

## Some Disturbances of Erythrocyte Metabolism in Galactosaemia

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Galactosaemia is an inborn error of metabolism involving a specific inability to metabolize galactose normally. The affected infants show failure to thrive, enlargement of the liver and spleen, vomiting, jaundice, proteinuria, aminoaciduria, a high blood-galactose level and consequent excretion of large quantities of this sugar in the urine. These disturbances may be avoided or overcome by the early withdrawal of all sources of dietary galactose. Thereafter, the administration of galactose in any form causes this sugar to reappear in blood and urine in significant amounts. If the condition should remain untreated for any length of time death may ensue, whilst the survivors may develop cataract, cirrhosis of the liver and mental retardation.

Published reports throw no light on the exact nature of the basic biochemical lesion which is responsible for the syndrome, nor on the possible connexion between the accumulation of galactose in the body and hepatic, renal, cerebral and other disturbances. In an attempt to study these aspects we have sought to use those cells of the patient which are most readily available, the erythrocytes. Since the mammalian erythrocyte is known to metabolize galactose (Katayama, 1926; Feigelson & Conte, 1954; Nossal, 1948) there was ground for the hope that the presence of a biochemical defect in hepatic parenchymal cells might be shared, at any rate to some degree, by the erythrocytes. We were thus led to compare the metabolism of galactosaemic red cells with that of normal cells under the same conditions.

### EXPERIMENTAL

*Respiration of erythrocytes.* Heparinized venous blood (7–10 ml.) was kept in ice until required, when the cells were separated by centrifugation, washed twice with ice-cold Krebs–Ringer phosphate buffer, pH 7.35, and made up to 21 ml. in ice-cold buffer containing adenosine triphosphate (ATP) and methylene blue (final concentrations mm and 3.57 mg./100 ml., respectively) (Feigelson & Conte, 1954). Measured fractions of the suspension (3.0 ml.) were transferred to Warburg flasks for use in respiration studies. The gas phase was air. The substrate (glucose or galactose) was added after an equilibration period of 15–20 min. during which the endogenous respiration ceased. The  $O_2$  uptake was measured at 37° for 1–3 hr., except where stated otherwise. During this time the respiration rate was constant. The cells were counted in a fraction of the sus-

pension and the  $O_2$  uptake was calculated for  $10^{10}$  cells, unless otherwise stated. No attempt was made to separate the red and white cells after it had been established that, in the presence of methylene blue, the  $O_2$  uptake of red cells alone was the same as that of red cells plus buffy coat.

*Incubation of erythrocytes prior to metabolic studies.* Blood was collected under sterile conditions and 6 ml. were placed in each of two siliconed screw-cap bottles containing 4.0 ml. sterile Krebs–Ringer phosphate buffer, pH 7.38, and 4 mg. glucose. In addition, one of the two bottles contained 16 mg. galactose. The bottles were kept at 6° for 6 days, with gentle inversion twice daily. In a second experiment, the quantities used were 6 ml. buffer, 12 mg. glucose and 24 mg. galactose, and the bottles were kept at 37° for 24 hr. After incubation the suspensions were centrifuged, the cells were washed with buffer and finally suspended in 10 ml. buffer. Portions were taken for measurement of respiration, phosphate studies and haemoglobin estimation, the last in order to confirm the presence of equal numbers of cells in the glucose and galactose solutions.

*Isolation and chromatography of phosphate esters.* Whole blood was deproteinized with 10% (w/v) trichloroacetic acid; erythrocytes were haemolysed with water by freezing and thawing, and the solution was deproteinized. The filtrate was fractionated into water-soluble and -insoluble Ba salts (LePage & Umbreit, 1946). The Ba salts were dissolved in 0.1 N-HCl, the  $Ba^{2+}$  was removed as  $BaSO_4$  and the solutions were neutralized to litmus with  $NH_4OH$ .

