a reducing substance, shown by chromatography to be galactose.

The condition of the infant deteriorated and intravenous plasma with N/5 saline and 5% dextrose was given: on January 2, 1954 (22nd day of life) the diagnosis of galactosaemia was made and a modified diet was instituted. This consisted of feeds of 5% glucose and 'casilan' and was given for several days; the child's general condition did not improve to any appreciable extent and early bilateral central cataracts were noted for the first time. In addition a reducing substance was still to be found in the urine.

The signs and symptoms regressed when the lactose-free diet was introduced and her general condition had improved greatly by the time she was transferred on February 12 to St. Mary's Hospitals for further studies.

On admission she weighed 8 lb. 5 oz., her general condition was fair, the liver was enlarged two fingers below the costal margin but the spleen was not felt. A small cataract was still to be seen in the right eye; the left eye was completely clear. Investigations were began and the child made a slow but steady improvement. By March 15 the cataract in the right eye had gone and her motor development seemed to be perfectly normal. The liver was still palpable and she had one large fluid stool daily. She continued in good health until April 12 (five days after the completion of an eight-day régime of milk feeding) when she had a sharp attack of gastro-enteritis which demanded intravenous plasma, 10% dextrose and Hartmann's solution. She was given streptomycin, 4 × 125 mg., and recovered quickly. Following this her progress was excellent and she returned home on May 4.

She has been under constant supervision since that date and has had two further admissions to hospital for additional observations.

She was seen on January 14, 1955 (aged 13 months), and her weight was 18½ lb. She walked and talked a little, was very active and happy and had a perfectly normal intelligence. Her general condition was good, the liver was not palpable, there was no evidence of cataracts and the urine examination revealed no abnormality.

She was also seen in July, 1955, a healthy, happy child weighing 23 lb. Her intelligence was tested at the time and found to be perfectly normal.

Routine Investigations of Case 2

The galactose tolerance test and glucose tolerance test are described in the sections on carbohydrate studies.

In February, 1954, thymol turbidity was 6 units, serum alkaline phosphatase 23 units/100 ml., serum albumin 3·74 mg. %, and serum globulin 2·61 mg. %. (The child was not fully treated at this stage.)

Full blood counts were all within normal limits and the clotting time, off milk, was 3 minutes, and on milk it was 5 minutes.

A radiograph of the skeleton was normal and the renal function tests will be discussed in the section on urinary investigations.

Carbohydrate Studies

We now present the results of investigations carried out on the two children while on milk or galactose feeding. The observations were made on five separate occasions (see Appendix).

Glucose Tolerance Tests. Glucose tolerance tests were carried out on both children whilst they were on milk-free diets (Table 1). Both curves are somewhat flat but the amount of glucose given was relatively small (1·25 g. per kg.).

TABLE 1
GLUCOSE TOLERANCE TESTS

E.F. November 28, 1951, Given 5·5 g. Glucose (mg. per 100 ml.)		A.K. February, 1954, Given 5·5 g. Glucose (mg. per 100 ml.)
Fasting	65	80
30 min.	95	95
60 ,,	83	95
90 ,,	79	85
120 ,,	73	60

Galactose Tolerance Tests. These were abnormal with galactose indices above 600 (Fig. 1). In one child (E.F.) the test was repeated at 2 years of age and showed no change for the better.

The depression of the blood glucose levels reported by several authors (Hudson *et al.*, 1954; Townsend *et al.*, 1951) was noted and on two occasions pallor, sighing respirations and sweating were observed during the investigation; in one case (A.K.) we are certain that the symptoms were related to a hypoglucosaemia (18 mg. per 100 ml.).

In the child A.K. the galactose tolerance test was repeated with the administration of 7 g. glucose simultaneously with the galactose (Fig. 2). There was a definite modification of the curve (Bruck and Rapoport, 1945; Greenman and Rathbun, 1948; Wagner, 1943) with a slower rise of galactose to a considerably lower level, and a smaller quantity of galactose was excreted

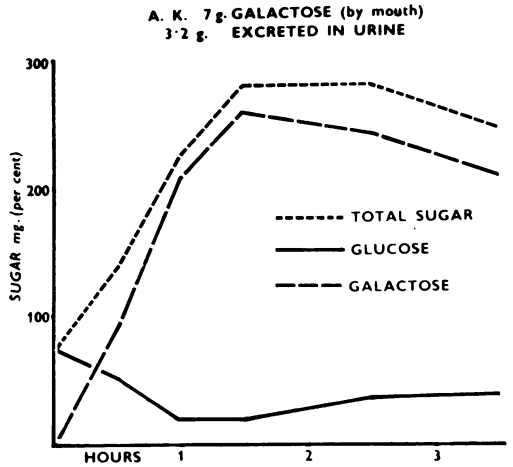


FIG. 1.—Curves of galactose tolerance test.

