occurrence of secondary oxidative changes (for his experiments were not carried out under anaerobic conditions), and by the probability that insufficient time was allowed for the reaction to go nearly to completion.

Extrapolation from the results obtained *in vitro* suggests that the rate of reduction of methaemoglobin, which could be expected with the concentrations of ascorbic acid likely to occur *in vivo*, would be small as compared with the rate of reduction of the pigment brought about by the enzymic mechanism of normal erythrocytes [Cox & Wendel, 1942]. The clinical use of ascorbic acid in the treatment of methaemoglobinaemia would thus appear to be limited to cases in which the normal enzymic mechanism for the removal of methaemoglobin is absent or has become suppressed.

SUMMARY

1. It has been confirmed that ferricyanide-methaemoglobin, as ordinarily prepared, is reduced more rapidly by ascorbic acid than is nitrite-methaemoglobin.

2. This difference is due to the catalytic effect of ferrocyanide on the reduction of methaemoglobin. There is thus no basis for the supposition that this effect indicates a chemical difference between the two forms of methaemoglobin.

3. Iron and copper salts produce similar catalytic effects.

4. The reaction between ascorbic acid and methaemoglobin is described by the equation for a bimolecular reaction.

The author wishes to thank Prof. D. C. Harrison for much helpful criticism and advice.

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The Assimilation of Glucose and Galactose in the Liver

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(*Received* 20 *September* 1943)

In this paper an attempt is made to examine the intermediary metabolism of glucose and galactose in the liver of the intact animal. *In vitro* experiments [Cori, Cori & Schmidt, 1939; Ostern, Herbert & Holmes, 1939] have proved that glucose-1-phosphate and hexose-6-phosphate are intermediaries in the glucose and glycogen metabolism of the liver. Both galactose-1-phosphate and glucose-1-phosphate have been found in the livers of rabbits assimilating galactose [Kosterlitz, 1937; 1943*a*]. In the present investigation, in addition to the phosphoric esters, glucose, galactose, and glycogen were estimated in the livers of rats at varying intervals after ingestion of glucose or galactose.

METHODS

General. Male rats from the Rowett Research Institute were kept on the stock diet. Prior to the experiment they were fasted for 24 hr., and then fed by stomach tube with either 1·5 ml. of 60% (w/v) galactose solution/100 g. body weight, or with 1·5 ml. of 55% (w/v) glucose solution. After 30, 60, 90 or 120 min. the rats were killed by stunning and the livers excised. After about 0·5 g. liver had been placed in 30% KOH for glycogen estimation, the remainder was frozen in liquid O_2; samples of the frozen liver were used for the various estimations.

Estimation of glucose and galactose. The $HgSO_4$-$BaCO_3$ method of West, Scharles & Peterson [1929] was used for the preparation of protein-free filtrates. Absence of traces of Ba^{++} and Hg^{++} was ensured by the addition of two drops of saturated solution of K_2SO_4 and of a small quantity of Zn powder. Glucose and galactose were estimated in the filtrate by the fermentation method previously described [Kosterlitz, 1937].

Estimation of glycogen. Our micro-adaptation of Pflüger's method was used [Kosterlitz, 1933], with 30% KOH as digestion fluid.

Estimation of reducing phosphoric esters, glucose-1-phosphate and galactose-1-phosphate. This method has previously been described [Kosterlitz & Ritchie, 1943]. The results are given under the following headings: (1) 'Initial reduction',