Portions equivalent to 0.2–0.3 ml. blood were placed on Whatman no. 1 or no. 54 paper, previously washed with dil. HCl or ethylenediaminetetraacetic acid. The chromatograms were run in *n*-propanol–ammonia–water (60:30:10, by vol.) (Hanes & Isherwood, 1949) for 24 hr., after stapling some folded filter paper, 32 cm. wide, to the bottom of the sheet to act as solvent absorber. The chromatogram was air-dried and then rapidly drawn through acid molybdate reagent in acetone (Burrows, Grylls & Harrison, 1952). It was heated for 3 min. in an oven at 85°, placed under an ultraviolet lamp for 10 min. and finally exposed to water vapour, when the blue spots due to hydrolysed phosphate appeared. The amounts of glucose phosphate and galactose phosphate were estimated visually by comparison with known amounts of glucose 1-phosphate run simultaneously. The method of visual estimation was considered to be accurate to  $\pm 25\%$ , but an attempt was always made to give minimum estimates of high values and maximum estimates of low values. Whereas the latter were generally accepted as representing hexose 1-phosphate, high values were always checked against visual estimates of the amounts of free sugars liberated by acid hydrolysis and chromatographed (see below). The lower of the two estimates was taken as the actual value, in spite of the small loss inevitably incurred in the hydrolysis and in the extraction of the dry residue for application to the paper chromatogram.

*Identification of sugar moiety of hexose 1-phosphate.* Portions of the water-soluble Ba salt fractions were hydrolysed with 0.1N-HCl for 7 min. at 100°; the solutions were neutralized, evaporated to dryness, and the residues extracted with pyridine and chromatographed in butanol-pyridine-water (3:2:1.5, by vol.) (Jeanes, Wise & Dimler, 1951). Alternatively, the phosphate fraction was chromatographed as described above, the hexose 1-phosphate area was eluted with water, the eluate hydrolysed, taken to dryness and the residue extracted with pyridine as above. The dry chromatogram was developed with the benzidine-trichloroacetic acid reagent in acetone (Harris & MacWilliam, 1954), and after heating for a few minutes at 100°, it was examined in ultraviolet light. In this way less than 0.5 µg. of hexose could be detected easily and estimated approximately by comparison with known amounts of glucose and galactose.

*Determination of hydrolysis constant of galactose 1-phosphate isolated from erythrocytes (Wilkinson, 1949).* The water-soluble Ba phosphates extracted from galactosaemic erythrocytes were chromatographed on a broad sheet of paper and the area containing the hexose 1-phosphate fraction was eluted with water. The eluate was heated for 30 min. with 0.1 vol. of N-KOH, neutralized and treated with barium acetate: the supernatant was treated with 2 vol. of ethanol and the precipitate, after one reprecipitation, was dissolved in water. After addition of HCl to 0.25N, the solution was incubated at 25° and the inorganic P determined in portions after 3 and 6 hr. The total amount of galactose 1-phosphate was determined by heating the solution at 100° for 7 min. A modification of the method of Fiske & Subbarow (1925), using a final volume of 3.2 ml., was adopted.

*Determination of inorganic and ester P in erythrocytes.* When only small volumes of blood were available for analysis (patient A.K., Table 3), inorganic P was determined by the method of Kuttner & Cohen (1927) and total acid-soluble P by the method of Fiske & Subbarow (1925), both in whole blood and in plasma. The ester P was taken as (total P) - (inorganic P). From these values and from the haematocrit the inorganic and ester P in erythrocytes were calculated. After incubation experiments, inorganic and total P were determined in portions of the saline suspension of the cells.

## RESULTS

### *Erythrocyte respiration*

Determination of the oxygen uptake of rat and human erythrocytes with galactose as substrate failed to achieve levels of respiration equal to those found by Feigelson & Conte (1954). These authors reported an oxygen consumption of rat cells on galactose as substrate amounting to 50% of the respiration on glucose as substrate. We have tried numerous variations of their method, but none yielded respirations with galactose greater than 5-6% of the glucose respiration, although the latter was always in agreement with the published values. However, a small but definite respiration on galactose substrate was invariably obtained with normal human red cells. On the other hand,

cells from a patient with galactosaemia did not respire in galactose (Table 1).