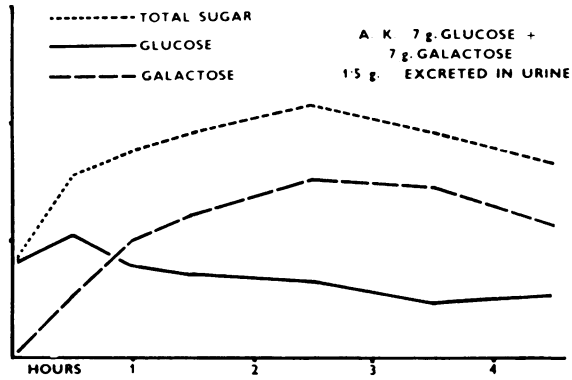


FIG. 2.—Curves of galactose plus glucose tolerance test.

in the urine. This probably was due to a slower absorption of galactose from the intestine in the presence of glucose (Cori, 1926), possibly permitting an improved utilization of galactose by the tissues.

Chromatography of Blood Sugars. On a galactose-free diet only glucose was detected but on a milk diet both glucose and galactose were found. No lactose was found in 0·15 ml. blood, indicating that the maximum blood lactose level was below 16 mg. per 100 ml.

Blood Glucose and Galactose Levels. No galactose was found either in the blood or in the urine when the children were on galactose-free diets, and the blood glucose levels, determined immediately before and two hours after feeds, ranged between 92 mg. and 113 mg. per 100 ml. blood.

An appreciable quantity of blood galactose was detected after 24 hours of milk feeding and the level continued to rise throughout the eight days of the observation (Table 2).

TABLE 2
BLOOD GLUCOSE AND GALACTOSE LEVELS IN A.K. ON A MILK DIET

Day	Date	Time	Glucose (mg./100 ml.)	Galactose (mg./100 ml.)
1	31/3	11 a.m.	97	56
	,,	4 p.m.	73	61
2	1/4	4 p.m.	89	47
3	2/4	10 a.m.	63	97
	,,	4 p.m.	76	91
6	5/4	10 a.m.	43	152
	,,	4 p.m.	61	162
7	6/4	10 a.m.	68	113
10	9/4	10 a.m.	100	Nil two days after cessation of milk

Blood collected by heel prick.
(a) at 10 a.m.—just before 10 o'clock feed.
(b) at 4 p.m.—two hours after last feed.

The blood glucose levels, although appreciably lower, were not as low as other writers have reported. This was probably due to the addition of glucose (1 drachm (3·9 g.) to 4 oz. feed) to the milk feeds.

Once the milk feeding ceased, the blood galactose levels dropped rapidly and in one observation the level was zero 15 hours after the termination of a 10-day spell of galactose feeding, while in the other child galactose was not detected either in the blood or urine 24 hours after the milk diet had ceased.

The amounts of galactose excreted in the urine are listed below (Table 3). There is a steady rise in the

TABLE 3
GALACTOSE INTAKE AND EXCRETION IN A.K. DURING EIGHT DAYS ON A MILK DIET

Day	Date	Galactose (g.) Taken by 3 p.m.	Galactose (g.) Excreted by 3 p.m.	% Excreted	% Retained
1	30–31/3	15·2	4·6	30	70
2	31–1/4	18·9	3·7	20	80
3	1–2	17·7	2·7	15	85
4	2–3	15·9	4·5	28	72
5	3–4	16·5	6·6	40	60
6	4–5	17·1	6·4	37	63
7	5–6	17·7	6·5	37	63
8	6–7	15·2	9·6	63	37
9	7–8	Nil			
10	8–9	Nil	Nil	—	—

excretion of the hexose until the end of the milk or galactose feeding whereafter there is a rapid fall in the amount of sugar in the urine. In one case no galactose was found in the urine during the second day after the test period. In both cases the blood galactose level returned to zero within 24 hours.

Assuming that the lactose is completely absorbed from the gut it is possible to assess the proportion of galactose retained or utilized by the patient (Table 3). It is immediately obvious that an appreciable proportion of the hexose is metabolized and this observation supports the statements of Bruck and Rapoport (1944) and Cusworth, Dent and Flynn (1955).

The high value on day 8 may be due to the timing of micturition in relation to the start and finish of the day's collection. The volume of urine passed on day 8

The amino-acid spots are drawn in the form of circles, the relative sizes of the circles giving some indication of the strength of ninhydrin colour and hence of the quantity of each amino-acid. The urine has been applied at the right-hand bottom corner. Phenol has been run as first solvent from right to left followed by collidine in an upward direction.

Identification of the spots.

1. Cysteic acid. 2. Aspartic acid. 3. Glutamic acid. 4. Serine. 5. Glycine. 6. Threonine. 7. Alanine. 8. Glutamine. 9. Tyrosine. 10. Phenylalanine. 11. Leucine. 12. Valine. 13. Alpha-amino-n-butyric acid. 14. Methionine. 15. Histidine. 16. Citrulline. 17. Beta-amino-isobutyric acid. 18. Methylhistidine. 19. Lysine. 20. Taurine.