which is an approximate measure of the reducing phosphoric esters present. Since the nitrogenous esters have been removed, this fraction is composed mainly of equilibrium ester (glucose-6-phosphate and fructose-6-phosphate), possibly together with small quantities of pentose-phosphates. If galactose-6-phosphate should be formed, it also would contribute to the 'initial reduction'; but so far there is no evidence that this ester is formed in the liver during galactose assimilation. (2) Increase in reducing power, calculated as glucose, after 3 min. hydrolysis at 100° ($Red._{100°}$) and after 30 min. hydrolysis at 50° ($Red._{50°}$), and increase in inorganic P (uncorrected) after 3 min. hydrolysis at 100° ($P_{100°}$) and 30 min. hydrolysis at 50° ($P_{50°}$). The values thus obtained are used for characterizing the 1-esters present: glucose-1-phosphate has $Red._{50°}/Red._{100°} = P_{50°}/P_{100°} = 0{\cdot}286$ and $Red._{100°}/P_{100°} = Red._{50°}/P_{50°} = 5{\cdot}81$, while galactose-1-phosphate has $Red._{50°}/Red._{100°} = 0{\cdot}775$, $P_{50°}/P_{100°} = 0{\cdot}765$, $Red._{100°}/P_{100°} = 4{\cdot}63$, and $Red._{50°}/P_{50°} = 4{\cdot}70$.

In experiments on the addition of 1-esters to CCl_3COOH extracts of liver only about 80% of the added material was recovered: in this paper no correction has been made for this loss of 20%.

Significance of results. The statistical methods described by Fisher [1941] were used.

found for $Red._{50°}/Red._{100°}$, $Red._{100°}/P_{100°}$, and particularly $Red._{50°}/P_{50°}$ suggested, however, that besides glucose-1-phosphate an unidentified substance was present, which was probably not a phosphoric ester. It was about as sensitive to acid hydrolysis as galactose-1-phosphate and there was no further increase in reducing power when the hydrolysis at 100° was extended from 3 to 20 min.

Some variation was found in the glycogen contents of the livers. There was, however, a remarkably close relationship between the glycogen and glucose contents, which varied in the same direction and were sometimes almost identical. This phenomenon was absent in rats fed either glucose or galactose. Similarly, Guest [1941] found in fasted but not in fed rats that the mean blood sugar concentrations were directly proportional to the logarithms of the liver glycogen concentrations.

Rats fed with glucose (Tables 1 and 2). The glycogen content of the liver increased with prolongation of the period of glucose absorption, but otherwise

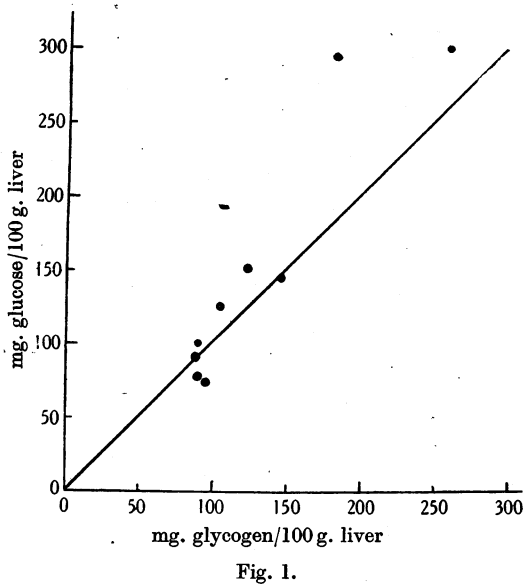

Fig. 1.

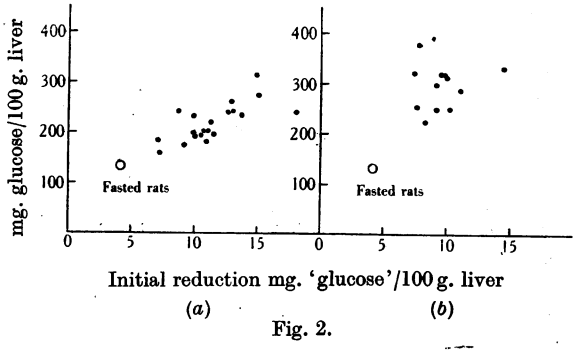

Fig. 2.

Fig. 1. Relationship between glucose and glycogen contents of livers of rats fasted for 24 hr.