Table 1. *Oxygen uptake of erythrocytes on glucose and galactose substrates*

Equal portions of cell suspension used for glucose and galactose respiration; 0.2 ml. 0.11M hexose solutions. All values represent means of duplicate determinations. The cells were not counted in these experiments. The O<sub>2</sub> uptake was measured at 15 or 30 min. intervals for 2-4 hr. For other details, see Experimental section.

Subject	O <sub>2</sub> consumption (µl./hr.)	
	Glucose	Galactose
Normal		
M.K.	35.7	2.1
V.S.	51.6	2.4
G.K.	56.0	2.3
T.B.	69.3	2.8
J.B.	48.5	2.0
M.G.	42.9	2.6
Galactosaemic		
A.K.	69.6	0

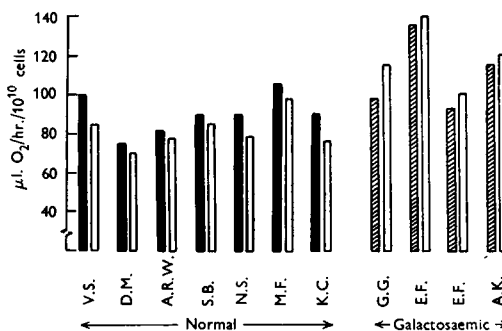


Fig. 1. Oxygen uptake of normal and galactosaemic erythrocytes with glucose (310 µg.) and glucose + galactose (310 µg. + 40 mg.) as substrates. In each case the glucose was tipped in when the endogenous respiration had ceased. The O<sub>2</sub> uptake was measured at 5 min. intervals until it stopped (45-65 min.), the time being the same in the presence and absence of galactose. □, Glucose; ■, glucose + galactose (normal); ▨, glucose + galactose (galactosaemic).

Next, the effect of massive amounts of galactose on the oxygen uptake of cells on a substrate of sub-optimal amounts of glucose was investigated. Galactose (40 mg.) was added to each of three flasks, and, when the endogenous respiration had ceased, glucose (310 µg.) was added to each flask, as well as to control flasks without galactose. In Fig. 1 are shown the results obtained with blood from seven controls and four galactosaemic patients.

Fig. 1 shows that even under these rather drastic conditions the galactose respiration of normal cells is only a small fraction of the glucose respiration. Nevertheless, a small increment due to galactose is invariably obtained with normal cells. The behaviour of galactosaemic cells, on the other hand, offers a striking contrast: in the presence of galactose less  $O_2$  is consumed than in its absence. There is thus a slight but definite inhibition of glucose respiration by galactose.

*Chromatography of erythrocyte phosphate esters*

The results so far described suggest a difference in carbohydrate metabolism between normal and galactosaemic erythrocytes. They prompted a comparison of the phosphorylated intermediates of such metabolism. In Fig. 2 is shown the chromatogram of the water-soluble barium salt fraction of

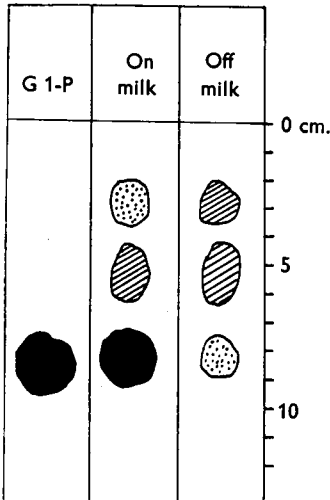


Fig. 2. Chromatogram of water-soluble barium salt fraction of phosphates of blood (A.K., aged 3 months) taken before and after 8 days on milk. The aliquots chromatographed represent equal volumes of blood. Glucose 1-phosphate is included in the chromatogram as a marker.

phosphate esters of red cells taken from a galactosaemic infant on a milk-free regimen and again after 8 days on a milk-containing diet (supplying 5 g. galactose/kg./day).

