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Glycolytic Isoenzymes and Glycogen Metabolism in Regenerating Liver from Rats on Controlled Feeding Schedules

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Intact rats trained on a controlled feeding and lighting schedule designated '8+16' exhibited diurnal oscillations in liver weight, glucokinase activity and liver glycogen content. Glucokinase activity expressed as units/g of liver decreased to 30% of that from unoperated controls during the first 48h after partial hepatectomy and returned to near normal values in 2 weeks. When the glucokinase activity was expressed as units/liver per 100g body wt., a decrease to 50% of control activity was observed between 24 and 48h after the operation. A similar pattern was found for pyruvate kinase type I. In contrast, pyruvate kinase type III activity increased after partial hepatectomy. It is suggested that the newly divided cells after partial hepatectomy do not synthesize glucokinase and pyruvate kinase I but do synthesize pyruvate kinase III. Glycogen was found to accumulate as early as 24h after partial hepatectomy, and normal concentrations were reached after 48h if the operation was performed at times other than during the feeding periods.

The regulation of synthesis of certain liver enzymes is probably related to the differentiated state of the individual cells of that organ. For example, certain isoenzymes of glycolytic enzymes such as ATP–hexose 6-phosphotransferase (hexokinase; EC 2.7.1.1), fructose 1,6-diphosphate–D-glyceraldehyde 3-phosphate lyase (aldolase; EC 4.1.2.13) and ATP–pyruvate phosphotransferase (pyruvate kinase; EC 2.7.1.40) are found predominantly in foetal liver whereas enzymes carrying out the same reaction but with different properties (isoenzymes) are found predominantly in adult liver (Walker & Potter, 1972). The developmental patterns for some of the above isoenzymes have been studied. Walker & Holland (1965) have shown that the activity of one of the ATP–hexose 6-phosphotransferase isoenzymes, glucokinase (EC 2.7.1.2), is absent from the liver of foetal rats. Enzyme activity of this isoenzyme begins to be detected approx. 15 days after birth and reaches the adult activity shortly after weaning. Pyruvate kinase type I is present at low activities in neonatal life and increases near the onset of weaning (Walker & Potter, 1972; Middleton & Walker, 1972). Therefore it appears that the synthesis of these adult isoenzymes occurs late in the neonatal period after most of the liver development is completed (Greengard, 1971). However, in hepatomas certain isoenzyme patterns are similar to those in foetal liver. For example, glucokinase has been found to be absent, or at least decreased, in most hepatomas (Farina *et al.*, 1968; Sato *et al.*, 1969; Knox *et al.*, 1970; Walker & Potter, 1972).

Partial hepatectomy has been used by many workers to study normal liver hyperplasia in adult animals, a condition normally present only in foetal and neonatal rats. Several reports have shown that the activity of glucokinase decreases after partial hepatectomy (Sato *et al.*, 1969; Lea *et al.*, 1970; Walker & Potter, 1972), although at least one report demonstrated no change in activity (Knox *et al.*, 1970). The electrophoretic pattern of pyruvate kinase isoenzymes has been shown to be altered after partial hepatectomy (Tanaka *et al.*, 1967; Walker & Potter, 1972). The aldolase isoenzyme profile has also been reported to change after the operation, with increases in the muscle type isoenzyme, aldolase A (Farron *et al.*, 1972). All of the above changes in isoenzyme profile after partial hepatectomy have been towards an isoenzyme pattern characteristic of foetal or neonatal liver. However, the published data concerning hexokinase, pyruvate kinase and aldolase isoenzyme patterns after partial hepatectomy in rats are quite variable and incomplete for several reasons: (1) the results are reported for operations performed at various times of the day; (2) the enzyme activities reported are for different times after the operation; (3) most

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of the data concern early changes in enzyme activity after the operation rather than the complete pattern of the changes, which must necessarily encompass the time required for the activity to return to normal.

A study of the effect of partial hepatectomy on enzymes involved in glucose metabolism should be correlated with measurement of glycogen to establish the physiological state of the animal. Potter & Ono (1961) reported a diurnal variation for glycogen in rats fed *ad libitum*, with the peak observed during the dark when the animals ate. Lea *et al.* (1970) reported that glycogen was depleted 24h after partial hepatectomy and reached 50% of normal values after 96h. It is possible that the latter study failed to consider the optimum times of the day for glycogen determinations.

A detailed study of certain glycolytic isoenzymes as a function of time after partial hepatectomy and a re-examination of glycogen metabolism with careful attention to normal diurnal rhythms co-ordinated with a study of DNA metabolism (H. A. Hopkins, H. A. Campbell, B. Barbiroli, V. R. Potter, unpublished results) might shed light on the possible relation of the shifting isoenzyme patterns to cell division.