Fig. 2. Correlation between glucose and reducing phosphoric ester levels in the livers of rats fed with (*a*) galactose, and (*b*) glucose. Correlation coefficients: (*a*) $r = +0{\cdot}6926$, $P < 0{\cdot}01$; (*b*) $r = +0{\cdot}1457$, $P > 0{\cdot}1$.

RESULTS

Rats fasted for 24 hr. (Fig. 1 and Table 2). Very small quantities of acid-labile phosphoric esters appear to be present in the liver. The ratio $P_{50°}/P_{100°}$ was 0·225, agreeing approximately with the value calculated for glucose-1-phosphate. The high values

the duration of the absorption had no influence on the results. For the purpose of comparing the reducing phosphoric ester and glucose-1-phosphate levels of the glucose-fed rats with those of the fasted ones, the results obtained after feeding glucose were pooled. There was a highly significant increase in both esters after glucose administration. On the

Table 1. *Data relating to carbohydrate and phosphoric ester contents of livers of rats given different sugars*

Sugar fed	Time elapsing between feeding of sugar and death of animal (min.)	No. of rats in group	Mean weight of group (g.)	Liver: Weight (g./100 g. body wt.)	Liver: Glycogen content (g./100 g.)	Liver: Glucose content (g./100 g.)	Liver: Galactose content (g./100 g.)	Liver: Initial reducing power (approx. measure of reducing, i.e. hexose-6-phosphoric esters) (mg. 'glucose'/100 g.)	Liver: Increase in reducing power or inorganic P content (approx. measure of hexose-1-phosphates) (mg./100 g.): After 3 min. hydrolysis at 100°: 'Glucose'	After 3 min. hydrolysis at 100°: Inorganic P	After 30 min. hydrolysis at 50°: 'Glucose'	After 30 min. hydrolysis at 50°: Inorganic P
Glucose	30	3	293 ±16	2·86 ±0·03	0·331 ±0·006	0·331 ±0·023	—	8·0 ±0·52	2·25 ±0·22	0·17 ±0·051	1·25 ±0·13	0·07 ±0·039
	60	3	278 ±21	2·85 ±0·05	0·741 ±0·102	0·284 ±0·017	—	9·5 ±1·02	2·7 ±0·09	0·185 ±0·044	1·4 ±0·14	0·045 ±0·035
	90	3	292 ±34	2·90 ±0·15	1·52 ±0·075	0·277 ±0·027	—	11·2 ±1·63	2·3 ±0·15	0·22 ±0·032	0·9 ±0·09	0·065 ±0·035
	120	3	280 ±25	3·11 ±0·09	2·06 ±0·173	0·285 ±0·031	—	9·15 ±0·48	2·65 ±0·12	0·245 ±0·018	1·5 ±0·24	0·065 ±0·023
Galactose	30	5	319 ± 9	2·75 ±0·06	0·259 ±0·047	0·218 ±0·028	0·181 ±0·013	10·5 ±1·61	4·35 ±0·26	0·56 ±0·036	2·7 ±0·28	0·315 ±0·010
	60	5	296 ±12	2·59 ±0·09	0·469 ±0·089	0·214 ±0·016	0·227 ±0·020	11·4 ±0·60	4·3 ±0·33	0·60 ±0·033	2·9 ±0·24	0·36 ±0·002
	90	5	301 ±19	2·70 ±0·07	0·719 ±0·112	0·239 ±0·011	0·281 ±0·026	11·5 ±1·13	5·0 ±0·15	0·57 ±0·048	3·05 ±0·17	0·34 ±0·020
	120	5	287 ±12	2·86 ±0·14	0·825 ±0·066	0·206 ±0·010	0·277 ±0·025	12·2 ±1·51	4·85 ±0·38	0·71 ±0·038	3·15 ±0·34	0·455 ±0·046

In the expression 293 ±16, 16 is the standard deviation of the mean.

