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Metabolic Studies in Experimental Liver Disease Resulting from D(+)-Galactosamine Administration

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1. In confirmation of previous work, administration of D(+)-galactosamine (0.5–0.75g/kg body wt.) to rats caused a hepatitis with histological evidence of liver damage and a 9-fold rise in aspartate aminotransferase activity in serum. 2. There was a significant elevation of blood lactate and pyruvate concentrations in 24h-starved rats treated with galactosamine but no change in the [lactate]/[pyruvate] ratio. 3-Hydroxybutyrate and acetoacetate concentrations in blood were decreased. 3. The changes in the concentrations of lactate, pyruvate and ketone bodies in the freeze-clamped liver were parallel to those observed in the blood. 4. In the livers of 24h-starved galactosamine-treated rats there were large increases in the concentrations of alanine (3-fold), citrate (5-fold), 2-oxoglutarate (4-fold), with smaller increases in malate, glutamate and aspartate. There was a 4-fold rise in the value of the mass-action ratio of the alanine aminotransferase system in the livers of galactosamine-treated rats when compared to controls. 5. There was a significant decrease in the activities of aspartate and alanine aminotransferases in the cytoplasm and the soluble fraction of sonicated homogenates of the livers of rats treated with galactosamine. The activity of phosphoenolpyruvate carboxylase was decreased by 75% of the control value. 6. Glucose synthesis from lactate in perfused livers from galactosamine-treated rats was inhibited 39% when compared with controls. 7. The results indicate that the conversion of lactate into glucose is decreased in the livers of galactosamine-treated rats and that this decrease may be due to the loss of phosphoenolpyruvate carboxylase from damaged hepatocytes.

Administration of the amino sugar D(+)-galactosamine to rats causes liver damage, which morphologically resembles acute viral hepatitis (Keppler *et al.*, 1968). The mechanism of the hepatotoxicity of D-galactosamine is as yet unknown, although certain biochemical defects have been reported. These include depletion of liver glycogen and adenine nucleotides (Keppler & Decker, 1969), and depletion of uridine phosphates with accumulation of UDP-hexosamines (Keppler *et al.*, 1970), as well as a decrease in protein synthesis (Koff *et al.*, 1971*b*).

As galactosamine treatment provides a model for human viral hepatitis a comparison of blood and liver metabolites and hepatic-enzyme activities in normal and galactosamine-treated rats has been made to assess whether any of the metabolic changes present in the human disease can be explained on the basis of this model. The results indicate that at gluconeogenesis from lactate is decreased in livers from galactosamine-treated rats and that this may be due to the lower activity of the key enzyme, phosphoenolpyruvate carboxylase (EC 4.1.1.32).

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Experimental

Animals

Male rats of the Wistar strain weighing 150–200g were used for all experiments. Rats were fed on a standard small-animal diet (Herbert Styles Ltd., Bewdley, Worcs., U.K.). D(+)-Galactosamine hydrochloride (0.5–0.75g/kg body wt.) was administered as a neutral solution (0.45–0.9M) by a single intraperitoneal or intravenous (tail-vein) injection. Intraperitoneal injection resulted in a variable degree of liver damage and the rats did not maintain their normal food consumption, whereas after intravenous injection the amount of food they consumed was not appreciably different from that consumed by control rats.

Reagents

The D(+)-galactosamine hydrochloride used in part of this work was a gift from E. Merck A.-G., Darmstadt, West Germany; a commercial preparation was supplied by Sigma (London) Chemical Co. Ltd., London S.W.6, U.K. All coenzymes and enzymes were obtained from Boehringer Corp. (London) Ltd., London W.5, U.K.

Methods

Treatment of blood and liver. Blood was collected into heparinized tubes after decapitation of the rats. Blood (0.2ml) was deproteinized with 3% (w/v) $HClO_4$ (2.0ml), denatured protein was removed by centrifugation, the supernatant fluid was neutralized with KOH and the $KClO_4$ removed by centrifugation. Plasma was obtained by centrifugation of whole blood.