Materials and Methods

Biological materials

Animals and feeding schedule. White male rats weighing approx. 50g were purchased from the Sprague–Dawley Co. (Madison, Wis., U.S.A.) at 21 days of age. The animals were placed two to a cage with constant temperature in windowless rooms and trained to a strict feeding and lighting schedule. An inverted light–dark cycle of 12h was used, with the dark period from 08:00h to 20:00h. The diet consisted of 30% protein and 61% carbohydrate (Watanabe *et al.*, 1968) and was available only during the first 8h of the dark period (08:00–16:00h). This regimen ('8+16') has been described (Potter *et al.*, 1968; Potter, 1970).

Preparation of cell extract. To ensure complete and even dispersion of the liver remnant for the various determinations to be described, the following procedure was adopted. Rats were killed by decapitation and the livers rapidly removed into cold 0.9% NaCl soln. Livers were blotted, weighed, wrapped in aluminium foil, frozen in liquid N_2 and stored at −70°C. For the preparation of homogenates, the livers were pulverized under liquid N_2 and several samples of the frozen powder were weighed out for the various determinations. For the enzyme assays, 2g portions of the powdered liver were kept cold on solid CO_2 until suspended with an equal volume of ice-cold homogenizing buffer (0.25M-sucrose, 0.5mM-dithiothreitol, and 0.075M-Tris–HCl, pH7.5). The sample was homogenized in a Potter–Elvehjem tissue homogenizer and centrifuged at 105 000*g* for 1h at 4°C in a Beckman type 50 titanium rotor in a Beckman L2 centrifuge. The clear supernatant was used for enzyme determinations. This treatment was found not to affect significantly the activities of pyruvate kinase, glucokinase or aldolase, compared with those of fresh tissue. However, the activities of the 'low K_m' hexokinases did appear to be slightly elevated when compared with freshly prepared homogenates.

Analytical methods

Assays. All substrates, coupling enzymes and cofactors used in the enzyme assays were purchased from the Boehringer Corp. (New York) Ltd. The isoenzymes of ATP–phosphohexose transferase were assayed by the method of Walker & Parry (1966). Glucokinase was determined as the 'high K_m' (0.1M-glucose) isoenzyme and the hexokinases (EC 2.7.1.1) were assayed as a group of 'low K_m' (0.5mM-glucose) isoenzymes. The isoenzymes of aldolase (fructose 1,6-diphosphate–D-glyceraldehyde 3-phosphate lyase; EC 4.1.2.13) were determined by a differential spectrophotometric assay, described by Blostein & Rutter (1963), with fructose 1,6-diphosphate and fructose 1-phosphate as substrates. The concentration of aldolase A and aldolase B in the mixture can be calculated from the fructose 1,6-diphosphate/fructose 1-phosphate activity ratio as described by Endo *et al.* (1970). The isoenzymes of pyruvate kinase were separated by starch-block zonal electrophoresis and quantitatively determined by elution and enzyme assay, as described by Tanaka *et al.* (1967) and previously discussed by Walker & Potter (1972). A unit of enzyme activity is the amount of enzyme required to metabolize 1 μmol of substrate/min at 25°C.

Determination of glycogen. Glycogen was extracted by the method of Roe *et al.* (1961) and measured with the anthrone reagent (Ashwell, 1957). A Polytron homogenizer (Kinematic GmbH, Luzern, Switzerland) was used to obtain complete cell breakage before extraction of glycogen from the tissue. This acid-extraction procedure was compared with extraction of glycogen with 1M-NaOH followed by precipitation of solubilized proteins with perchloric acid (Roe & Dailey, 1966). The two extraction procedures yielded equivalent results for glycogen.

Determination of DNA. DNA was extracted by an adaptation of the procedures of Schmidt & Thannhauser (1945) and of Schneider (1945), which gives optimum release of DNA and minimal losses during washings and through oxidation of deoxyribose (Hopkins *et al.*, 1973*b*). DNA was determined by a modification of the method of Burton (1956) in which sulphuric acid was omitted, the diphenyl-

amine content increased to a 2g/100ml and perchloric acid was present at a final concentration of 0.4M. Highly polymerized DNA (Sigma Chemical Co.) was used as the standard.

Partial hepatectomy and sham operations

After 3–4 weeks on the controlled feeding and lighting schedule, the animals weighed 150–170g. At this time 70% partial hepatectomies were performed under ether by the technique of Higgins & Anderson (1931) at 18:00h±45min on the same day unless indicated otherwise. This particular time of operation was chosen so as not to interrupt the feeding period of the animals on the day of the operation and because previous experiments suggested a higher degree of synchrony with this protocol. The animals were returned to their respective cages and killed at various times after the operation. The 'zero-time' control animals were not operated on but were removed from the cages with the rest of the experimental animals, returned to their cages and killed 24h after the experiment began. Sham operations consisting only of an abdominal incision and closure were also performed at 18:00h±45min.