Fig. 3

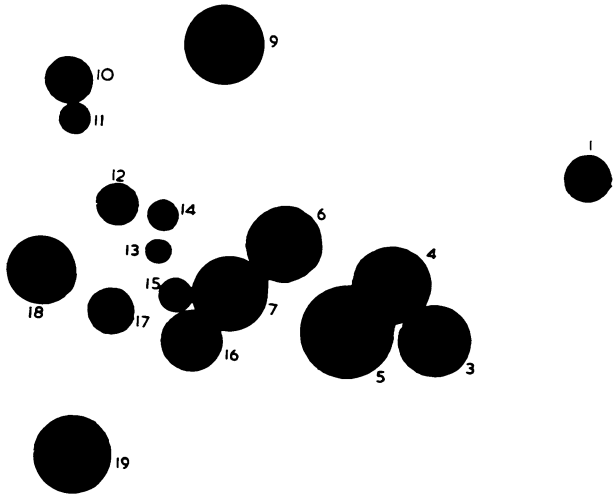


FIG. 3.—Urine amino-acid chromatogram in an untreated case of galactosaemia.

Fig. 4

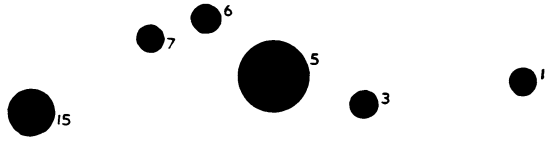


FIG. 4.—Urine amino-acid chromatogram before galactose feeding in A.K. aged 3 years 4 months.

Fig. 5

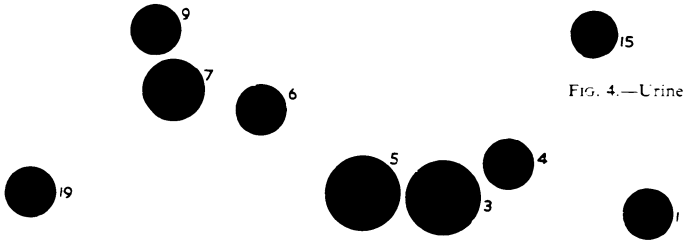


FIG. 5.—Urine amino-acid chromatogram after four days galactose feeding: 24 g. a day added to the diet.

Fig. 6

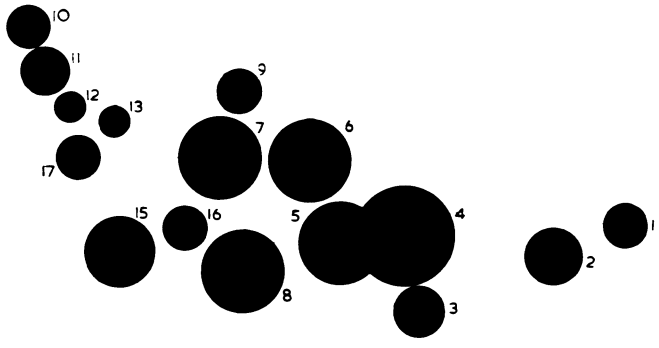


FIG. 6.—Urine amino-acid chromatogram after seven days of galactose feeding: 24 g. a day added to the diet.

Fig. 7

FIG. 7.—Urine amino-acid chromatogram two months after return to milk-free diet.

(413 ml.) is greater than that on day 7 (315 ml.) and the previous daily average (326 ml.); hence we would expect more galactose to be passed.

Results of Urinary Investigations

The presence of a gross aminoaciduria in untreated cases of galactosaemia was first described in 1952 (Holzel, Komrower and Wilson), and several workers have confirmed these observations (Bickel and Hickmans, 1952; Bickel and Thursby-Pelham, 1954; Darling and Mortensen, 1954; Cusworth *et al.*, 1955; Hsia, Hsia, Green, Kay and Gellis, 1954). The amino-acid pattern has been essentially similar in all cases, with a predominance of the neutral simple aliphatic chain type, i.e., serine, glycine, alanine, threonine, glutamine and valine; in addition phenylalanine, lysine, cystine, glutamic acid, methylhistidine, tyrosine, and amino-iso-butyric acids have been detected in the urine of these infants (Fig. 3).

In both our cases it was possible to restore the urine amino-acid pattern to normal by offering the infant a lactose- or galactose-free diet, and also to reproduce the abnormal picture by introducing adequate amounts of milk or galactose into the feed. In one child (E.F.) this observation was repeated at the age of 3 years 4 months after an interval of two years (Figs. 4, 5, 6, 7).

The serial urine chromatograms and daily estimations of total urinary amino-nitrogen show that the outpouring of amino-acids begins on the fifth or sixth day of milk or galactose feeding and continues to increase up to the tenth day (no investigations have continued longer than this). The amino-aciduria declines quickly once the milk feeding ceases and the chromatogram picture takes approximately one week to return to normal (Fig. 8).