It is apparent that the 'on milk' sample contains a phosphate which is almost completely lacking in the 'off milk' sample. The  $R_f$  of this substance is very close to that of glucose 1-phosphate (G1-P), thus suggesting a hexose phosphate. The amount was estimated visually to correspond to 19 mg. hexose phosphate/100 ml. blood, whereas the 'off milk' sample contained approximately 3 mg./100 ml. Another aliquot of each phosphate

fraction was chromatographed side by side with some G 1-P, and the areas containing the hexose phosphate were eluted and hydrolysed with 0.1N-HCl for 7 min. at 100°. The liberated sugars were chromatographed, with the results depicted in Fig. 3.

It will be seen that the sugar liberated from the 'on milk' sample travels just behind glucose, its  $R_g$  (glucose = 1.0) being that of galactose. The 'off milk' sample, on the other hand, yielded small quantities of sugars whose  $R_g$  values are those of glucose and galactose respectively. The approximate amounts of these hexoses were again estimated visually and corresponded to 22 mg. galactose 1-phosphate (Gal 1-P)/100 ml. blood ('on milk'

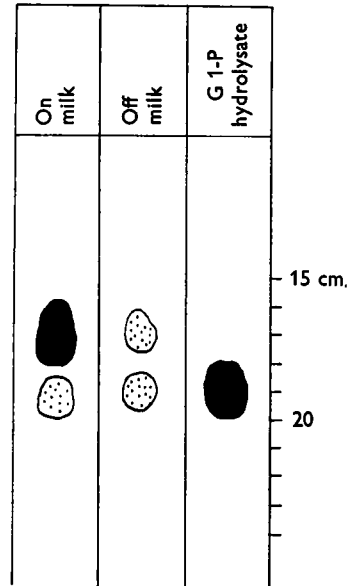


Fig. 3. Chromatogram of sugars liberated by 7 min. hydrolysis with 0.1N-HCl at 100° from phosphates eluted from hexose phosphate area. Descending chromatogram.

sample), and 5 and 4 mg. of Gal 1-P and G1-P respectively per 100 ml. ('off milk' sample). Thus the whole of the phosphate apparent on the chromatogram in Fig. 2 in the hexose 1-P position is accounted for as glucose and galactose phosphates.

In view of the ready hydrolysis of the galactose phosphate it was likely to be the 1-isomer. This was confirmed by the hydrolysis constant which was determined on the chromatographically separated and chemically purified material; a value of  $0.93 \pm 0.16 \times 10^{-3}$  was obtained. Kosterlitz (1939) obtained a value for synthetic  $\alpha$ -D-Gal 1-P of  $0.89 \times 10^{-3}$ . Since the total phosphate available for

this determination corresponded to less than 10  $\mu\text{g}$ . of phosphorus, the agreement can be considered satisfactory. After the completion of these experiments, an authentic sample of Gal 1-P was found to have the same  $R_f$  value as G 1-P.

It was clear that the change-over from a milkless to a milk or galactose diet resulted, after only a few days, in the accumulation of considerable amounts of Gal 1-P in the erythrocytes of the galactosaemic patient. A similar observation was made on two subsequent occasions and again on another patient. Estimation of total hexose phosphate in the red cells of normal infants on a milk diet showed that no such accumulation of Gal 1-P occurred in healthy infants (Fig. 4).

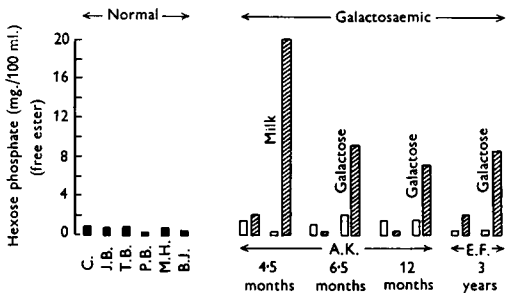


Fig. 4. Hexose 1-phosphates in blood of normal and galactosaemic infants. Normal infants on ordinary milk feeds. Galactosaemic infants on milk or galactose, as indicated, pairs of measurements referring to sugar phosphate levels before and after 8 days on milk or galactose. ■, G 1-P + Gal 1-P; □, G 1-P; ▨, Gal 1-P.