For measurements of hepatic metabolites rats were killed by cervical dislocation and the livers were removed within 10s and freeze-clamped (Wollenberger *et al.*, 1960). Further treatment of the frozen tissue was as described by Williamson *et al.* (1967*a*). In separate experiments livers were removed for determination of dry-weight/wet-weight ratios and liver/body-weight ratios. The dry weights of the livers were obtained after drying the liver overnight at 100°C.

For determination of activities of hepatic enzymes rats were killed by cervical dislocation and a portion of liver (about 1g) was removed, wrapped in Parafilm, and placed in crushed ice (10–15min). The liver was then cut into small pieces, mixed with 4vol. of ice-cold 0.25M-sucrose in 10mM-tris–HCl buffer, pH7.4 and homogenized by hand in a loose-fitting Potter–Elvehjem glass homogenizer. A portion of this homogenate was exposed to ultrasonic treatment (100W model; Measuring and Scientific Equipment Ltd., London S.W.1, U.K.) for two periods of 15s (15kHz) with an interval of 1min in between, while being cooled in an ice-bath. This sonically treated homogenate and an untreated portion of the homogenate were centrifuged at 30000*g* for 30min; the resulting supernatant fluids were considered to represent the soluble fraction of sonicated homogenates of the liver and the cytosol respectively.

Liver perfusion. Liver perfusion was done by the method of Hems *et al.* (1966), except that the rats used were males and were fed on a different diet. The rats were starved for 48h before perfusion.

Determination of metabolites. The following metabolites were determined by standard enzymic methods: glucose (Slein, 1963); glucose 6-phosphate and ATP (Lamprecht & Trautschold, 1963); pyruvate, L(+)-lactate, L(−)-malate and L(−)-glycerol 3-phosphate (Hohorst *et al.*, 1959); phosphoenolpyruvate, 2- and 3-phosphoglycerate (Czok & Eckert, 1963); acetoacetate and D(−)-3-hydroxybutyrate (Williamson *et al.*, 1962); L-glutamate (Bernt & Bergmeyer, 1963); 2-oxoglutarate (Bergmeyer & Bernt, 1963*a*); L-glutamine (Lund, 1970); L-aspartate (Pfleiderer, 1963); citrate (Möllering & Gruber, 1966); L-alanine (Williamson *et al.*, 1967*b*); ADP and AMP (Adam, 1963); plasma triglycerides (Eggstein & Kreutz, 1966).

Plasma free fatty acids were determined colorimetrically (Itaya & Ui, 1965).

Determination of enzyme activities. Alanine aminotransferase (EC 2.6.1.2) and aspartate aminotransferase (EC 2.6.1.1) were determined spectrophotometrically by the methods of Bergmeyer & Bernt (1963*b*,*c*), and lactate dehydrogenase (EC 1.1.1.27) by the method of Bergmeyer *et al.* (1963). The activity of phosphoenolpyruvate carboxylase (EC 4.1.1.32) was measured in the direction of oxaloacetate synthesis by the method of Holten & Nordlie (1965). Glutamate dehydrogenase (EC 1.4.1.3) was determined by a modification of the method of Schmidt (1963) in which ADP (1.6mM) was included in the assay mixture.

The units of enzyme activity are expressed as μmol of substrate transformed/min (measured at 30°C) and the specific activity as units/g fresh wt. of liver. Serum enzyme activities are expressed as units/litre of serum (measured at 25°C).

Determination of protein. Protein was determined by the colorimetric method of Lowry *et al.* (1951). Bovine serum albumin (fraction V powder) was used as a protein standard.

Results

Evidence of liver damage

There was a 9-fold increase (from 285 to 2700 units/l) in serum aspartate aminotransferase activity 48h after galactosamine administration (0.75g/kg), with a smaller increase at 24h and a return to normal at 72h. The plasma of galactosamine-treated rats was frequently icteric. Histology of the livers 24 and 48h after galactosamine administration showed changes similar to those described by Keppler *et al.* (1968) (see the Discussion section).