The daily urine total nitrogen value remains fairly constant as does the urine urea-nitrogen (Fig. 9); there is, however, a marked increase in the daily urine amino-nitrogen output during the feeding experiment.

A small number of plasma and blood α amino-nitrogen levels were estimated (Table 4); some have been normal

TABLE 4
BLOOD AND PLASMA AMINO-NITROGEN LEVELS

E.F. aged 6 months	Plasma Amino-nitrogen (mg. per 100 ml.)	Urine
Milk diet	3·8	Chromatogram very abnormal
aged 18 months (Milk-free diet)	5·5	Chromatogram normal
A.K. aged 3 months	**Blood Amino-nitrogen (mg. per 100 ml.)**	**Urine Amino-nitrogen**
(Milk-free diet)	9·1	32·1 mg. per 24 hours
(Milk diet for eight days)	10·1	520 mg. per 24 hours

We are indebted to Dr. C. E. Dent for the estimations of plasma α amino-nitrogen (method of Hamilton and Van Slyke, 1943).

but the blood levels estimated by the method of Frame, Russell and Wilhelmi (1943), which is not completely specific, were slightly raised. There is, however, final evidence (Hsia *et al.*, 1954; Cusworth *et al.*, 1955) to confirm the original suggestion (Komrower, 1953) that there is a renal amino-aciduria with normal plasma levels of α amino-nitrogen.

Proteinuria. This has been reported in all the early cases of galactosaemia described in the literature; in our observations it appeared without fail within 48 hours of milk or galactose feeding and increased steadily until the end of the régime, substantial quantities of protein being excreted. The proteinuria diminished rapidly following the cessation of the milk feeding and disappeared after four or five days (Fig. 10). Electrophoresis of these urines revealed the normal range of plasma proteins.

Blood Urea. Blood urea determinations were made on three occasions and in each instance the result was within normal limits (24 mg.-33 mg. per 100 ml. blood).

Specific Gravity. The specific gravity of the urines varied considerably and revealed an ability to dilute and concentrate normally (Child E. F., fasting, sp. gr. 1018 and 1011; one hour after a feed sp. gr. 1001. We only obtained random determinations on child A.K. and the range was sp. gr. 1001-1013). The daily excretion of creatinine was not affected by the giving of milk or galactose (Fig. 11).

Excretion of Sodium, Potassium and Salt. A single study was made on child A.K. on November 19, 1954. The same daily diet was given all through the period of observation and she did not vomit on any of the three collection days; all the diet was taken each day. The child's motions were normal, apart from three loose stools during the last two hours of the final day.

There does not appear to be any significant alteration in the urinary excretion of sodium or potassium during the feeding experiment (Table 5).

TABLE 5
URINARY EXCRETION OF POTASSIUM, SODIUM AND SALT DURING A FEEDING EXPERIMENT USING GALACTOSE IN A.K. AGED 1 YEAR

Day	24 hour Urine Volume (ml.)	K (g.)	Na (g.)	NaCl (g.)
0	510	0·66	1·7	3·2
5	451	0·5	1·5	1·8
10	515	0·52	1·9	2·8

Acidification of the Urine. Both children showed a normal renal mechanism of acidification when given ammonium chloride (Table 6).

All these investigations suggest that there is no established renal damage in cases of galactosaemia that are treated efficiently and in these two children, who are respectively 4 years and 20 months old, there is no clinical evidence of renal impairment nor do the usual laboratory tests suggest it.

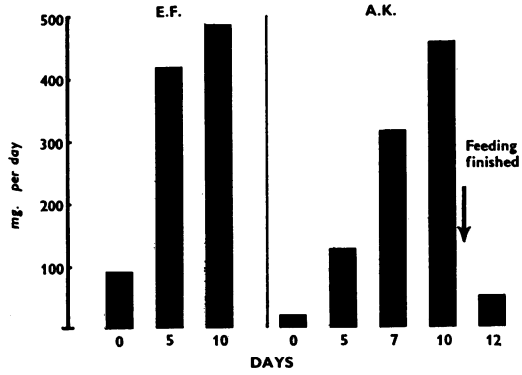


FIG. 8.—Daily urinary amino-nitrogen levels during feeding observations with galactose (E.F.) and milk (A.K.).

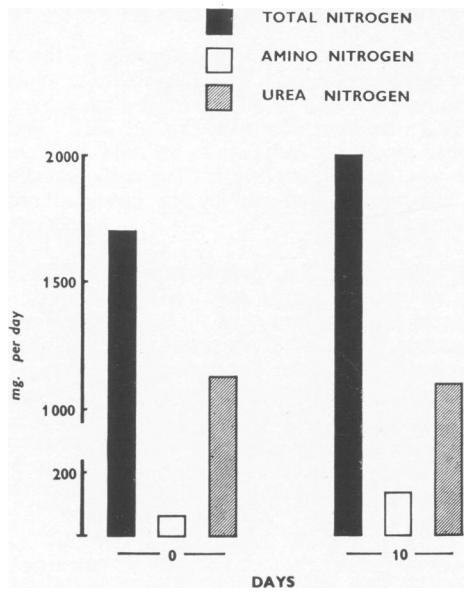


FIG. 9.—Comparison of daily urinary total nitrogen, urea-nitrogen and amino-nitrogen during a period of administration of galactose in the diet (E.F.).