#### Incubation of erythrocytes *in vitro*

Since factors other than the direct or indirect action of galactose might influence the metabolic state of erythrocytes *in vivo*, the effect of galactose

was investigated by *in vitro* incubation. When erythrocytes from galactosaemic patients were incubated in galactose-containing media, accumulation of Gal 1-P occurred. The total hexose 1-P (glucose + galactose) present in the cells of galactosaemic infants and normal controls after incubation under different experimental conditions is given in Table 2.

There is no doubt that the presence of galactose in the medium, *in vivo* or *in vitro*, results in the accumulation of considerable amounts of Gal 1-P in galactosaemic erythrocytes. At the same time the normal phosphate ester content of the cells is reduced, as can be seen from the chromatogram in Fig. 2. A comparison of the two phosphate ester fractions of the blood cells forming water-soluble and water-insoluble barium salts respectively before and after administration of galactose to a galactosaemic infant shows that after galactose the phosphate esters of both fractions (with the exception of Gal 1-P) are reduced (Fig. 5). On the other hand, both phosphate ester fractions of cells collected after galactose contain an unidentified component (not shown in Fig. 5) appearing below, i.e. travelling faster than G 1-P, which is not present in the cells obtained before galactose administration. This component has not been observed on chromatograms obtained previously, with cells of the same patient or on chromatograms of other galactosaemic children.

Quantitative determination of inorganic and total acid-soluble phosphorus and, by difference, ester phosphorus in whole blood and plasma, has confirmed the fall in red-cell phosphate esters (other than Gal 1-P) which occurs after galactose has been given (G. M. Komrower & V. Schwarz, unpublished work). Table 3 shows the changes in cellular phosphorus due to administration of galactose to a galactosaemic infant. The ester

Table 2. Hexose 1-phosphate in erythrocytes after incubation with and without galactose

Estimated visually on chromatograms, as indicated in Experimental section, and calculated as hexose phosphate (free ester).

Subject	Galactose in substrate† (g./100 ml.)	Period of incubation (hr.)	Temp. (°)	Hexose 1-phosphate (mg./100 ml. blood)
E.F.*	1.3	1.25	37	5.6
V.S.	1.3	1.7	37	2.3
A.K.*	0.2	4.25	37	7.5
K.C.	0.2	5	37	3.0
S.B.*	0	144	6	2.5
S.B.*	0.16	144	6	10.0
V.S.	0	144	6	<1
V.S.	0.16	144	6	2.7
C.S.*	0	24	37	0.7
C.S.*	0.2	24	37	6.7
K.C.	0	24	37	<1
K.C.	0.2	24	37	1.4

\* Galactosaemic patients; the remainder were normal controls.

† All substrates contained glucose.