Effect of galactosamine on the concentrations of metabolites in blood

There was a significant elevation ($P<0.05$) of [lactate] and [pyruvate] in 24h-starved rats treated with galactosamine (0.5g/kg body wt.) (Table 1) but there was no appreciable change in the [lactate]/[pyruvate] ratio. The [3-hydroxybutyrate] and [acetoacetate] were decreased by 34 and 56% respectively, but the [3-hydroxybutyrate]/[acetoacetate] ratio, although higher than that of the

Table 1. *Effects of* D(+)*-galactosamine on the concentrations of metabolites and the ratios of metabolites in rat blood or plasma*

The rats were injected intravenously with D(+)-galactosamine (0.5g/kg), starved for 24h and then killed. Control rats were not injected. For other experimental details see the text. Concentrations of metabolites are expressed in μmol/ml of whole blood, except free fatty acids (μequiv./ml of plasma) and triglycerides (mg/100ml of plasma), as means ± S.E.M. with numbers of observations in parentheses. The metabolite ratios are calculated from the mean values of the concentrations of the reactants. Values for galactosamine-treated rats which are statistically (Student's *t* test) different from controls are indicated by: $*P<0.05$; $**P<0.01$.

Metabolite or metabolite ratio	Control (5)	Galactosamine-treated (5)
Lactate	1.83 ±0.19	3.28 ±0.38*
Pyruvate	0.150±0.033	0.328±0.042*
[Lactate]/[Pyruvate]	12.2	10.0
3-Hydroxybutyrate	1.43 ±0.13	0.95 ±0.10*
Acetoacetate	0.497±0.049	0.219±0.021**
Total ketone bodies	1.93 ±0.17	1.17 ±0.09**
[3-Hydroxybutyrate]/[Acetoacetate]	2.8	4.3
Glucose	3.59 ±0.13	3.62 ±0.13
Free fatty acids	0.98 ±0.06	1.10 ±0.09
Glycerol	0.293±0.028	0.379±0.45
Triglycerides	111 ±6	209 ±40

controls, was not significantly different. Blood [glucose], plasma [free fatty acids] and blood [glycerol] did not differ between the two groups, but plasma [triglycerides] were elevated in four of the five galactosamine-treated rats (Table 1).

Effect of galactosamine on concentrations of metabolites in liver

The increase in blood [lactate] and [pyruvate] in the starved rats treated with galactosamine suggested either a decreased rate of gluconeogenesis from these precursors or an increased rate of lactate production by the peripheral tissues. To decide between these possibilities the metabolite patterns in freeze-clamped liver were determined (Table 2; Fig. 1).

Tricarboxylic acid-cycle intermediates and glucose precursors. There was a significant increase in [lactate] ($P<0.01$) and [pyruvate] ($P<0.05$) in the livers of 48h-starved rats treated with galactosamine (Table 2). There were also increases in [alanine] (3-fold; $P<0.001$), in [citrate] (5-fold; $P<0.001$), [2-oxoglutarate] (4-fold; $P<0.05$) and a smaller rise in [malate]. [Glutamate] and [aspartate] were almost doubled in the galactosamine-treated group. There was a marked change in the value of the mass-action ratio of the alanine aminotransferase system as calculated from the concentrations of the reactants, the value for the galactosamine-treated group being 4-fold higher than that of the control group.

[Glucose], [glucose 6-phosphate], [phosphoenolpyruvate] and [2- and 3-phosphoglycerate] (Table 2) were not different in livers of the control and galactosamine-treated rats. The resultant metabolite pattern (Fig. 1) can be interpreted as indicating a rate-limiting step at the stage of oxaloacetate conversion into phosphoenolpyruvate.

Ketone bodies. There was a significant decrease in [acetoacetate] and [total ketone bodies] ($P<0.05$) in galactosamine-treated rats but there was no change in the [3-hydroxybutyrate]/[acetoacetate] ratio.

Adenine nucleotides. In agreement with the findings of Keppler *et al.* (1968) [ATP] decreased (42%; $P<0.001$) and there was a similar decrease in the concentration of total adenine nucleotides. [ADP] and [AMP], although less than control values, were not significantly decreased and there was no appreciable change in the value of the mass-action ratio of the myokinase system.

Effect of galactosamine on glucose synthesis from lactate in the isolated perfused liver

The increases in [lactate] and [pyruvate] in the blood and livers of rats treated with galactosamine were consistent with an impairment of the conversion of these metabolites into glucose. To confirm this possibility the livers of control and galactosamine-treated rats (48h-starved) were perfused with lactate.

