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Phospholipin Metabolism in Rabbit-liver Cytoplasm

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The recent work of Fishler, Entenman, Montgomery & Chaikoff (1943) and of Entenman, Chaikoff & Zilversmit (1946) demonstrated that liver is the principal tissue in the body concerned with the production and removal of plasma phospholipins. However, it is not known whether synthesis and decomposition of phospholipins take place uniformly throughout the liver cell or whether these functions are associated with a particular type of particle in the cell. The present work deals partly with the technique of liver-cell fractionation and partly with the study of the rates of renewal of phospholipins in liver-cell fractions. Hevesy (1945) showed that the rate of renewal of phospholipin phosphorus in liver nuclei is slower than in the cytoplasm. His observation is confirmed, and it is shown that there are at least three different rates of renewal in different fractions of the liver cytoplasm.

1. ANALYSIS OF LIVER FRACTIONS

There have been numerous attempts to fractionate liver into components which can be directly related to visible granules in the liver cell. Several methods, e.g. those of Marshak (1941–2), Dounce (1943), Mirsky & Pollister (1946), have been used to prepare liver-cell nuclei, while methods have been described by Claude (1946*a*), and more recently by Hogeboom, Schneider & Pallade (1948) for the fractionation of liver cytoplasm. Claude described the preparation of three fractions: (1) large granules, corresponding to the mitochondria and secretory granules of the histologist; (2) microsomes (small granules), composed of particulate elements of submicroscopic size; and (3) a supernatant, containing the particles and molecules which remain in solution after the first two fractions had been removed. Hogeboom *et al.* (1948) have criticized Claude's (1946*a*) procedure on the ground that the granules isolated in fraction 1 are not identical morphologically or in staining reactions with the mitochondria seen in the intact cell, and they have described a method of isolating these intact.

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MATERIAL AND METHODS

In an earlier (unpublished) series of experiments normal rabbit liver was fractionated by Claude's (1946*a*) procedure. The presence of glycogen, however, interfered seriously with the fractionation, as has also been observed by Claude. In subsequent experiments this effect was largely eliminated by withholding food from the animals the night before the removal of the liver.

In view of the evidence of Hogeboom *et al.* (1948) that the large granules prepared by the use of a concentrated solution of sucrose more closely resemble the mitochondria of the intact cell, their method (in a slightly modified form) was used for most of the present investigation. In our work the first consideration has been the purity of the fractions, necessarily at the expense of information about the amount of each present.

Fully grown (2·3–2·8 kg.) rabbits were used. They were anaesthetized with nembutal and ether, and after withdrawal of blood from the aorta, the liver was excised. All fractionation procedures were carried out at or near 0°.

Liver fractionation. Hogeboom *et al.* used 0·88 M-sucrose solution in their procedure. For the sedimentation of the large and small granules in a reasonably short time from a solution of such high density, centrifugal forces of 24,000 and 41,000 *g* respectively are needed. A refrigerated centrifuge developing such forces was not available, and the following modifications were introduced to separate the particles at lower centrifugal forces.

The liver was pressed through a 1 mm. mesh screen; 15–20 g. of the resultant pulp were ground for 5 min. in a mortar and 100 ml. of 0·88 M-sucrose solution added over a period of 10 min. with continuous grinding. The suspension was centrifuged at 600 *g* for 10 min. and the deposit of intact liver cells, debris and nuclei discarded. The supernatant was centrifuged twice more at the same speed, the small sediments being discarded.

The suspension of cytoplasmic constituents, usually divided in two to four tubes, was now centrifuged at 18,000 *g* for 45 min. to throw down the large granules. The supernatant, SII, was set aside. The deposits were pooled with the help of the small amounts of SII left behind and

spun again to separate a little more SII. The residue of large granules was suspended in the sucrose solution and centrifuged at 18,000 *g* for 40 min.; this was repeated three times, the supernatants being discarded. As previously observed by Hogeboom *et al.*, streaming birefringence could be readily demonstrated in the suspensions of large granules in sucrose solutions. After the purification, the preparation under the phase contrast microscope was seen to consist largely of rod-like particles. For chemical analysis, the large granules were rapidly suspended in a saline bicarbonate solution (0·85% (w/v) NaCl containing 0·05 M-$NaHCO_3$), centrifuged at 18,000 *g* for 10 min. and the deposit immediately dried from the frozen state.