Results

Isoenzyme patterns in foetal and adult liver

The isoenzyme compositions of ATP–D-hexose 6-phosphotransferase, fructose 1,6-diphosphate–D-glyceraldehyde 3-phosphate lyase and ATP–pyruvate phosphotransferase in foetal liver (17 days) and adult liver are shown in Table 1. The foetal liver isoenzyme profile is characterised by the absence of glucokinase and the presence of aldolase A and a low activity of pyruvate kinase type I, which is in agreement with other workers (Walker & Holland, 1965; Vernon & Walker, 1968; Walker & Potter, 1972). In contrast, glucokinase represents the major ATP–hexose 6-phosphotransferase isoenzyme and pyruvate kinase type I the major ATP–pyruvate phosphotransferase isoenzyme in adult liver. In addition, only aldolase B is present in adult liver. It should be noted that Walker & Potter (1972) suggested that pyruvate kinase type II and aldolase A were present in foetal liver in the haemopoietic cells, which disappear shortly after birth.

Liver growth after partial hepatectomy

The daily rhythm of liver weight for unoperated control animals trained to the '8+16' schedule is shown in Fig. 1(*a*) and for sham-operated animals in Fig. 1(*b*). The diurnal change in weight for normal animals is presumed to be due mainly to changes in glycogen and water (Leveille & Chakrabarty, 1967). For the experiments reported here the total liver protein was 16–19% of the wet weight. Protein/liver per 100g body wt. was only 15% higher at 20:00h (maximum value) than at 08:00h. After partial hepatectomy there was a slight gain in liver weight as a function of body weight by 24h (Fig. 1*c*). The liver continued to increase in weight, reaching 4.51g/100g body wt. in 7 days, which was approx. 86% of the unoperated control values. Two weeks

Table 1. *Isoenzyme activities of some enzymes of glycolysis*

The enzyme activities for foetal liver are values taken from Walker & Potter (1972). Adult liver values are an average of the activities found in three rats killed at 08:00h. M, Muscle-type isoenzymes; L, liver-type isoenzymes.

	Activity (units/g of liver)	
Isoenzyme	Foetal liver (17 day)	Adult liver
1 ATP–hexose 6-phosphotransferase		
(a) 'High K_m' glucokinase	0.00	0.80
(b) 'Low K_m' hexokinases	0.18	0.15
2 Fructose 1,6-diphosphate–D-glyceraldehyde 3-phosphate lyase		
(a) Aldolase A (M)	0.82	<0.10
(b) Aldolase B (L)	1.48	3.50
3 ATP–pyruvate phosphotransferase		
Total activity	14.4 (100%)	63.0 (100%)
Type I (L)	1.5	58.0
Type II (M_1)	4.3	0.0
Type III (M_2)	8.6 (60%)	5.0 (8%)

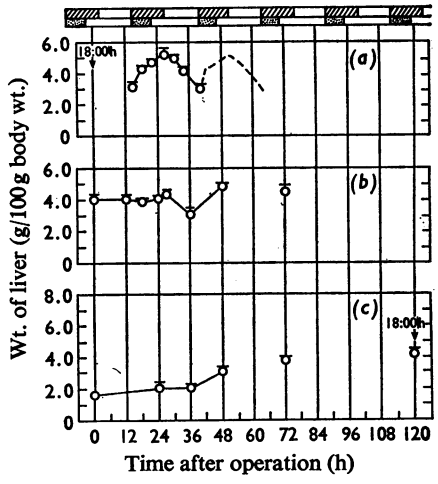

Fig. 1. *Liver weight as a percentage of body weight at various times of the day for rats on the* '8+16' *feeding schedule* (*stippled part of the top scale in this and subsequent figures*).

The feeding begins at the onset of the dark period (hatched part of the top scale in this and subsequent figures) at 08:00h. (*a*) Unoperated control rats. (*b*) After a sham operation. (*c*) After partial hepatectomy performed at 18:00h. Each point represents the mean ±S.E.M. for five animals in (*a*) and three animals in (*b*) and (*c*).

after partial hepatectomy the weight was 4.07g/100g body wt., indicating that liver growth had reached a plateau in 7 days.

Enzyme activities

Glucokinase activity. The activity of glucokinase in rat liver at various times of the day of the controlled feeding and lighting schedule can be seen in Fig. 2(*a*). The results are plotted in two ways, to better observe the enzyme-activity changes. Results plotted as units/g of liver are not only influenced by changes in actual enzyme activity but also by variations in liver weight due to increases in content of glycogen, fat, water and protein (Hopkins *et al.*, 1973*a*; Leveille & Chakrabarty, 1967). These weight fluctuations are corrected for by multiplying units/g by liver weight. Finally, the results are normalized for changes in rat weight over the 2 weeks experimental period by dividing the units/liver by the body weight.