Table 2. *Interpretation of carbohydrate and phosphoric ester analyses*

	Liver: Glucose (g./100 g.)	Liver: Hexose-6-phosphates. Initial reducing power (mg. 'glucose'/100 g.)	Liver: Hexose-1-phosphates: Increase in reducing power or inorganic P content (mg./100 g.): After 3 min. hydrolysis at 100°: 'Glucose' (=$Red._{100°}$)	After 3 min. hydrolysis at 100°: Inorganic P (=$P_{100°}$)	After 30 min. hydrolysis at 50°: 'Glucose' (=$Red._{50°}$)	After 30 min. hydrolysis at 50°: Inorganic P (=$P_{50°}$)	$Red._{50°}/Red._{100°}$	$P_{50°}/P_{100°}$	$Red._{100°}/P_{100°}$	$Red._{50°}/P_{50°}$
Mean of 10 fasted rats	0·133 ±0·026	4·13 ±0·40	1·54 ±0·09	0·089 ±0·009	0·84 ±0·09	0·020 ±0·007	0·545	0·225	17·3	42·0
Mean of 12 glucose-fed rats	0·294 ±0·012	9·45 ±0·56	2·48 ±0·09	0·205 ±0·019	1·28 ±0·10	0·060 ±0·015	0·516	0·293	12·1	21·3
Mean of 20 galactose-fed rats	0·219 ±0·009	11·4 ±0·60	4·63 ±0·15	0·610 ±0·023	2·94 ±0·13	0·367 ±0·017	0·635	0·602	7·6	8·0
Mean of glucose-fed minus mean of fasted rats	0·161	5·32	0·94	0·116	0·44	0·040*	0·468	0·345	8·1	11·0
Mean of galactose-fed minus mean of fasted rats	0·086	7·27	3·09	0·521	2·10	0·347	0·680	0·666	5·93	6·05
Mean of galactose-fed minus mean of glucose-fed rats	−0·075	1·95*	2·15	0·405	1·66	0·307	0·772	0·758	5·31	5·41
Calculated for: Glucose-1-phosphate							0·286	0·286	5·81	5·81
Galactose-1-phosphate							0·775	0·765	4·63	4·70

In the expression 0·133 ±0·026, 0·026 is the standard deviation of the mean.

* The differences of the means are all significant, the values marked by an asterisk having P between 0·05 and 0·02 and the others $P<0·01$ [Fisher, 1941].

other hand, no correlation was found between liver glucose, glycogen, reducing phosphoric esters and glucose-1-phosphate. The ratios Red.$_{50°}$/Red.$_{100°}$ and Red./P were somewhat smaller than those in the fasted rats but still greater than the values calculated for glucose-1-phosphate. Similarly the values obtained from the differences of the means of the glucose-fed and fasted rats were still too high for glucose-1-phosphate. This may indicate an increase in the unknown acid-labile substance already found in the livers of fasted animals, but the number of observations is too small to make any definite statement on this point.