TABLE 6
ACIDIFICATION OF URINE ON MILK-FREE AND GALACTOSE DIETS

	Urine *p*H			
E.F. Test dose NH4Cl. 2 g.	Before Test Dose	After 1 Hour	After 2 Hours	After 3 Hours
Milk-free diet ..	6·4	5·4	5·6	5·2
Galactose diet (20 g. daily) ..	6·7		5·6	5·4
A.K. Test dose NH4Cl. 0·5 g. Milk-free diet ..	6·4	5·4	5·3	5·2

Acidosis. Although acidosis is included among the results of routine investigations by some workers, its presence was not commented upon until Arthurton and Meade (1954) suggested that it might be of renal origin. In the case described by Bruck and Rapoport (1945) a hyperchloraemic acidosis was noted but not discussed further.

An acidosis was noted in the elder child (E.F.) when she was 12 months of age and in the second child (A.K.) when she was 3 months old. The blood bicarbonate level returned to normal when a milk- or galactose-free diet was resumed (see Table 7). The urine *p*H determinations suggested the possibility of a renal acidosis similar to that found in hyperchloraemic renal acidosis, and we repeated the investigations at 3 years 4 months (E.F.) and 12 months (A.K.) hoping that this point would be clarified.

The results were unsatisfactory as we were unable to reproduce the acidosis in the 10-day period of galactose feeding to which we were limited. (We considered that any longer period would be unsafe.)

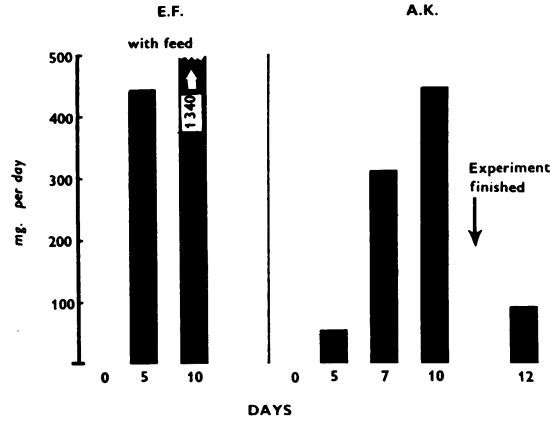


FIG. 10.—Daily proteinuria during feeding observations with galactose (E.F.) and milk (A.K.).

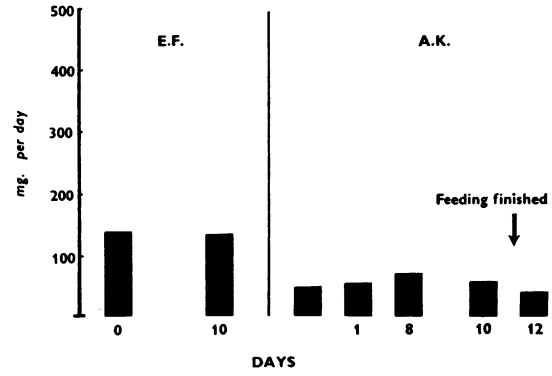


FIG. 11.—Daily excretion of urinary creatinine during feeding observations with galactose (E.F.) and milk (A.K.).

TABLE 7

PLASMA CO_2-COMBINING POWER ON MILK-FREE AND MILK DIETS

		Plasma CO_2 Combining Power (vol. per 100 ml.)	Urine *p*H
E.F.			
Galactose 20 g. daily × 10	June 27	26	
	June 28	29	6·2
Milk/galactose-free diet	Aug. 1	45	6·6
A.K.			
Milk/galactose-free diet	Mar. 10	62	6·42
Milk diet 8 days ..	Apr. 6	25	7·00
Milk/galactose-free diet	Apr. 27	50	5·63

The results, nevertheless, do give evidence (E.F., October 22, 1954) of an increase of serum chloride, diminished urine titratable acidity and ammonia content, with an increased urinary bicarbonate, suggesting that the acidosis may well be of renal origin (Table 8).

