Phospholipid Metabolism during Regeneration of Rat Liver

By GÖRAN FEX

*Department of Chemistry, Section on Physiological Chemistry,
University of Umeå, S 901 87 Umeå, Sweden*

(Received 1 May 1970)

The concentration and composition of phospholipids and mitotic activity in regenerating rat liver were studied. (1) The total amount of liver phospholipid increased approximately linearly during 48h after operation but without change in the relative concentrations of individual phospholipids. (2) The appearance of mitoses 30h after operation was accompanied by an increased incorporation of ^{32}P into the liver phospholipids. (3) The regenerating livers incorporated a higher percentage of the label into the phosphatidylserine + phosphatidylinositol fraction than those of control rats. The percentage of the label incorporated into phosphatidylethanolamine in these livers increased but decreased in the phosphatidylcholine.

After a two-thirds partial hepatectomy in the rat the remaining liver promptly starts to grow. During the first 24h after the operation the cells increase in size but not in number (Harkness, 1957), but after this lag period there is a relatively synchronized burst of mitotic activity during which 3-5% of the cells may be in mitosis at the same time. Maximal mitotic activity is usually observed at about 28h after the operation (Cater, Holmes & Mee, 1956; Weinren, 1959). Cell division is undoubtedly associated with the synthesis of new membrane and, as phospholipids are integral parts of all cell membranes, it might be supposed that cell division is accompanied by alterations in the metabolism of membrane phospholipids.

The present study was undertaken to determine whether changes in the metabolism of liver phospholipids are concomitant with the first outbreak of mitotic activity and persist while the mitotic activity in the liver is high.

EXPERIMENTAL

Carrier-free $^{32}\text{P}[\text{P}_i]$ in HCl solution, pH 2-3, was obtained from The Radiochemical Centre, Amersham, Bucks., U.K. It was diluted with 2 vol. of 0.9% NaCl and used directly for injections.

Male Sprague-Dawley rats weighing 150-220g were kept in a dark and quiet room and given free access to food and water during the week preceding the experiments. Partial hepatectomy was performed by the procedure of Higgins & Anderson (1931). All operations were performed between 8 and 11 a.m. Unoperated rats were used as controls (Gurr, Prottey & Hawthorne, 1965). Up to the time of death the rats were kept in the dark room where they had free access to water but no food. At 3h

before the rats were killed $^{32}\text{P}[\text{P}_i]$ solution containing approx. $50\mu\text{Ci}$ was injected intravenously via an exposed neck vein.

The rats were killed by exsanguination through the abdominal aorta. The livers were excised, rinsed in water, freed of necrotic tissue, blotted and weighed. A small piece of the central part of the right anterior lobe was excised and placed in 10% formalin. A 1g portion of liver tissue was taken for the extraction of acid-soluble phosphorus (Gurr *et al.* 1965). The rest was weighed and homogenized in 20 vol. of chloroform-methanol (2:1, v/v). Paraffin sections of formalin-fixed liver tissue were stained with iron haematoxylin. Then 1000 nuclei were counted for each liver and the number of mitotic nuclei was recorded. Only nuclei in typical anaphase or metaphase were counted. In unoperated control rats the proportion of mitotic nuclei never exceeded 0.2%.

A chloroform extract of the liver lipids was prepared by the procedure of Folch, Lees & Sloane-Stanley (1957). Neutral lipids and phospholipids were separated on silicic acid columns (Olivecrona, 1962). Individual phospholipid classes were separated by t.l.c. by the procedure of Skipski, Peterson & Barclay (1964). Phosphatidylserine and phosphatidylinositol were not well separated by this method. The plates were sprayed with 0.2% dichlorofluorescein in ethanol and viewed under u.v. light, and the spots corresponding to different phospholipid fractions were scraped off, transferred to small columns and eluted with chloroform-methanol-water-formic acid (10:9:1:1, by vol.). Lysophosphatidylcholine was eluted with chloroform-methanol-water-formic acid (2:7:1:1, by vol.). For determination of the concentration of individual phospholipids the procedure of Arvidson (1968) was used. The recoveries of both radioactivity and phosphorus from the chromatoplates were always better than 93%. Lipid phosphorus was determined by the method of Chen, Toribara & Warner (1956). Radioactivity was measured in a Packard model 3320 liquid-scintillation spectrometer. Quenching was corrected for by the channels-ratio procedure.