It can be seen in Fig. 2(*c*) that the activity of glucokinase plotted as units/g of liver began to decrease approx. 12h after the operation and reached a minimum between 36 and 48h, which was less than 30% of the unoperated controls. At 48h the protein

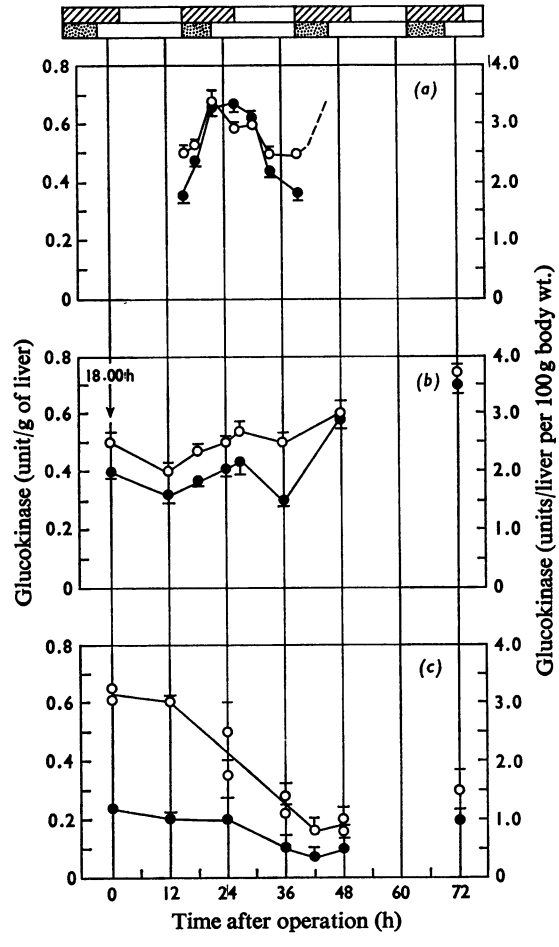

Fig. 2. *Glucokinase activity in liver at various times of the day for rats on the* '8+16' *feeding schedule*

(*a*) Unoperated control rats. (*b*) After a sham operation. (*c*) After a partial hepatectomy performed at 18:00h. Each point represents the mean ±S.E.M. for three animals. Where two points are shown for the same time, these are means of values for two separate experiments. Activity is expressed as unit/g of liver (○) or as units/liver per 100g body wt. (●). The zero-time point after partial hepatectomy for glucokinase activity expressed as units/liver per 100g body wt. is calculated as 30% of the unoperated controls.

concentration was only 10–12% less than at zero time (two experiments, results not shown). When the results are plotted as units/liver per 100g body wt. the activity of glucokinase does not appear to be significantly decreased until after 24h. Sham-operated animals (Fig. 2*b*) did not show a decrease in glucokinase but did show a decreased daily oscillation for the first 24h.

The activity of the 'low K_m' hexokinase group of enzymes did not vary significantly with time of day or food intake in normal or sham-operated animals (now shown). The activity of hexokinase increased from 0.12 unit/g of liver at zero time to 0.200 unit/g 72h after partial hepatectomy. However, this does not preclude larger changes in concentrations of the individual hexokinase isoenzymes, which were not measured here.

The possibility that the decrease in glucokinase after partial hepatectomy is due to an increase in concentration of an enzyme inhibitor is unlikely since extracts prepared from rats 48h after they had been partially hepatectomized did not inhibit glucokinase activity in control extracts (unpublished data). Also, it does not appear that this decrease in glucokinase was a result of lack of food intake by the animals. Stomach weights indicated that the animals had at least some solid food in their stomachs as early as 24h after the operation and normal stomach weights were observed after 48h.

An attempt was made to demonstrate more clearly that the decrease in glucokinase activity after partial hepatectomy was due to the operation itself and not to severe hypoglycaemia. The results in Table 2 show that, after partial hepatectomy, glucokinase activity decreased to 30% of the control values even in the presence of excess of glucose and insulin. Blood glucose measurements in animals given glucose by tube showed in all cases an increased concentration of glucose in these animals compared with animals that were partially hepatectomized but not given glucose. These results also indicate that hypoglycaemia is not an explanation for the decrease in glucokinase activity after partial hepatectomy.

The complete pattern of glucokinase activity after partial hepatectomy is shown in Fig. 3. The activity of glucokinase appeared to reach a minimum 42–48h after the operation but returned to 86% of the

Table 2. *Effect of glucose by intubation followed by insulin on the changes in isoenzyme pattern*

Partial hepatectomies were performed as described. One-half of the group of rats were intubated under ether anaesthesia every 12h with 300mg of glucose/100g body wt. followed by intraperitoneal injection of 5 units of insulin/100g body wt. 1h later. Both groups of rats were fed with the usual diet and killed at various times up to 48h. The enzyme was assayed in high-speed supernatants prepared from fresh tissue. Each value is the mean±S.E.M. for three animals.