TABLE 8

SERUM AND URINE BIOCHEMICAL FINDINGS IN A GALACTOSE FEEDING OBSERVATION

		11.10.54 No Galactose	18.10.54 5 Days Galactose	22.10.54 10 Days Galactose
Serum	Cl. (mg. per 100 ml.)	608		632
	CO_2-combining power (vol. per 100 ml.) ..	54		50
Urine	NH_4 (mg. per 100 ml.)..	134	79	45
	Titratable acidity (Folin) (ml./100 ml.)	81	41	34
	$NaHCO_3$ (mg./100 ml.)	57	52	138
	Volume (ml.) per day ..	290	400	445

Blood and Plasma Phosphate Determinations

Quantitative estimations of inorganic and total acid-soluble phosphate in whole blood and in plasma were made on one occasion before and during galactose feeding.

The methods used were those of Kuttner and Cohen (1927) for the inorganic P and of Fiske and Subbarow (1925) for total acid-soluble P; the difference between these figures gave us the ester P figure. Thus it was possible to calculate the inorganic and ester P of the erythrocytes based on an assumed haematocrit of 45%. The selection of this figure for purposes of calculation was felt to be justified because, on the one hand, there was no evidence of haemoconcentration or any reason to suspect it and, on the other, any changes in the P content of the erythrocytes would be underestimated (Table 9).

The figures show a definite reduction in the amounts of inorganic and ester P in the erythrocytes after a régime of galactose feeding (Fig. 12).

The galactose-l-phosphate was estimated at the same time (Schwarz, Golberg, Komrower and Holzel, 1956) and showed a considerable increase (Table 9).

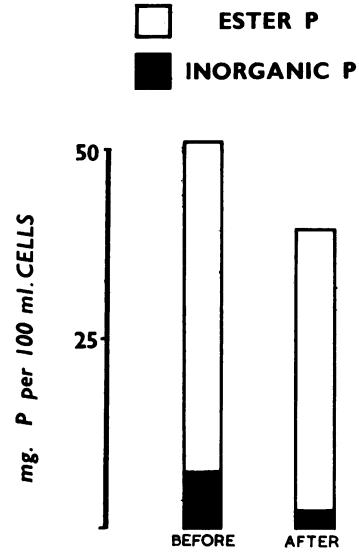


FIG. 12.—Inorganic and ester P in the erythrocytes before and after a period of milk diet (A.K.).

TABLE 9

BLOOD AND PLASMA P DETERMINATIONS IN A.K. (12 MONTHS) BEFORE AND AFTER ADMINISTRATION OF GALACTOSE

		Before Galactose (mg. per 100 ml.)	After Galactose (mg. per 100 ml.)
Blood	Inorganic P ..	6·62	4·14
	Total P	26·65	21·49
	Ester P	20·03	17·35
Plasma	Inorganic P ..	6·35	5·79
	Total P	6·55	7·25
	Ester P	0·20	1·46
	assuming a haematocrit of 45%, erythrocytes would contain		
R.B.C.	Inorganic P ..	6·96	2·13
	Ester P	44·27	36·78
Blood	Galactose-l-phosphate (free ester) ..	3·0	19·0

Studies of Erythrocyte Metabolism

A detailed description of the work carried out on certain aspects of erythrocyte metabolism in galactosaemia has been published (Schwarz *et al.*, 1956), and a summary of the findings will suffice here.

The oxygen uptake of normal erythrocytes is increased by the addition of galactose to a medium containing sub-optimal amounts of glucose. No such increase is observed with the erythrocytes of the galactosaemic patient.

The child's red cells after milk feeding have a lower oxygen uptake on a glucose substrate than the erythrocytes of the same individual before milk feeding is started (Fig. 13). Thus there is a partial inhibition of metabolism of red cells on the milk diet; furthermore, an accumulation of galactose-l-phosphate was found in these erythrocytes and this observation was paralleled

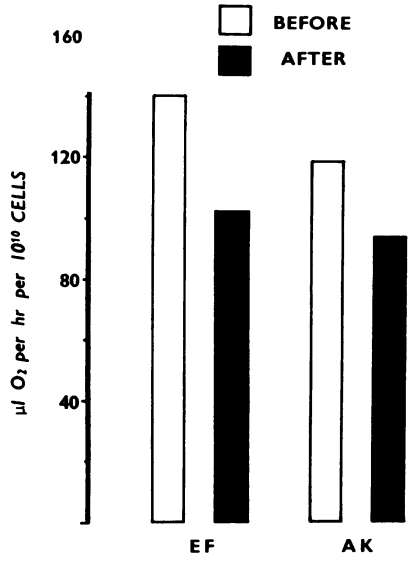


FIG. 13.—Respiration of the erythrocytes from galactosaemic patients before and after milk or galactose feeding.

in vitro when erythrocytes from galactosaemic patients were incubated in galactose-containing media (Fig. 14).

We have already stated that quantitative determination of inorganic and total acid-soluble phosphorus and, by difference, ester phosphorus in whole blood and plasma has revealed a fall in inorganic phosphate and total red cell phosphate esters and these changes lend support to the suggestion that normal metabolic processes are partially inhibited.