Table 2. *Effects of* D(+)*-galactosamine on the concentrations of metabolites in livers of starved rats*

Starved rats (24h) were injected with D(+)-galactosamine (0.5–0.75g/kg body wt.) intraperitoneally, starved for a further 24h, then killed. Concentrations are expressed in μmol/g fresh wt., as means±S.E.M., with the numbers of observations in parentheses. The metabolite ratios are calculated from the mean values for the concentrations of the reactants. Other experimental details are given in the text. Values for galactosamine-treated rats that are statistically (Student's *t* test) different from controls are indicated by: $*P<0.05$; $**P<0.01$.

Metabolite or metabolite ratio	Galactosamine-treated (6)	Control (5)
Lactate	0.475±0.075**	0.178±0.018
Pyruvate	0.044±0.005*	0.027±0.004
3-Hydroxybutyrate	1.20 ±0.21	1.97 ±0.33
Acetoacetate	0.547±0.077*	0.857±0.066
Total ketone bodies	1.78 ±0.20*	2.80 ±0.36
Glucose	4.81 ±0.31	3.96 ±0.27
Glucose 6-phosphate	0.064±0.012	0.067±0.007
3-Phosphoglycerate	0.137±0.015	0.141±0.016
2-Phosphoglycerate	0.036±0.006	0.031±0.006
Phosphoenolpyruvate	0.064±0.008	0.077±0.014
Malate	0.597±0.114	0.170±0.011
Citrate	1.22 ±0.16**	0.239±0.022
2-Oxoglutarate	0.321±0.076*	0.088±0.016
Glutamate	3.71 ±0.31**	2.12 ±0.15
Glutamine	7.37 ±1.14	5.52 ±0.22
Aspartate	0.931±0.080**	0.528±0.055
Alanine	0.676±0.040**	0.221±0.043
ATP	1.57 ±0.05**	2.71 ±0.19
ADP	1.17 ±0.16	1.40 ±0.10
AMP	0.332±0.092	0.389±0.069
Total adenine nucleotides	3.07 ±0.17**	4.50 ±0.07
$\frac{[ATP][AMP]}{[ADP]^2}$	0.38	0.53
$\frac{[Lactate]}{[Pyruvate]}$	10.9	6.6
$\frac{[3\text{-Hydroxybutyrate}]}{[Acetoacetate]}$	2.1	2.3
$\frac{[Alanine][2\text{-Oxoglutarate}]}{[Pyruvate][Glutamate]}$	1.3	0.34

The rate of formation of glucose from lactate by the livers of galactosamine-treated rats was 39% less than that in control rats (Table 3). Similarly, lactate removal was 31% less in galactosamine-treated rats and a smaller proportion of the lactate removed was converted into glucose (59% compared with 67%).

Effect of galactosamine on the activity of certain liver enzymes

The established leakage of liver enzymes into the serum of galactosamine-treated rats (Keppler *et al.*, 1968) and the inhibition of protein synthesis (Koff *et al.*, 1971*b*) suggested the possibility that metabolic changes observed in the liver after galactosamine treatment might in part be mediated by lower activities of certain key hepatic enzymes. To test this possibility the activities of aspartate and alanine aminotransferases (concerned in the intrahepatic transport of carbon and reducing equivalents), phosphoenolpyruvate carboxylase (possible rate-limiting step in gluconeogenesis), glutamate dehydrogenase (a mitochondrial marker enzyme) and lactate dehydrogenase were determined in the cytoplasm and the soluble fraction of sonicated homogenates of liver (Table 4).