Time after operation (h)	Glucokinase (units/g of liver)	
	No additions	Glucose+insulin
0	1.00±0.09	1.11±0.02
24	0.54±0.03	0.63±0.06
36	0.37±0.04	0.57±0.04
48	0.30±0.05	0.42±0.09

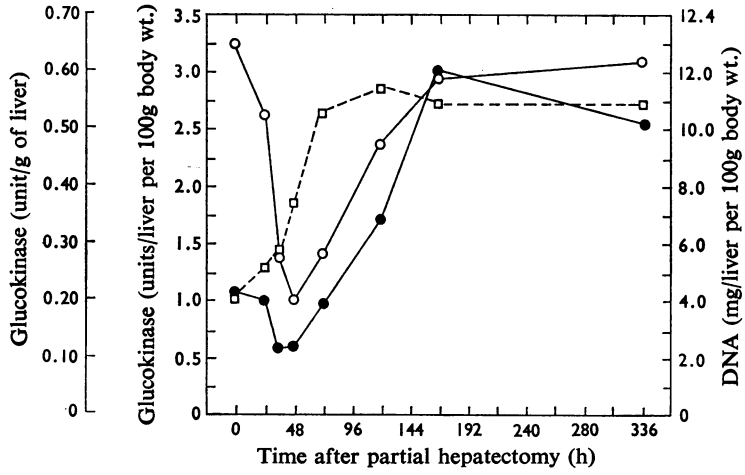

Fig. 3. *Pattern of glucokinase activity in rat liver after partial hepatectomy*

Each point represents the mean ±S.E.M. for three animals. Glucokinase activity is expressed as unit/g of liver (○) or units/liver per 100g body wt. (●). The DNA (□) was determined in the same liver remnants as were used for enzyme assays.

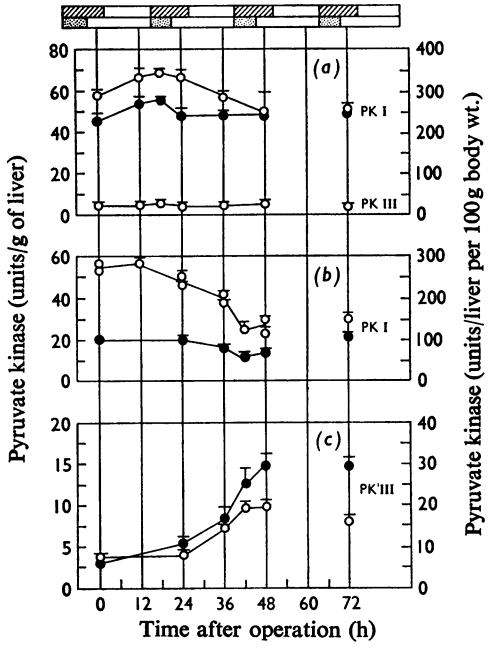

Fig. 4. *Pyruvate kinase activity in rat liver*

(*a*) Pyruvate kinase isoenzymes (PKI, PKIII) after a sham operation. The two isoenzymes were separated by starch-block zonal electrophoresis and quantitatively determined. (*b*) Pyruvate kinase type I (PKI) activity after partial hepatectomy. (*c*) Pyruvate kinase type III (PKIII) activity after partial hepatectomy. Each point represents the mean ±S.E.M. for three animals. Pyruvate kinase activity is expressed as units/g of liver (○) or units/liver per 100g body wt. (●).

unoperated controls after 7 days. However, in contrast, the amount of DNA/liver per 100g body wt. reached a plateau level at about 72h, which was 88% of the unoperated control value. Therefore it appears that cell replacement was nearly complete after 72h but the reattainment of adult glucokinase activities lagged by possibly as much as 96h.

Pyruvate kinase isoenzyme patterns. The total pyruvate kinase activity in rat liver does not vary appreciably with time of day on the '8+16' schedule. The activities fluctuated between 56 (08:00h) and 68 (16:00h) units/g of liver (300–380 units/liver per 100g body wt.).

The pyruvate kinase isoenzyme pattern remained essentially unchanged after sham operations (Fig. 4*a*). However, after partial hepatectomy (Fig. 4*b*) a decrease in pyruvate kinase type I expressed as units/g of liver was found to begin 12h after the operation and to reach a minimum at 42h. The decrease in units/liver per 100g body wt. began 24h after the operation. This pattern in strikingly similar to that of glucokinase (Fig. 2*c*).

Pyruvate kinase type III activity began to increase 24h after the operation, reached a maximum after 48h and remained elevated to 72h (Fig. 4*c*).

The pyruvate kinase type I activity profile over a 2 weeks period after partial hepatectomy is shown in Fig. 5. In a profile similar to that of glucokinase, pyruvate kinase type I activities did not return toward control values in parallel with increased DNA content.