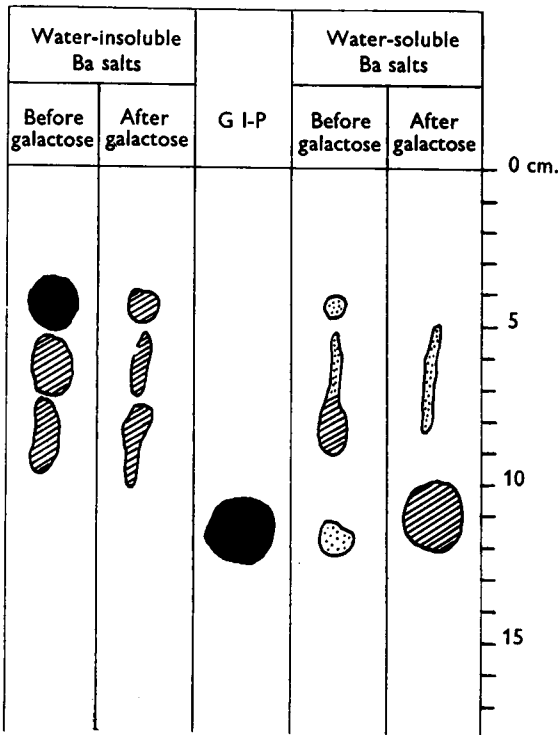


Fig. 5. Chromatogram of fractions of phosphate esters of blood cells forming water-soluble and water-insoluble barium salts respectively (A.K., aged 11 months) before and after administration of galactose. The portions chromatographed represent equal volumes of blood.

Table 3. Changes in P content of erythrocytes taken before and after administration of galactose to a galactosaemic infant (A.K.)

For the methods employed see Experimental section.

	Erythrocyte P content	
	Inorganic P (mg./100 ml. cells)	Ester P (mg./100 ml. cells)
Before galactose	6.96	44.3
After galactose	2.13	36.8

Table 4. Respiration and Gal 1-P content of red blood cells from two galactosaemic patients

Blood was taken before and after 8 days on a galactose-containing diet. The cells were washed with buffer as indicated in the Experimental section and the  $O_2$  uptake was measured on 310  $\mu\text{g}$ . of glucose/flask as substrate until respiration ceased. Readings were taken at 5 min. intervals. The total  $O_2$  consumed during the actual period of respiration was calculated in each case on the basis of  $\mu\text{l}$ . consumed/hr. The means of the determinations are given in the table, together with their deviations. Gal 1-P was estimated in the cells from 1 ml. of blood, taken at the end of the 8-day period on galactose feeds. The amount of Gal 1-P found in the cells before commencing galactose was negligible. The results are expressed as mg. free ester/100 ml. packed cells.

Patient	Respiration ( $\mu\text{l. O}_2/\text{hr./}10^{10}$ cells)		Gal 1-P in cells (mg./100 ml. cells).
	Before galactose	After galactose	
E.F.	139.7 $\pm$ 0.1	100.7 $\pm$ 0.4	20
A.K.	119.4 $\pm$ 4.0	94.4 $\pm$ 1.9	17

Table 5. Effect of galactose on galactosaemic and normal erythrocytes in vitro

The cells were incubated as shown in the table. After washing with saline, portions were taken for determination of inorganic and total P and Gal 1-P, as described in the Experimental part. The remainder of the cell suspension was used for respiration measurements. The  $O_2$  uptake was measured in each case on glucose as substrate, 3.39 mg./flask, giving a final concentration of 109 mg./100 ml. Readings were taken at 15 min. intervals for 3 hr. During this time the uptake was steady in all cases. The recorded values for  $O_2$  consumption are the means of three flasks. The effect of galactose is expressed as % reduction (-) or increase (+) of the respiratory rate of the glucose-incubated cells.

Subject	Incubation medium		Incubation		Glucose respiration ( $\mu\text{l. O}_2/\text{hr./}10^{10}$ cells)	Change of respiratory rate due to galactose (%)	Phosphate fractions (mg./100 ml. cells)		
	Glucose† (mg./100 ml.)	Galactose (mg./100 ml.)	Time (hr.)	Temp. ( $^{\circ}$ )			Gal 1-P (free ester)	Inorganic P	Ester P
S.B.*	100	0	144	6	96.8 $\pm$ 1.8	-19	—	3.6	47.1
S.B.*	100	160	144	6	78.7 $\pm$ 1.7		22	3.6	42.6
V.S.	100	0	144	6	54.8 $\pm$ 2.3	(+2.6)	—	4.1	55.9
V.S.	100	160	144	6	56.2 $\pm$ 2.0		6	2.2	57.8
C.S.*	150	0	24	37	53.9 $\pm$ 1.2	-30	—	18.9	28.6
C.S.*	150	200	24	37	37.8 $\pm$ 0.2		15	19.8	20.9
K.C.	150	0	24	37	44.8 $\pm$ 0.4	+13.4	—	25.8	25.5
K.C.	150	200	24	37	50.8 $\pm$ 0.4		3	23.8	36.2

\* Galactosaemic patients.