There was a 64% loss of alanine aminotransferase activity from both the cytoplasm and the sonicated homogenate of livers of 24h-starved rats treated with galactosamine (0.5g/kg body wt.). In the cytoplasm

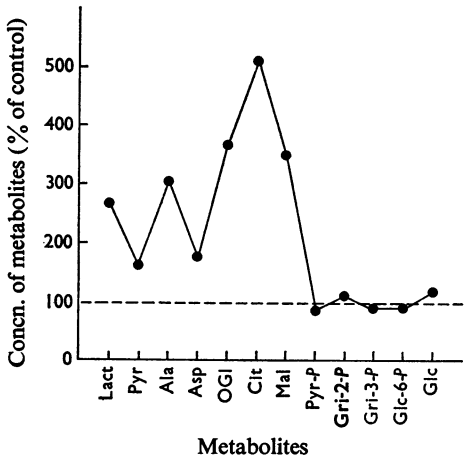

Fig. 1. *Pattern of metabolites in rat liver after administration of galactosamine*

The values shown are the percentage changes in concentration in the livers of galactosamine-treated rats compared with control animals. The absolute concentrations are given in Table 2. For other experimental details see the Experimental section. Abbreviations for metabolites: Lact, lactate; Pyr, pyruvate; Ala, alanine; Asp, aspartate; OGl, oxoglutarate; Cit, citrate; Mal, malate; Pyr-*P*, phosphoenolpyruvate; Gri-2*P*, 2-phosphoglycerate; Gri-3-*P*, 3-phosphoglycerate; Glc-6-*P*, glucose 6-phosphate; Glc, glucose.

the activity of aspartate aminotransferase was decreased by 53% and in the sonicated homogenate by 38%. There was a 29% decrease in the activity of glutamate dehydrogenase in the sonicated liver homogenate, but the low activity in the cytoplasm, which is usually attributed to leakage from the mitochondria during preparation of the homogenate, was 65% lower. The activity of the phosphoenolpyruvate carboxylase was decreased by 74% in the cytoplasm of galactosamine-treated rats. Galactosamine (20mM) had no effect on the activity of phosphoenolpyruvate carboxylase *in vitro*. The lower activities of these enzymes, especially of phosphoenolpyruvate carboxylase, could account for the accumulation of lactate and pyruvate in the blood and liver of galactosamine-treated rats and the decreased synthesis of glucose from lactate in the perfused liver.

The protein concentrations of both liver fractions were not significantly decreased after galactosamine treatment, nor was the activity of the lactate dehydrogenase.

Table 3. *Effects of previous administration of* D(+)-*galactosamine on synthesis of glucose in perfused rat liver*

All rats were starved for 48h before perfusion of liver. One group was injected intraperitoneally with D(+)-galactosamine (0.5g/kg) 24h before perfusion. L(+)-Lactate (10mM) was added to the perfusion medium 40min after the start of perfusion and the perfusion was continued for a further 90min. The results are expressed in μmol/min per g fresh wt., as means±S.E.M. with the number of observations in parentheses. For other experimental details see the text. Values for galactosamine-treated rats that are statistically (Student's *t* test) different from controls are indicated by: **P*<0.05; ***P*<0.01.

	Control (10)	Galactosamine-treated (8)
Lactate removed	1.90±0.07	1.32±0.21*
Glucose formed	0.63±0.03	0.39±0.06**

Change in liver composition after galactosamine treatment

Any change in liver composition that may occur in a pathological situation could alter the interpretation of measurements of metabolites and enzyme activities made on the liver. To examine possible changes in liver composition in the present experiments liver triglycerides, dry and wet weight of livers and body weights were measured in normal fed and 24h-starved rats and in equivalent galactosamine-treated rats. As reported by Koff *et al.* (1971*a*) there was an accumulation (10-fold higher) of neutral fat in the livers of starved rats treated 24h previously with galactosamine, whereas in fed rats galactosamine actually caused a 33% decrease in liver triglycerides (Table 5). This infiltration of fat in the livers of the 24h-starved rats resulted in a 20% rise in the wet liver wt./body wt. ratio (Table 5). Assuming no change in total activity/liver, the activity of any enzyme would therefore be underestimated if expressed as units/g wet wt. and this could be corrected for by expression of the results as units/100g body wt. This method of expression for the enzyme results was not adopted because it was thought that any apparent underestimation was at least partly if not entirely compensated for by liver wasting and that this was obscured by fat-infiltration. Evidence for this is the fact that in fed galactosamine-treated rats where no fat-infiltration of the liver occurs there is a 25% decrease in the dry weight of the livers (Table 5).