The complete pattern of pyruvate kinase type III activity after partial hepatectomy is shown by Fig. 6. In contrast to glucokinase and pyruvate kinase type I, the increase in activity of pyruvate kinase

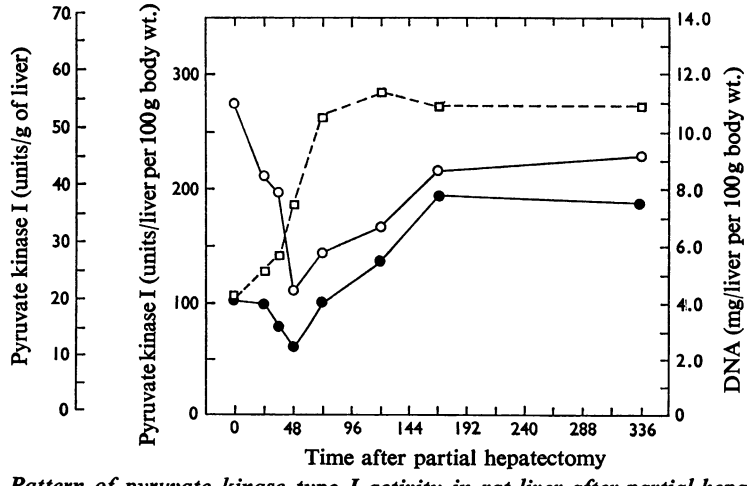

Fig. 5. *Pattern of pyruvate kinase type I activity in rat liver after partial hepatectomy*

Each point represents the mean ±S.E.M. for three animals. Pyruvate kinase activity is expressed as units/g of liver (○) or units/liver per 100g body wt. (●). □, DNA content.

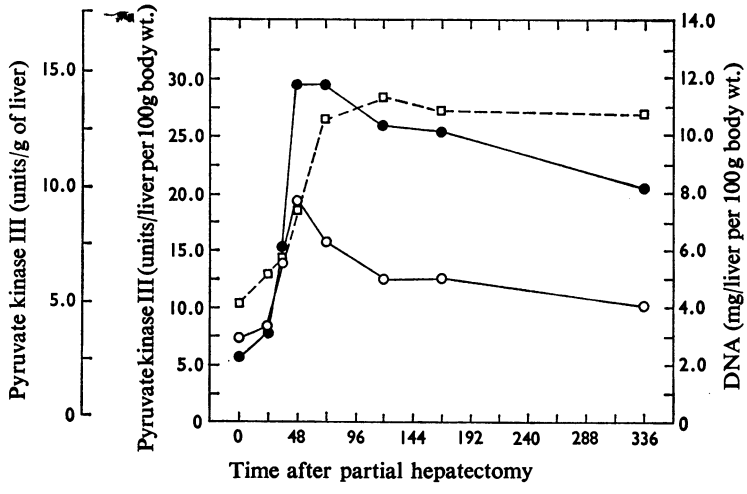

Fig. 6. *Pattern of pyruvate kinase type III in rat liver after partial hepatectomy*

Each point represents the mean ±S.E.M. for three animals. Pyruvate kinase activity is expressed as units/g of liver (○) or units/liver per 100g body wt. (●). □, DNA content.

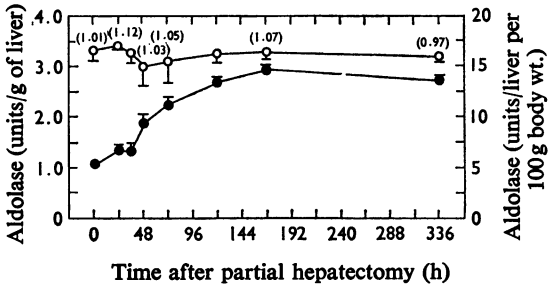

Fig. 7. *Pattern of aldolase B activity in rat liver after partial hepatectomy*

The activity of aldolase with fructose 1-phosphate as substrate is shown as units/g of liver (○) or units/liver per 100g body wt. (●). The values in parentheses are the aldolase activity ratios (activity with fructose 1,6-diphosphate as substrate/activity with fructose 1-phosphate as substrate). Each point represents the mean ±S.E.M. for three animals.