RESULTS

The number of mitotic nuclei rose sharply between 24 and 30h after the operation and remained high for the rest of the period investigated (Table 1). The increase in mitotic activity was not accompanied by any abrupt change in the liver phospholipid content (Fig. 1). The total hepatic content of phospholipid increased almost linearly during the first 48h after the operation at a rate of about $20\mu\text{mol}$ of lipid phosphorus in 24h per liver and 200g body wt. The rate of increase in wet liver weight during the same time was somewhat more rapid: about 1g in 24h per liver and 200g body wt. The discrepancy between the rate of increase in phospholipid content and the gain in wet weight was probably due to the deposition of

triglyceride (Fex, 1970; Glende & Morgan, 1968). No alteration in the proportions of the major phospholipid classes occurred during the first 48h after partial hepatectomy (Table 2), these results being similar to data for normal rats (Rouser, Nelson & Fleisher, 1968).

Since previous work has shown that haemodynamic alterations occur after partial hepatectomy (Menyhart & Simon, 1966a,b), the incorporation of ^{32}P into liver phospholipids as a function of time after intravenous injection of $[^{32}\text{P}]\text{P}_i$ was studied. Less ^{32}P was recovered in the regenerating liver phospholipids at all times after the injection. However, because of the lower liver weight and the lower concentration of phospholipid per g of liver, the specific radioactivity of the regenerating liver phospholipids was equal to or higher than in the controls. For the further experiments an exchange period of 3h for the ^{32}P was chosen (Fig. 2). The rate of incorporation of ^{32}P into liver phospholipids was taken as a measure of the rate of phospholipid synthesis. The relative specific radioactivity was calculated as the ratio of the specific radioactivity of the phospholipids to that of the acid-soluble phosphorus. The specific radioactivity of the latter was consistently higher in the partially hepatectomized rats. A 1.0 ratio means that the relative specific activities were the same in the partially hepatectomized and in the control rats, whereas a ratio greater than 1.0 means that relatively more radioactivity had been incorporated into the liver phospholipid of the partially hepatectomized rats. The results show that there was an increase in the relative specific radioactivity of the phospholipids of the regenerating liver (Table 3) coincident with the rise in mitotic activity (Fig. 1). The percentage distribution of label among the three major phospholipid fractions (Table 4) demonstrates a different labelling pattern in the regenerating livers. At all times but one more label was incorporated in the phosphatidylserine+phosphatidylinositol fraction in the regenerating livers. The percentage of label incorporated into phosphatidylethanolamine in these livers was increased at 24, 30 and 33h, whereas the percentage of the label recovered in phosphatidylcholine was decreased at almost all times, compared with the control rats. The radioactivity of the minor phospholipid fractions (lysophosphatidylcholine, sphingomyelin and cardiolipin+phosphatidic acid) is not shown in Table 4 as it was very low and did not differ in partially hepatectomized rats and unoperated control rats.

Table 1. *Mitotic activity in the liver after partial hepatectomy*

Results are given as means $\pm$ S.D.

Time after operation (h)	No. of animals	Mitotic nuclei per 1000 nuclei
18	5	<1
24	4	1 ± 1
30	4	15 ± 7
33	5	8 ± 3
36	3	21 ± 2
39	3	17 ± 2
48	3	20 ± 1

Fig. 1. Phospholipid content of liver after partial hepatectomy. Means $\pm$ S.D. for five rats are shown.

DISCUSSION

My results demonstrate that the rate of incorporation of ^{32}P into the phospholipids of the regenerating rat liver increased at the same time as

Table 2. Liver phospholipid content and distribution of lipid phosphorus 30h after partial hepatectomy

Results are given as means $\pm$ S.D. Abbreviations: PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; PC, phosphatidylcholine.