The changes in the dry liver weight/wet liver weight ratios in the experiments in which the liver was freeze-clamped were not appreciable (Table 5) and

Table 4. *Effects of* D(+)*-galactosamine on the activities of certain enzymes in cytoplasm and soluble fraction of sonicated homogenates of rat liver*

Treatment of the rats was as described in Table 1. For other experimental details see the text. The activities are expressed in units/g fresh wt., as means±S.E.M. with the number of observations in parentheses. Values for galactosamine-treated rats which are statistically (Student's *t* test) different from controls are indicated by: $*P<0.05$; $**P<0.01$.

Enzyme	Fraction		Enzyme activity: Control	Enzyme activity: Galactosamine-treated
Aspartate aminotransferase	Cytoplasm	(5)	49.7 ± 4.3	23.5 ± 3.4**
	Whole homogenate	(5)	186 ±12.3	116 ±13.5*
Alanine aminotransferase	Cytoplasm	(5)	12.9 ± 2.5	4.7 ± 0.4*
	Whole homogenate	(5)	18.7 ± 2.2	6.8 ± 0.8**
Glutamate dehydrogenase	Cytoplasm	(7)	2.6 ± 0.5	0.9 ± 0.2*
	Whole homogenate	(7)	226 ±12.1	161 ±14.0*
Phosphoenolpyruvate carboxylase	Cytoplasm	(6)	2.15± 0.36	0.56± 0.19**
Lactate dehydrogenase	Cytoplasm	(5)	255 ±34.3	205 ±37.5

Table 5. *Effects of* D(+)*-galactosamine on liver composition*

Rats were injected intravenously with D(+)-galactosamine (0.5g/kg body wt.). One group was starved for 24h and the other fed *ad libitum* for 24h. The control rats were not injected. The results are means±S.E.M. An average mol.wt. of 800 was assumed for the triglycerides.

Treatment of rats	Triglycerides (μmol/g fresh wt.)	Fresh liver (g/100g body wt.)	Dried liver (g/100g body wt.)	Dry wt./wet wt. ratio
Fed rats	23.8±2.7	4.2±0.2	1.2±0.06	27.9±0.3
Galactosamine-treated, fed rats	15.9±2.2	3.7±0.1	0.9±0.02	24.6±0.4
Starved rats (24h)	7.1±0.8	3.5±0.1	1.0±0.04	28.4±0.2
Galactosamine-treated, starved rats (24h)	73.0±5	4.4±0.2	1.3±0.1	29.6±0.5

therefore no correction has been made for this in calculating the concentrations of liver metabolites.

Discussion

Dose of galactosamine

In the present study a single dose of galactosamine of 0.5–0.75g/kg body wt. was used to produce a hepatitis 24h later. This is one-third to one-half of the dose given by Keppler *et al.* (1968), who used 1.5g/kg body wt. in six divided doses over 24h. The histological appearance of the liver essentially resembled that described by these authors, though the extent of the liver damage was much less marked, probably no more than 10% of the liver parenchyma being necrotic. Administration of galactosamine at a dose of 0.5g/kg body wt. followed by starvation for 24h resulted in marked eosinophilic change throughout the liver, with small collections of inflammatory cells, but extensive foci of necrosis as described by Keppler *et al.* (1968) and Koff *et al.* (1971*a*) obtained by using a single dose of 1.5g/kg body wt. were not seen. With galactosamine-treated rats which were starved for 24h there was marked accumulation of fat in the liver, confirming the findings of Koff *et al.* (1971*a*).

Site of inhibition of glucose synthesis

The accumulation of lactate and pyruvate in the blood and in the livers of galactosamine-treated starved rats and the decreased rates of glucose synthesis from these precursors in perfused livers raises the question of the site(s) of inhibition of gluconeogenesis. The large increases in hepatic [malate], [aspartate], [citrate] and [2-oxoglutarate] with no significant changes in [phosphoenolpyruvate], [2-phosphoglycerate] and [3-phosphoglycerate] gives a metabolic profile (Fig. 1) similar to that found after administration of quinolinic acid (Veneziale *et al.*, 1967; Williamson *et al.*, 1971), an inhibitor of phosphoenolpyruvate carboxylase. In the present experiments the activity of this key enzyme in glucose