The density of supernatant SII in four experiments was found to vary between 1·1091 and 1·1098 at 2°. This solution was dialysed under pressure against distilled water for 20–24 hr. The volume of the supernatant usually doubled during the dialysis, the density of the final solution varying between 1·019 and 1·011 at 2°. The dialysed supernatant, SII, was first clarified by centrifugation at 2000 *g* for 15 min., the small deposit discarded, and the clear supernatant from this centrifuged at 18,000 *g* for 2 hr. The sediment, a reddish brown transparent pellet, will be referred to as the small granule fraction. The supernatant from this was centrifuged at 18,000 *g* for 2 hr. and the very small sediment which was usually deposited added to the first sediment. The final supernatant from this centrifugation, SIII, was dried from the frozen state.

The sedimented small granules were resuspended in the saline-bicarbonate solution and centrifuged at 18,000 *g* for 2 hr. This was repeated, and the final deposit dried from the frozen state.

Chemical analysis. The purified granule fractions were analysed for total lipid, phospholipin, nucleic acid P and total N. Whole liver tissue (a dried sample of the liver pulp) was analysed for total lipid and phospholipin, and the supernatant fraction (SIII) for phospholipin.

The dried large and small granule fractions and the whole liver samples were extracted four times by boiling with an ethanol-ether mixture (3:1, v/v) and finally once with ether. All the lipid was considered to be removed by this treatment. The fat-free residues of the large and small granule fractions were dried at 37°; the difference in dry weights before and after extraction was taken as the total lipid content of the sample, and was expressed as a percentage of the dry weight before extraction.

The combined extracts from each sample were evaporated to dryness under reduced pressure (20–40 mm. Hg) with N_2 passing through the extract. The dry residue was extracted three times with light petroleum (b.p. 40–60°). Since the animals were injected with inorganic radioactive phosphate (see Part 2), it was necessary to get rid of traces of contaminating radioactive phosphate from the phospholipins. Ten drops of a saturated aqueous solution of Na_2HPO_4 were therefore added during the light petroleum extraction and the extracts vigorously shaken. It has been shown by Fries, Schachner & Chaikoff (1942) that shaking an ethereal solution of phospholipins with saturated Na_2HPO_4 solution effectively removed traces of radioactive inorganic $PO_4^{\equiv}$ from the phospholipins. The extract was cleared by centrifugation, any particulate matter and inorganic phosphate being discarded in the lower aqueous phase. The clear extract was concentrated to about 2 ml., and the phospholipin precipitated by adding acetone and a saturated solution of ethanolic $MgCl_2$ as described by Bloor (1929). The phospholipins were dissolved in moist ether, and phospholipin P estimated colorimetrically by Allen's (1940) method. Instead of $HClO_4$ as described by Allen, 5 N-H_2SO_4 and H_2O_2 ('100 vol.') were used for the digestion. (The micro-analytical reagent grade H_2O_2, supplied by British Drug Houses Ltd., was used throughout this work.) The dried supernatant SIII was similarly analysed.

The P and N contents of the fat-free, dry, liver fractions were determined on 10 mg. samples in duplicate. The N content was determined by a micro-Kjeldahl method; 50% (v/v) H_2SO_4 containing 1% (w/v) SeO_2 and H_2O_2 ('100 vol.') were used for the digestion.

It was assumed that all low molecular weight P compounds were removed in the purification process and therefore the P remaining in the fat-free residue must be nucleic acid P. This P was multiplied by 1·7 to give nucleic acid N and by 9·01 to give the nucleic acid value. The nucleic acid N was subtracted from the total N to give protein N and this was multiplied by 6·25 to give total protein.