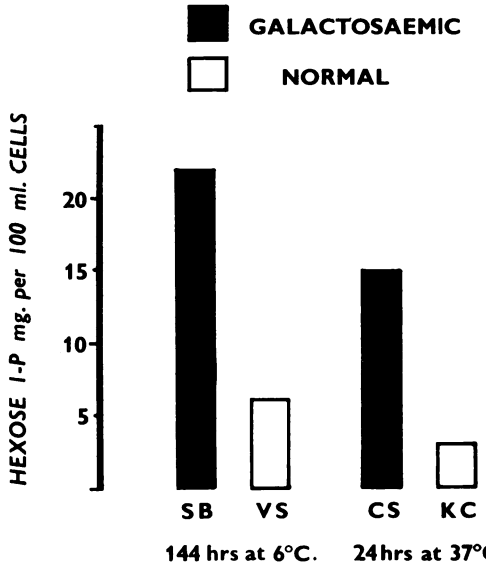


FIG. 14.—The accumulation of galactose-l-phosphate in normal and galactosaemic erythrocytes after incubation with galactose *in vitro*.

Discussion

It has frequently been suggested that galactose is a toxic substance (Cox and Pugh, 1954; Mitchell and Dodge, 1935; Bray *et al.*, 1952; Bruck and Rapoport, 1945; Donnell and Lann, 1951; Guha, 1931; Nelson, 1954) and that the clinical symptoms of galactosaemia are the result of galactose poisoning due to the accumulation of the sugar in the blood and tissues. It is difficult to verify this *in vivo* as it has been found impossible to produce blood levels of galactose comparable with those in galactosaemia by feeding the sugar to normal adults or near normal infants. From the work presented in this communication it seems possible that the classical symptoms of the disease may result from a disturbed cell metabolism due to the accumulation of galactose-l-phosphate in the tissues.

The presence of amino-aciduria, proteinuria and metabolic acidosis indicates a disturbance of the renal tubule which is, however, quickly corrected when milk or galactose is removed from the diet. The renal tubular upset does not extend to the excretion of potassium, sodium and water: the only careful observation we have been able to make of the salt and water excretion does not show any significant variation in the daily urinary excretion of these substances. One may postulate either a dysfunction of a particular portion of the renal tubule or a generalized disturbance of such a degree that only certain functions are affected.

It is also necessary to take into account the maturation of the kidney; the fact that, as the children grow older, it has proved easy to produce the proteinuria and amino-aciduria but not the acidosis, suggests a more rapid maturation of the tubule in respect of acid-base balance which is not paralleled in respect of protein and amino-acid re-absorption. In this context it is interesting to note that the average age of onset of idiopathic renal acidosis is approximately 6 months and that the disorder has corrected itself by the end of the second year of life (Latner and Burnard, 1950).

The figures for galactose excretion and retention indicate that an appreciable amount of hexose is utilized by the affected individual. This would suggest that the metabolic block is incomplete and hence it is likely that the severity of the defect will vary from one patient to the next. This is borne out in our clinical experience as there is considerable variation in the time of onset of symptoms and also in their severity. The two infants described here became ill during the first 10 days of life and, in our opinion, would have died had they not been treated promptly; there are other cases in our experience and in the literature where the symptoms did not

appear until 3 or 4 months of age or where the child presented in the second year of life with mental retardation, hepatomegaly and cataracts.

The question arises as to the mechanism by which the galactose is metabolized in these children. It is possible that the metabolism proceeds along the normal pathway although this channel is incapable of coping with the absorbed galactose quickly enough to prevent an elevation of the blood galactose level with consequent urinary excretion and accumulation in the tissues. Alternatively, a different metabolic pathway might be involved if the normal mechanism is completely inoperative, but this, too, is unable to deal with galactose at a satisfactory rate.

Hypoglycaemia has been reported by several investigators to follow the administration of milk or galactose to a galactosaemic patient (Bruck and Rapoport, 1945; Cusworth *et al.*, 1955; Hudson *et al.*, 1954; Mason and Turner, 1935; Norman and Fashena, 1943). We have shown that the blood glucose may fall precipitously during a galactose tolerance test, but that on an ordinary milk diet the degree of hypoglycaemia is slight. It is very unlikely that any of the symptoms of galactosaemia could result directly from this mild hypoglycaemia, when similar symptoms are not apparent in conditions of more severe hypoglycaemia. On the other hand, the relative deficiency of glucose might well aggravate a condition arising from the partial inhibition of glucose metabolism. The aetiology of the hypoglycaemia associated with galactosaemia is still obscure, but in accordance with our hypothesis the galactose-l-phosphate, which would be expected to accumulate in the liver more rapidly than elsewhere, might well inhibit glycogenolysis and thus lead to a lowering of the blood sugar.

On account of the possibly disastrous fall in blood glucose during a galactose tolerance test, the diagnosis of galactosaemia should be made by other means whenever this can be done and the galactose tolerance test only used when the diagnosis is still in doubt.