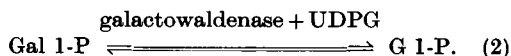
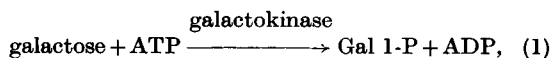
† The glucose concentration makes allowance for the glucose content of the blood used.

phosphorus is reduced by some 17%, and, if allowance were made for the presence of Gal 1-P, the reduction of ester phosphorus would amount to about 25%. The inorganic phosphorus is reduced by nearly 70%. It is obvious that the metabolism of the red cells is greatly affected, an observation which is borne out by the respiratory rate of erythrocytes taken from galactosaemic infants before and after galactose feeding (Table 4). Very similar results are obtained for the respiration of cells incubated with and without galactose at 6° for 6 days or at 37° for 24 hr. (Table 5).

It is clear from Table 5 that the metabolism of galactosaemic erythrocytes is strikingly affected by *in vitro* incubation with galactose. The respiration of normal cells may be enhanced, and this effect is reflected in the ester phosphorus values which are increased by 3 and 42%, whereas the reduced respiratory rate of the galactosaemic cells is accompanied by a fall in ester phosphorus of 14 and 27%.

### DISCUSSION

The pathway of galactose metabolism in the normal mammal has not been definitely established. Leloir and his school (Caputto, Leloir, Cardini & Paladini, 1950) have elucidated the mechanism of galactose degradation in some micro-organisms. Kosterlitz (1937) has observed the accumulation of Gal 1-P in the livers of galactose-fed rabbits and Leloir & Cardini (1953) have shown that the mammalian liver contains galactokinase. Uridine diphosphate glucose (UDPG) is present in various mammalian tissues and increases in the liver of galactose-fed chicks (Rutter & Hansen, 1953). It seems reasonable to suppose, therefore, that the transformation of galactose into glucose in animal tissues may proceed according to the scheme discovered by Leloir and co-workers in *Saccharomyces fragilis*:

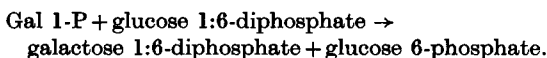


The abnormal behaviour of galactosaemic erythrocytes in galactose media indicates that these cells do, in fact, share in the characteristic inability of the organism as a whole to metabolize galactose normally. The accumulation of Gal 1-P in the red cells suggests that it is reaction (2) in the scheme above which is partly or completely blocked.

The absence of any substantial amount of Gal 1-P from the red cells of healthy infants receiving normal milk feeds may be accounted for (a) by the low blood-galactose level and (b) by the intracellular transformation of galactose into glucose. The *in vivo* accumulation of Gal 1-P in

galactosaemic cells might, therefore, be attributed entirely to the high blood-galactose levels rather than to a defective mechanism in these cells, were it not for the *in vitro* experiments in which much more Gal 1-P accumulated in galactosaemic than in normal cells exposed to the same concentration of galactose. It is not possible to carry out this test *in vivo*, since in a normal subject even large doses of galactose fail to produce a sufficiently prolonged rise in the blood galactose level.