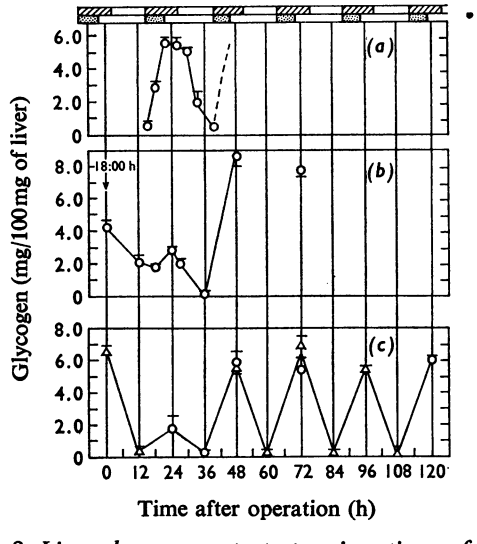

Fig. 8. *Liver glycogen content at various times of the day in rats on the '8+16' feeding schedule*

(*a*) Unoperated control rats. (*b*) After a sham operation. (*c*) After partial hepatectomy performed at 18:00h. Each point represents the mean ±S.E.M. for five (*a*) or three (*b* and *c*) rats. Results for experiments (○, △) are shown in (*c*).

type III appears to parallel or even to precede the increase in DNA content after partial hepatectomy. The zero time-point represents 30% of control values. Therefore it can be seen that the maximum activity of pyruvate kinase type III reached at 72h represents an elevated activity of total units/liver per 100g body wt. compared with intact liver.

Aldolase isoenzyme patterns. After partial hepatectomy, the activity of aldolase B expressed as units/g of liver showed a slight decrease at 48h but was otherwise constant (Fig. 7). The concentration of aldolase A present in the homogenates

prepared at various times after partial hepatectomy was calculated from the ratio of activity with the two substrates fructose 1,6-diphosphate and fructose 1-phosphate (Endo *et al.*, 1970). The ratios of 0.97–1.12 throughout the experiment demonstrate that only aldolase B was present. It should be noted that this is in disagreement with Farron *et al.* (1972), who reported a significant amount of aldolase A at 48h after partial hepatectomy. However, Weber & Shapira (1972) reported that there was not a very significant change in the aldolase isoenzyme pattern during liver regeneration.

Glycogen metabolism

The diurnal variation of liver glycogen content when rats are trained to the '8+16' schedule is shown in Fig. 8(*a*). The peak value is observed at the end of the 8h feeding and represents an approximately 12-fold change in the daily amounts. This rhythm is similar in pattern to that described by Potter & Ono (1961) for rats fed *ad libitum*.

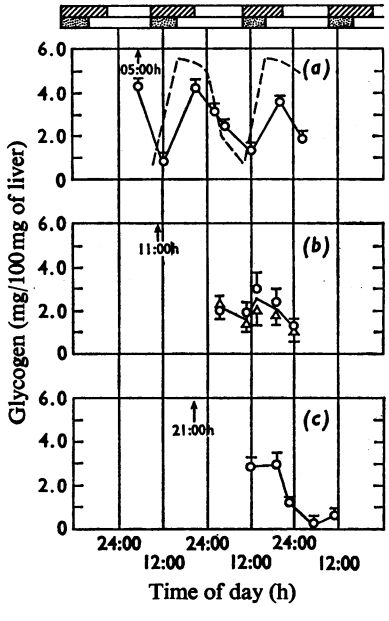

Fig. 9. *Liver glycogen content in rats after partial hepatectomy at various times of the day*

Partial hepatectomies were performed (*a*) at 05:00h, (*b*) at 11:00h or (*c*) at 20:00h. Each point represents the mean value ±S.E.M. for six rats. In (*a*) the broken line indicates the normal diurnal rhythm for liver glycogen content in rats on the '8+16' feeding schedule. In (*b*) results for two experiments are shown (○, △).

This diurnal rhythm of liver glycogen content was markedly diminished 24h after a sham operation performed at 18:00h but returned to normal after 48h (Fig. 8*b*). This decreased oscillation may have resulted from a decreased food intake during the 8h feeding period after the sham operation. Stomach weights at 06:00h indicated that the animals did not empty their stomachs normally and low stomach weights at 18:00h indicated that the animals did not eat the normal amount of food during the first 24h.

After partial hepatectomy in the rats, the diurnal rhythm for glycogen was still observed during the first 24h but was decreased in amplitude (Fig. 8*c*). The normal pattern was restored on the second and ensuing days of the experiment.

To demonstrate the importance of controlling the time of operation with respect to the diurnal oscillation of the parameter to be measured, liver glycogen contents were examined after partial hepatectomies performed at three different clock times. When the operation was performed at the time of day when the daily glycogen was approaching its lowest value (05:00h) the liver was able to store glycogen during the first 24h (Fig. 9*a*) but to decreased extent. However, if the operation was performed during the middle of the feeding period (11:00h), which is the time that the glycogen value was increasing, the daily rhythm was not observed over the following 24h period (Fig. 9*b*). When the operation was performed at 21:00h (Fig. 9*c*) a diminished daily rhythm was again observed.