synthesis was decreased by about 75% in galactosamine-treated rats and this change in activity may be the sole factor responsible for the inhibition of gluconeogenesis from lactate. Other metabolic changes after galactosamine treatment that might decrease the rate of glucose synthesis are the lower activities of aspartate aminotransferase and alanine aminotransferase in the cytoplasm (Table 4). The main function of cytoplasmic aspartate aminotransferase in gluconeogenesis is probably the conversion of aspartate (transported from the mitochondria) into oxaloacetate (Lardy, 1965) whereas that of alanine aminotransferase is presumably the conversion of alanine into pyruvate. Evidence that the decreased activity of alanine aminotransferase may be important is the altered ratio of the reactants of the system after galactosamine treatment (Table 2). The lower [ATP] in the galactosamine-treated rats might be expected to decrease the rate of glucose synthesis, but there is no evidence for this from the metabolite profile.

The lower activities of several hepatic enzymes could be the result of leakage from cells owing to membrane damage. Evidence for this suggestion is the appearance of aminotransferases and glutamate dehydrogenase in the serum after galactosamine treatment (Keppler *et al.*, 1968; the present paper) and the membrane damage noted by Medline *et al.* (1970) as a feature of the ultrastructural abnormalities caused by galactosamine. Alternatively, a decrease in protein synthesis as reported by Koff *et al.* (1971*b*) could be responsible, although this explanation seems less likely, as the cytoplasmic and sonicated homogenate soluble protein of galactosamine-treated rats did not differ significantly from controls. Similarly there was no significant decrease in the hepatic activity of lactate dehydrogenase (cytoplasm) in the galactosamine-treated group, although it is not possible to rule out effects on the synthesis of specific proteins. Increased rates of intracellular degradation of the enzymes or conversion into inactive forms are other possible explanations of the findings.

Interpretation of metabolite concentrations and enzyme activities in damaged tissues

Measurements of metabolite concentrations and enzyme activities in damaged tissues represent values for a mixed population of normal, dead or dying and inflammatory cells. Thus any metabolite or enzyme profile may be distorted by changes occurring predominantly in necrotic cells and may bear little relation to events in surviving tissue. Although this could in part explain the changes in metabolite concentrations in freeze-clamped tissue after administration of galactosamine the available evidence suggests that this is not so. The alteration in hepatic enzyme activities, notably that of phosphoenolpyruvate carboxylase, would suggest that over 50% of the parenchymal cells had been affected by galactosamine administration, whereas necrotic cells accounted for probably no more than 10% of the liver, and although inflammatory cells were present they were few in number.

On completion of the present work our attention was drawn to the studies of Monier & Wagle (1971) who found a decrease in the activity of phosphoenolpyruvate carboxylase in the livers of fed rats after treatment with 1.5g/kg of galactosamine. They also report an inhibition of gluconeogenesis from various precursors in rat liver slices.

Comparison with human liver disease

The liver damage that results from galactosamine as administered in this study is acute, relatively mild and more constant than the varied spectrum of liver damage that occurs in human viral hepatitis; nevertheless it is of interest to make a comparison of the metabolic changes. Common features are the increase in the activites of the aminotransferases and glutamate dehydrogenase in serum and the increase in serum bilirubin. Impaired gluconeogenesis resulting in hypoglycaemia after overnight starvation has been reported in human acute viral hepatitis (Felig *et al.*, 1970), this complication having previously only been recognized in acute massive hepatic necrosis (Marks & Rose, 1965). In other forms of human liver disease the findings include elevated concentrations of blood lactate (Tranquada, 1964) and 2-oxoglutarate (Seligson *et al.*, 1952; Dawson *et al.*, 1957) together with increased blood pyruvate in hepatic coma (Dawson *et al.*, 1957), all of which may indicate impaired gluconeogenesis.