The mechanism whereby the metabolism of the galactosaemic erythrocyte is partially inhibited is not yet established; but this inhibition is clearly not due to galactose itself, since the metabolism of normal cells exposed to similar concentrations is, in fact, slightly enhanced. There is thus no evidence that galactose itself exerts a 'toxic' action on normal cells. Since galactosaemic cells display a reduced metabolism in the presence of galactose, the inhibitory agent is likely to be an intermediate of galactose metabolism which accumulates as a result of the metabolic block. This intermediate may well be Gal 1-P, although other possibilities cannot be ruled out at this stage. Thus it is not impossible that the accumulation of Gal 1-P gives rise to the actual inhibitor. It is interesting to note in this context that Posternak & Rosselet (1954) have found that phosphoglucomutase promotes the conversion of Gal 1-P into the 6-isomer. This reaction is 400 times slower than the corresponding isomerization of G 1-P, but it proceeds by the same mechanism, involving glucose 1:6-diphosphate as a coenzyme. It is conceivable that Gal 1-P competitively inhibits the transformation of G 1-P, or that the normal coenzyme is gradually replaced by galactose 1:6-diphosphate, by the reaction:



The possibility must not be ignored that Gal 1-P accumulation in the cells is but one, perhaps the first, of a series of derangements which involve the intracellular phosphate distribution. There is a striking difference between the changes in ester phosphate content which occur in galactosaemic and in normal erythrocytes on incubation in a galactose medium.

The metabolic defect which the present investigations have served to demonstrate in galactosaemic erythrocytes may well be shared by a variety of other tissue cells in this condition. A partial inhibition of the normal metabolism of such cells and particularly of those whose functions are known to suffer most readily in consequence of anoxia, may serve to explain many of the manifestations of galactosaemia. Thus evidence of impaired function is displayed by the renal tubular epithelium—aminoaciduria and, probably, metabolic acidosis;

by the cerebral tissues—mental retardation or convulsions; by the lens—cataract formation; by the liver—fatty change, icterus and cirrhosis.

Experimentally, convulsions occur in chicks fed on diets containing more than 15% of galactose (Lecoq, Chauchard & Mazoué, 1943; Dam, 1944; Rutter, Krichevsky, Scott & Hansen, 1953; Golberg & Schwarz, unpublished work). The lens depends for its supply of energy almost entirely on the combustion of carbohydrate (Fischer, 1930), and it has been shown recently (Nordmann, Mandel & Achard, 1954) that inhibition of glucose metabolism of the lens results in cataract formation. It would, therefore, seem that a partial inhibition of the normal lenticular metabolism might eventually lead to structural changes and the development of opacity.

The postulated inhibition of liver, kidney, brain or lens tissue by Gal 1-P or another intermediate of galactose metabolism is, at present, purely speculative. It is not yet known whether Gal 1-P accumulates in these tissues, and if so, whether the effect is similar to that observed in blood cells. Experiments designed to throw light on this aspect, and on the more fundamental problem of the mechanism of the inhibition, are now under way.

*Note added in proof.* Since this paper was completed we have demonstrated the presence of Gal 1-P in cataractous lenses of galactose-fed rats [*Biochim. biophys. Acta* (1955), **18** (in the Press)].

### SUMMARY

1. The nature of the biochemical lesion in galactosaemia has been investigated by comparing the metabolic behaviour of the erythrocytes from normal individuals and from cases of the disorder.

2. Galactosaemic erythrocytes, in contrast to normal red cells, do not respire on galactose substrates. Oxygen uptake on glucose substrates is partially inhibited by the presence of galactose.

3. Galactosaemic erythrocytes, on exposure to galactose, *in vivo* or *in vitro*, accumulate galactose 1-phosphate. Values of up to 20 mg. galactose 1-phosphate/100 ml. blood have been obtained. Much smaller amounts accumulate in normal red cells exposed to high galactose concentrations *in vitro*, and very little galactose 1-phosphate is found in normal infants on a milk diet.

4. After exposure to galactose the  $O_2$  uptake of galactosaemic erythrocytes is considerably re-

duced, while that of normal cells is slightly enhanced. This effect is reflected in the level of phosphorylated intermediates: galactose lowers the ester phosphorus (other than galactose 1-phosphate) of galactosaemic cells and raises that of normal cells.

5. The toxic effect of galactose on blood cells and other tissues is discussed in the light of these findings. Some of the wider implications are touched upon.

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