Rats fed with galactose (Tables 1 and 2; Fig. 2). There was a gradual increase in the liver galactose and glycogen with increased duration of galactose absorption, which had no other significant influence on the results, with the possible exception of the 120 min. P values. The glucose levels were lower than those in the rats fed glucose. The reducing phosphoric esters (pooled results on 20 rats) were slightly higher in the animals fed galactose than in those fed glucose. There was a highly significant positive correlation between glucose and reducing phosphoric esters which was absent in the glucose-fed rats (Fig. 2). The 1-phosphates were considerably higher than in the fasted and the glucose-fed animals. Both ratios, Red.$_{50°}$/Red.$_{100°}$ and $P_{50°}/P_{100°}$, were near 0·6, a value indicating a mixture of approximately 1 part glucose-1-phosphate (0·203 mg. P/100 g. liver) and 2 parts galactose-1-phosphate (0·406 mg. P/100 g. liver) [Kosterlitz & Ritchie, 1943]. The ratios Red./P were too high for either glucose-1-phosphate or galactose-1-phosphate. This possibly indicates an increase in the unknown acid-labile substance, already noted in the case of the glucose-fed animals. If the ratios were calculated from values obtained by deducting the means of the values for the fasted rats from those for the galactose-fed ones, a small increase of the ratios Red.$_{50°}$/Red.$_{100°}$ and $P_{50°}/P_{100°}$ was found, and a decrease of the Red./P ratios. The data thus obtained indicated a mixture of approximately 1 part glucose-1-phosphate (0·104 mg. P/100 g. liver) and 4 parts galactose-1-phosphate (0·416 mg. P/100 g. liver). From these calculations, and the values given for the glucose-fed rats (Table 2), one may conclude that in the galactose-fed rats 0·41 mg. galactose-1-phosphate P and 0·2 mg. glucose-1-phosphate P were present in 100 g. liver, and that in both glucose-fed and galactose-fed rats the same quantity of glucose-1-phosphate was formed, namely, that corresponding to 0·11 mg. P. This conclusion is further supported by the fact that the ratios calculated from the differences between the mean values for the galactose-fed and those for the glucose-fed rats were very close to the ratios calculated for galactose-1-phosphate: Red.$_{50°}$/Red.$_{100°}$ was 0·772 and $P_{50°}/P_{100°}$ 0·758 instead of 0·775 and 0·765 respectively, and Red.$_{100°}$/$P_{100°}$ 5·31 and Red.$_{50°}$/$P_{50°}$ 5·41 instead of 4·63 and 4·7 respectively; the P content was 0·405 mg./100 g. liver.

DISCUSSION

Rats assimilating glucose. Besides a deposition of glycogen and an increase in glucose, the administration of glucose caused an increase in the reducing phosphoric ester and glucose-1-phosphate levels of the liver. This is in agreement with the generally accepted theory that glycogen formation from glucose is preceded by the formation of glucose-6-phosphate and glucose-1-phosphate [Colowick & Sutherland, 1942]. Apart from glycogen, no metabolite accumulated in the liver during the assimilation of glucose.

Rats assimilating galactose. The deposition of glycogen was much smaller in the galactose-fed than in the glucose-fed rats, a fact already frequently observed [for literature see Deuel, 1936].

The glucose content of the liver was higher than in the fasted rats but lower than in the glucose-fed ones. The rise in liver glucose after galactose administration demonstrates the conversion of galactose to glucose. Harding, Grant & Glaister [1934] could find no significant increase in liver glucose after the administration of galactose, but the quantities given were much smaller than those used here.

Although the experimental evidence available is incomplete, it is improbable that the reducing phosphoric ester fraction isolated from the liver during galactose assimilation contains galactose-6-phosphate. This fraction has not yet been specially examined for the presence or absence of galactose-6-phosphate; however, prolonged acid hydrolysis did not liberate more galactose from the hexosemonophosphate fraction than a brief period of hydrolysis which is sufficient to hydrolyze galactose-1-phosphate but which hardly affects the difficultly hydrolyzable galactose-6-phosphate [Kosterlitz, 1937]. Further, Grant [1935] could not find any evidence for galactose-6-phosphate as an intermediary in the fermentation of galactose by galactose-adapted yeast.

The positive correlation between liver glucose and reducing phosphoric esters in the galactose-fed rats is remarkable, particularly as no such correlation was found in the glucose-fed animals. In the conversion of galactose to glucose, the reducing phosphoric esters may play an important role, glucose-6-phosphate being the most probable precursor of glucose.