Discussion

To obtain a greater understanding of fluctuations in liver enzyme activities after various manipulations, one must take into account their normal diurnal rhythms, which are a function of the feeding and lighting regimen of the experimental animals. In addition, when the enzyme concentrations are calculated as units/g of liver, variations in liver weight (Fig. 1) due to increases in water, glycogen, fat and protein content could result in misleading conclusions. After partial hepatectomy, the growth of the liver also appears to be influenced by the normal diurnal variation resulting in a stepwise replacement of the liver weight.

The activity of glucokinase in units/g of liver has been reported by Knox *et al.* (1970) not to change after partial hepatectomy. However, Lea *et al.* (1970) reported a decrease in glucokinase activity/g of liver to 25% of that in unoperated controls 48h after partial hepatectomy. This discrepancy has not been explained. We have also observed a decrease to 25–30% of that in controls in activity/g and a 50% decrease in units/liver per 100g body wt. In addition, the total glucokinase activity was found to return to

near adult values within 2 weeks after the operation. However, its return lagged more than 2 days behind the increase in liver DNA content.

A pattern of activity after partial hepatectomy similar to that for glucokinase was found for pyruvate kinase type I. Both glucokinase and pyruvate kinase type I activities did not decrease significantly until 24h after the operations; both reached a minimum at 42h and both returned to near control values at approximately the same time after the operation. DNA/liver per 100g body wt. was increased after 24h and continued to increase during the next 48h. Glucokinase activity (units/g) decreased to 30% of control values and pyruvate kinase type I activity decreased to approx. 40% of control values, which is greater than the decrease in these activities when expressed as enzyme activity/liver per 100g body wt. Therefore it is possible that the decreases in activity of enzymes characteristic of the highly differentiated liver cell represents dilution by new liver cells, which do not synthesize these enzymes. When these new cells 'differentiate' they become able to synthesize glucokinase and pyruvate kinase type I and the total liver activity of these enzymes returns to control values along the path described by Figs. 3 and 5. However, the decreases in activity of these enzymes characteristic of the highly differentiated liver cell are only partially explained by dilution with new, foetal-like liver cells that lack these enzymes, since the total activities in the liver remnants, expressed as units/liver per 100g body wt., are decreased after partial hepatectomy. It would appear that the synthesis or degradation of these enzymes is also altered after partial hepatectomy.

In contrast, pyruvate kinase type III, which constitutes a major proportion of total pyruvate kinase activity when the liver is engaged in cell division (such as before birth and shortly after birth; Walker & Potter, 1972), was found to increase after partial hepatectomy. The activity of this isoenzyme began to increase 24h after the operation, reached a maximum after 48–72h and began to return to control values by 120h. The increase in type III enzyme activity follows a pattern similar to the increase in DNA, indicating that this isoenzyme might be synthesized in dividing cells or in the new cells shortly after division. At some time in the life of a new individual liver cell after partial hepatectomy a change from synthesis of pyruvate kinase type III to synthesis of type I may occur similar to that seen in developing liver (Middleton & Walker, 1972). It should be noted that a similar change in pyruvate kinase isoenzyme pattern after partial hepatectomy was reported by Tanaka *et al.* (1967), but the magnitude of change of type III with respect to type I was not as great in the study reported here.

Crisp & Pogson (1972) reported that pyruvate kinase type III and 'low K_m' hexokinase were found in the non-parenchymal liver cells and that pyruvate kinase type I and glucokinase were found exclusively in the parenchymal cells of mouse liver. This has been verified in rat liver (unpublished results). However, it is not known whether pyruvate kinase type III is synthesized in the parenchymal cells after partial hepatectomy.

A further indication that the decrease in glucokinase and pyruvate kinase type I seen 48h after partial hepatectomy is not due to hypoglycaemia is the fact that glycogen is synthesized as early as 24h after the operation and exhibits a normal response to food after 48h. It is noteworthy that near normal glycogen metabolism was observed after the operation despite a decrease in glucokinase activity. Lea *et al.* (1970) reported that glycogen was greatly depleted 24h after partial hepatectomy and only restored to 50% of the control values after 96h. In their study the animals were housed in the light from 06:00h to 19:00h, the operations were performed between 11:00h and 12:00h and animals were presumably killed at the same time of day 24, 48 and 96h later. From the known diurnal glycogen variation for rats fed *ad libitum* (Potter & Ono, 1961), glycogen content would be decreasing at this time, if the feeding behaviour was not altered by the operation. A true indication of glycogen metabolism after partial hepatectomy can only be determined with animals killed at several times of day; otherwise, it is impossible to tell whether glycogen is being deposited or being mobilized.

Oncogenesis in many tissues and especially in liver might involve the conversion of a differentiated non-dividing cell to a malignant dividing cell. Thus there is a need for study of normal, controlled tissue hyperplasia, so that characteristics associated with dividing cells may be distinguished from those characteristics which lead to transformation. The regenerating liver induced by 70% hepatectomy is a suitable model system for such studies.

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