	No. of animals	Phospholipid P (μ mol/g wet wt. of liver)	Phospholipids (% of total lipid P)		
			PE	PI+PS	PC
Partial hepatectomy	9	35.9 $\pm$ 4.2	28.3 $\pm$ 0.8	10.9 $\pm$ 0.8	52.5 $\pm$ 4.5
No surgery	8	39.2 $\pm$ 3.5	29.3 $\pm$ 1.8	11.3 $\pm$ 0.5	54.3 $\pm$ 1.8

Fig. 2. Incorporation of ^{32}P into liver phospholipids of partially hepatectomized and control rats. Partial hepatectomy was performed 30h before injection of ^{32}P P_i . Each point is the mean of two determinations. O, Control; ●, partial hepatectomy.

the increase in mitotic rate. The elevated rates of phospholipid labelling and mitotic activity persisted throughout the experimental period. The results agree well with those obtained in previous investigations (Johnson & Albert, 1952) although their experiments are not directly comparable with ours.

Gurr *et al.* (1965), however, did not find any increase in the rate of incorporation of ^{32}P into the

liver phospholipids 36h after partial hepatectomy, although they state that the mitotic activity was probably high at this time. It is not possible to say why my results differ from those of Gurr *et al.* (1965), but there are some experimental differences that may contribute to the discrepancy. In their experiments both the partially hepatectomized and control rats had free access to food after the operation. Since partially hepatectomized rats eat very little, whereas the control ones eat normally, there may well have been a difference in the nutritional state of the two groups. The route of administration of ^{32}P P_i is probably also important, as is the length of the exchange period of ^{32}P . I used a 3h exchange period and injected the ^{32}P P_i intravenously, whereas Gurr *et al.* (1965) used a 6h exchange period and injected the ^{32}P P_i intraperitoneally. The latter route of administration results in a much higher radioactivity of the liver phospholipids than the former (McMurray & Dawson, 1969). In spite of this, I chose to inject the label intravenously as changes in splanchnic blood flow induced by the partial hepatectomy (Menyhart & Simon, 1966a,b) might give rise to altered resorption from the peritoneal cavity.

The total amount of liver phospholipid increased approximately linearly during the first 48h after partial hepatectomy (Fig. 1). No abrupt change was observed coincident with the increase in mitotic activity or with the time when the mitotic activity was high. The percentage distribution of phospholipid phosphorus among the three major phospholipid fractions (Table 4) was constant throughout the entire experimental period. Thus the net synthesis of each fraction was proportional to its contribution to the liver phospholipid pool.

The values for the specific radioactivities of the three fractions were always in the order phosphatidylethanolamine > phosphatidylcholine > phosphatidylserine + phosphatidylinositol. This is in accord with previous investigations (Gurr *et al.* 1965) and suggests that the rate of synthesis of phosphatidylserine + phosphatidylinositol was low in comparison with those of phosphatidylethanolamine and phosphatidylcholine (Åkesson, 1969).

Table 3. *Relative specific radioactivities and ratio of relative specific radioactivities of liver phospholipids of partially hepatectomized and control rats at various times after operation*

[^{32}P]P_i was injected 3 h before the times indicated. Results are given as means $\pm$ s.d.

Time after operation (h)	No. of animals	Relative specific radioactivities		Ratio (partial hepatectomy/control)
		Partial hepatectomy	Control	
18	5	26.2 $\pm$ 4.9	27.9 $\pm$ 5.6	0.99 $\pm$ 0.35
24	5	22.6 $\pm$ 4.5	23.0 $\pm$ 4.2	1.04 $\pm$ 0.44
30	5	24.3 $\pm$ 2.0*	17.6 $\pm$ 4.2	1.46 $\pm$ 0.42
33	5	32.0 $\pm$ 2.6*	27.0 $\pm$ 1.8	1.18 $\pm$ 0.08
36	5	30.7 $\pm$ 5.4*	24.2 $\pm$ 4.1	1.30 $\pm$ 0.32
39	5	25.6 $\pm$ 1.5*	21.7 $\pm$ 2.1	1.19 $\pm$ 0.17
48	5	27.4 $\pm$ 4.6*	21.7 $\pm$ 2.2	1.28 $\pm$ 0.29

* Significantly higher than control ($P < 0.05$).