Both galactose-1-phosphate and glucose-1-phosphate were found in the livers of rats assimilating galactose; this confirms previous findings on the rabbit [Kosterlitz, 1937; 1943*a*]. Similar quantities

of glucose-1-phosphate were formed during galactose and glucose assimilation, corresponding to 0·11 mg. P or 0·6 mg. glucose/100 g. of liver. If the values for glucose-1-phosphate found during the assimilation of galactose by the rabbit [Kosterlitz, 1943*a*] are expressed in similar terms, a value of 0·16 mg. P/100 g. of liver is obtained. The galactose-1-phosphate content of the rat livers corresponded to 0·41 mg. P or 2·4 mg. galactose/100 g. and that of the rabbit livers to 0·9–2 mg. P or 5–12 mg. galactose/100 g. No explanation can be offered for the difference in reaction of rabbit and rat; the quantity of galactose-1-phosphate found in the liver is possibly related to the galactose tolerance of the species, which is very low in the rat. Kjerulf-Jensen [1942] determined galactose-1-phosphate in the livers of rats assimilating galactose; his values were much higher than those given in the present paper. His method of estimation, however, was not specific for hexose-1-phosphates; the acid-labile P was determined in the fraction of water-soluble Ba-salts isolated from the liver, without removal of the interfering acid-labile P compounds precipitable by Hg acetate [Kosterlitz & Ritchie, 1943]; there was no determination of the simultaneous increase in reducing power, and no estimation of the rate of acid hydrolysis.

No progressive accumulation of galactose-1-phosphate was found in the liver during galactose assimilation, a fact which makes it improbable that the rate of conversion of galactose-1-phosphate to a yet unknown metabolite is slower than that of galactose phosphorylation. Galactose-1-phosphate is very much more resistant to the enzymes present in liver slices, minced liver or aqueous liver extracts than glucose-1-phosphate [Kosterlitz & Ritchie, 1941]. Although the presence of galactose-1-phosphate, the increases in glucose-1-phosphate and reducing phosphoric ester levels, and particularly the correlation found to exist between reducing phosphoric esters and glucose after administration of galactose, suggest that all these substances take part in the conversion of galactose to glucose in the liver, the connecting link between the galactose and glucose compounds is still missing. In an attempt to formulate a tentative hypothesis of galactose fermentation [Kosterlitz, 1943*b*], galactose-1-phosphate was assumed to be converted to hexose-6-phosphate (Robison ester), probably by way of glucose-1-phosphate. This hypothesis was mainly based on the finding that galactose-1-phosphate and glucose-1-phosphate were fermented at almost identical rates by galactose-adapted yeast. Possibly a similar process takes place in the liver during galactose assimilation, the glucose phosphates then forming glycogen and glucose.

SUMMARY

1. The livers of fasting rats, and of those fed with glucose or with galactose, were analyzed for glucose, galactose, glycogen, glucose-1-phosphate, galactose-1-phosphate and reducing phosphoric ester (mainly glucose-6-phosphate and fructose-6-phosphate).

2. In the livers of fasting rats, reducing phosphoric esters and a small quantity of glucose-1-phosphate (0·09 mg. P/100 g.) were present. Further, there was evidence of the presence of a compound which was probably not a phosphoric ester and which yielded reducing substances on acid hydrolysis. In fasted rats, but not in fed ones, there was a very close direct relationship between the glycogen and glucose contents of the livers.

3. In the livers of rats fed with glucose there was an increase in the reducing phosphoric ester and glucose-1-phosphate (+0·11 mg. P/100 g.) levels.

4. During the assimilation of galactose the livers contained galactose-1-phosphate (0·41 mg. P/100 g.). Glucose-1-phosphate was present in quantities similar to those found after glucose administration, the ratio, glucose-1-phosphate formed to galactose-1-phosphate, being approximately 1/3·5. The livers contained less glucose and slightly more reducing phosphoric esters than those of glucose-fed rats. There was a high correlation between the glucose and reducing phosphoric ester levels, which was absent in glucose-fed animals. The significance of these findings for the mechanism of the conversion of galactose to glucose is discussed.

The expenses of this research were partly defrayed by a grant (to H. W. K.) from the Medical Research Council and a grant from the Royal Society, to whom grateful acknowledgement is made.

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