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References

Adam, H. (1963) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), p. 573, Academic Press, New York and London

Bergmeyer, H. U. & Bernt, E. (1963*a*) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), p. 324, Academic Press, New York and London

Bergmeyer, H. U. & Bernt, E. (1963*b*) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), p. 837, Academic Press, New York and London

Bergmeyer, H. U. & Bernt, E. (1963*c*) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), p. 846, Academic Press, New York and London

Bergmeyer, H. U., Bernt, E. & Hess, B. (1963) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), p. 736, Academic Press, New York and London
Bernt, E. & Bergmeyer, H. U. (1963) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), p. 384, Academic Press, New York and London
Czok, R. & Eckert, L. (1963) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), p. 224, Academic Press, New York and London
Dawson, A. M., De Groote, S., Rosenthal, W. M. & Sherlock, S. (1957) *Lancet* **i**, 392
Eggstein, M. & Kreutz, F. H. (1966) *Klin. Wochenschr.* **44**, 262
Felig, P., Brown, W. V., Levine, R. A. & Klatskin, G. (1970) *N. Engl. J. Med.* **283**, 1436
Hems, R., Ross, B. D., Berry, M. N. & Krebs, H. A. (1966) *Biochem. J.* **101**, 284
Hohorst, H. J., Kreutz, F. H. & Bücher, T. (1959) *Biochem. Z.* **332**, 18
Holten, D. D. & Nordlie, R. C. (1965) *Biochemistry* **4**, 723
Itaya, K. & Ui, M. (1965) *J. Lipid Res.* **6**, 16
Keppler, D. & Decker, K. (1969) *Eur. J. Biochem.* **10**, 219
Keppler, D., Lesch, R., Reutter, W. & Decker, K. (1968) *Exp. Mol. Pathol.* **9**, 279
Keppler, D., Rudigier, J. F. M., Bischoff, E. & Decker, K. (1970) *Eur. J. Biochem.* **17**, 246
Koff, R. S., Gordon, G. & Sabesin, S. M. (1971*a*) *Proc. Soc. Exp. Biol. Med.* **137**, 696
Koff, R. S., Fitts, J. J. & Sabesin, S. M. (1971*b*) *Proc. Soc. Exp. Biol. Med.* **138**, 89
Lamprecht, W. & Trautschold, I. (1963) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), p. 543, Academic Press, New York and London
Lardy, H. A. (1965) *Harvey Lect.* **60**, 261
Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. S. (1951) *J. Biol. Chem.* **193**, 265
Lund, P. (1970) in *Methoden der Enzymatischen Analyse* (Bergmeyer, H. U., ed.), 2nd. edn., p. 1671, Verlag Chemie, Weinheim
Marks, V. & Rose, F. C. (1965) in *Hypoglycaemia*, p. 166, Blackwell Scientific Publications, Oxford.
Medline, A., Schaffner, F. & Popper, H. (1970) *Exp. Mol. Pathol.* **12**, 201
Möllering, H. & Gruber, W. (1966) *Anal. Biochem.* **17**, 369
Monier, D. & Wagle, S. R. (1971) *Proc. Soc. Exp. Biol. Med.* **136**, 377
Pfleiderer, G. (1963) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), p. 381, Academic Press, New York and London
Schmidt, E. (1963) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), p. 752, Academic Press, New York and London
Seligson, D., McCormick, G. J. & Sborov, V. (1952) *J. Clin. Invest.* **31**, 661
Slein, H. W. (1963) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), p. 117, Academic Press, New York and London
Tranquada, R. E. (1964) *Calif. Med.* **101**, 450
Veneziale, C. M., Walter, P., Kneer, N. & Lardy, H. A. (1967) *Biochemistry* **6**, 2129
Williamson, D. H., Mellanby, J. & Krebs, H. A. (1962) *Biochem. J.* **82**, 90
Williamson, D. H., Lund, P. & Krebs, H. A. (1967*a*) *Biochem. J.* **103**, 514
Williamson, D. H., Lopez-Vieira, O. & Walker, B. (1967*b*) *Biochem. J.* **104**, 497
Williamson, D. H., Mayor, F. & Veloso, D. (1971) in *Regulation of Gluconeogenesis* (Söling, H. D. & Willms, B., eds.), p. 92, Academic Press, New York and London
Wollenberger, A., Ristau, O. & Schoffa, G. (1960) *Pflügers Arch. Gesamte Physiol.* **270**, 339