Table 4. *Percentage distribution of ^{32}P radioactivity among major liver phospholipid fractions*

Results are given as means $\pm$ s.d. of three animals in each group. Abbreviations: PE, phosphatidyl-ethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; PC, phosphatidylcholine.

Time after operation (h)	Partial hepatectomy			No surgery		
	PE	PI+PS	PC	PE	PI+PS	PC
18	37.2 $\pm$ 3.1	6.4 $\pm$ 0.9	51.8 $\pm$ 3.2	37.9 $\pm$ 2.4	3.9 $\pm$ 0.6	54.5 $\pm$ 3.5
24	41.6 $\pm$ 1.3	6.5 $\pm$ 1.1	47.7 $\pm$ 2.4	35.8 $\pm$ 1.0	3.8 $\pm$ 0.9	56.5 $\pm$ 1.0
30	39.3 $\pm$ 1.3	5.7 $\pm$ 0.4	51.6 $\pm$ 1.4	35.5 $\pm$ 0.9	3.8 $\pm$ 0.5	56.6 $\pm$ 0.6
33	37.2 $\pm$ 2.8	6.6 $\pm$ 2.2	52.1 $\pm$ 0.6	33.8 $\pm$ 0.6	3.8 $\pm$ 0.4	58.4 $\pm$ 0.5
36	35.9 $\pm$ 3.1	5.3 $\pm$ 0.7	54.9 $\pm$ 3.8	34.9 $\pm$ 1.3	3.2 $\pm$ 0.1	58.4 $\pm$ 1.9
39	33.2 $\pm$ 1.3	8.6 $\pm$ 2.8	52.9 $\pm$ 2.8	32.8 $\pm$ 0.4	3.9 $\pm$ 0.8	58.4 $\pm$ 0.5
48	32.7 $\pm$ 1.3	4.5 $\pm$ 0.6	56.3 $\pm$ 5.3	32.5 $\pm$ 0.7	3.7 $\pm$ 0.4	58.8 $\pm$ 1.5

Thus, as the proportions of the three phospholipid fractions remained constant during the first 48 h of liver regeneration, the rate of synthesis of phosphatidylserine+phosphatidylinositol probably has to increase more than rate of synthesis of the two other phospholipids. If this were true one would expect the phosphatidylserine+phosphatidylinositol fraction to incorporate ^{32}P at an increased rate in the regenerating liver, which was also the case.

The rate of incorporation of ^{32}P into the liver phospholipids increased at the same time as the increase in mitotic activity, 30 h after the operation (Table 3). As there was no abrupt change in the phospholipid content of the liver at this time it is probable that this increased rate of incorporation of ^{32}P represents either the synthesis of small amounts of phospholipids with high specific radioactivity or an increased rate of phospholipid turnover.

Nothing is known about the possible relation of the increased ^{32}P incorporation and cell division in mammalian cells. However, studies on synchronized bacterial populations (Daniels, 1969; Lubochinsky & Burger, 1969) have shown that cell

division is accompanied by an increased rate of incorporation of lipid precursors into phospholipids, which was attributed to the formation of the phospholipids of the dividing septum (Daniels, 1969). It is possible that the increased ^{32}P incorporation that I observed associated with mitotic activity in the regenerating liver represents a similar phenomenon, i.e. the formation of the plasma-membrane and/or the nuclear-membrane phospholipids in the dividing cells. The increased incorporation of ^{32}P might, however, also be accounted for in terms of new endoplasmic reticulum (Tata, 1970).

This work was supported by a grant from the Swedish Cancer Society. The skilful technical assistance of Miss K. Lindberg is gratefully acknowledged.

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