We have not found any galactose in the blood of treated cases (c.f. Hartmann, Grunwaldt and James, 1953), and believe that the absence of the sugar is attributable to the careful exclusion of all lactose from the diet in our cases.

It has been shown elsewhere that the metabolic defect of galactosaemia is present in erythrocytes (Schwarz *et al.*, 1955). Recently Kalckar, Anderson and Isselbacher (1956) have determined the uridyl transferase content of the erythrocytes from normal and galactosaemic infants, and have established that the enzyme is absent from the red cells of the latter. It is very likely, therefore, that it is the deficiency of this enzyme which is responsible for the faulty metabolism of galactose in the liver. The presence of the specific defect in erythrocytes suggests that many other tissues may be similarly affected. Since exposure of galactosaemic erythrocytes to galactose, *in vivo* or *in vitro*, results in the accumulation of galactose-l-phosphate and in a partial inhibition of the normal glucose metabolism of these cells, a similar process may be imagined to take place in other tissues. In this way a curtailment of energy-yielding reactions may account for the observed dysfunction of kidney, liver, brain and lens tissues.

The evidence obtained from experiments *in vitro* with erythrocytes suggests that galactose itself has no toxic action on normal cells. It may well be that the accumulation of galactose-l-phosphate, which is peculiar to galactosaemic cells, is responsible for the partial inhibition of glucose metabolism. Thus the actual toxic agent may prove to be galactose-l-phosphate, or a metabolite derived from it. This would explain why some of the signs of galactosaemia, e.g., the amino-aciduria, persist for several days after the cessation of galactose feeding, although the blood galactose level has already returned to zero. Similarly, the amino-aciduria does not appear until four or five days after the beginning of galactose feeding, although blood levels are built up rapidly and galactose is excreted in the urine from the first day.

According to this concept it is the relative amounts of galactokinase and of the uridyl transferase and galactowaldenase system in a particular tissue which determine the rate of accumulation and disappearance of galactose-l-phosphate, and since there may be local variations in the ratio of these enzymes, one would expect a greater accumulation in a tissue where the ratio is high and hence a disturbance of function of that particular tissue.

The hypothesis presented appears to give a reasonable explanation of the widespread and frequently reversible symptoms and signs of galactosaemia which are described in our two cases and in those previously reported by other authors.

Summary

Two further cases of galactosaemia are presented with the results of clinical and biochemical investigations.

The effect of milk or galactose feeding for eight- or 10-day periods is described, with particular reference to blood glucose and galactose levels, galactose excretion, renal function and blood and plasma phosphorus levels.

Reference is made to the work carried out in

certain aspects of erythrocyte metabolism in this disease.

The causation of the signs and symptoms of the disease is discussed in the light of the findings reported in this communication. The hypothesis is put forward that the varied manifestations of galactosaemia may be attributed to localized accumulation of galactose-l-phosphate within the tissues, and that the extent to which such accumulation occurs—and consequently the resulting lesion or impairment of function—is determined by the ratio of local concentration of enzymes concerned with the early stage of galactose metabolism.

We are particularly indebted to Mr. H. Varley (Manchester Royal Infirmary) and Miss V. K. Wilson (Royal Manchester Children's Hospital) for carrying out certain biochemical investigations, to Dr. S. W. Stanbury for his help and valuable criticism and to the Department of Medical Illustration for photographs and reproductions; also to Dr. Margaret Egan who so kindly sent to us the second case. We wish to thank Miss Anne Walton, recently sister-in-charge Infants' Ward, St. Mary's Hospitals, Manchester, who was in charge of these children throughout the investigations. Finally, we wish to record our appreciation of the support we received from Professor A. C. P. Campbell and Professor W. F. Gaisford.

One of us (V.S.) was assisted by a grant from Cow and Gate Limited, Guildford, Surrey.

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APPENDIX

The following are details of the feeding régimes during the periods of observation.

E.F.

I Jan. 1953 (aged 18 months). Wt. 19 lb. 1 oz. 20 g. of galactose added daily to lactose-free toddlers' diet.

II 11.10.54 (aged 3 years 4 months). Wt. 23 lb. 24 g. of galactose daily given in divided doses for 10 days.

A.K.

III 30.3.54 (aged 14 weeks). Wt. 9 lb. 14 oz. Half-cream National dried milk: 5½ oz. × 5 daily, for 8 days (1 drachm (3·9 g.) glucose added to each 5½ oz. feed).

IV 21.6.54 (aged 6 months). Wt. 12 lb. 5 oz. Galactose 15 g. daily added in divided doses to the special feed for six days. The special feed contained egg, 'farex' and dextrimaltose and had a high carbohydrate content.

V 20.11.54 (aged 11 months). Wt. 17 lb. 8 oz. Galactose 20 g. daily for four days, increased to 24 g. daily for a further six days, while child was on a weaning diet plus special feed described above.

On all occasions a detailed statement was made about the food intake and rejection; all the galactose was given and any vomited was replaced.