RESULTS

In Table 1 are given the values obtained by chemical analyses of whole liver and of the large and small granule fractions from it. The total lipid and more particularly the total phospholipin contents of whole liver are seen to be reasonably constant. In view of this, it is surprising that the total lipid of the large granule fractions is quite variable, which suggests that this fraction is not homogeneous. In appearance the large granule sediment was never homogeneous, two layers being always present. Attempts to separate these by further differential centrifugation, however, were not successful. The total lipid content of the small granules is greater and more constant than that of the large granules. This greater uniformity suggests that the fraction might be homogeneous. This was supported by preliminary electrophoretic experiments in a potassium phosphate buffer, of ionic strength $I = 0·2$ and pH = 8·0, in which a single boundary was apparent. The large granule fraction was not similarly examined.

It is suggested here that the centrifugal separation of the large from the small granules may depend mainly on the differences in their respective lipid contents, and hence on the differences in the density of the particles, rather than on the differences in their size.

The nucleic acid values of the large and small granule fractions show the greatest variation, the averages, however, being approximately the same in each. The variation may be due to the method of estimation, depending as it does on the assumption that all low molecular weight phosphorus compounds are removed in the purification process. Estimation of the ribonucleic acid content of these fractions by colorimetric methods based on ribose was unsatisfactory because of other interfering compounds, e.g. glucose from glycogen breakdown.

Table 1. *Lipid, nucleic acid and protein content of whole liver and of large and small granules obtained from it*

(All results are expressed as percentage of dry weight. L. = liver; L.G. = large granules; S.G. = small granules. 'Total' (last two columns) = sum of total lipid, nucleic acid and protein.)

Exp. no.	Total lipid			Phospholipin			Nucleic acid		Protein		Total	
	L.	L.G.	S.G.	L.	L.G.	S.G.	L.G.	S.G.	L.G.	S.G.	L.G.	S.G.
20	15·8	14·1	42·3	12·6	13·1	31·0	—	3·33	—	45·6	—	91·2
21	16·5	30·6	41·9	13·5	17·5	31·8	2·34	3·00	63·8	50·5	96·7	95·4
22	15·6	27·5	45·2	13·6	18·5	34·0	2·95	2·38	62·0	49·4	92·5	97·0
23	21·4	36·0	44·7	12·0	13·7	26·2	3·92	3·24	49·0	51·0	88·9	99·0
26	—	29·6	44·2	—	19·6	35·7	2·45	2·9	61·6	50·8	93·7	97·9
27	—	28·0	41·6	—	19·0	34·3	2·45	2·81	64·3	54·0	94·8	98·5
29	17·5	41·0	45·0	—	17·8	—	3·0	2·6	49·6	49·7	94·6	97·3
30	18·2	37·2	43·0	14·0	17·2	—	3·4	1·25	52·3	53·8	92·8	98·1
32	18·3	30·9	42·6	13·6	17·8	30·2	2·5	2·64	61·5	43·2	94·9	88·4
33	18·8	25·6	40·1	13·1	19·4	29·6	2·75	1·74	64·4	—	92·8	—
34	18·7	28·2	43·7	14·8	17·6	28·2	1·8	2·9	65·3	55·1	95·3	101·7
35	19·4	26·3	46·2	15·3	18·5	—	2·1	2·0	67·5	57·0	95·4	99·2
Mean	**18·0**	**29·6**	**43·4**	**13·6**	**17·5**	**31·2**	**2·7**	**2·6**	**60·2**	**50·4**	**94·0**	**96·6**
S.D.	1·76	6·82	1·77	1·02	2·14	2·97	0·57	0·62	6·3	3·5	2·1	3·8

Table 2. *Comparison of the analytical data obtained for the large and small granule fractions of rabbit liver with those reported by other investigators*

	Large granules					Small granules					
	Total lipid	Phospholipin	Total N	Total P	N.A.P.* (μg.)/total N (mg.)	Total lipid	Phospholipin	Total N	Total P	N.A.P.* (μg.)/total N (mg.)	Animal species
	(% dry wt.)					(% dry wt.)					
Claude (1946*b*)	25	16	10–12	0·9–1·3	—	40–45	29	9·15	1·51	—	Pig
								8·95	1·74	—	Rat
Hogeboom *et al.* (1948)	—	—	—	—	15·5	—	—	—	—	58	Rat
Present author	29·6	17·5	10·5	1	70	43·4	31·2	9	1·5	80	Rabbit

(N.A.P. = Nucleic acid phosphorus.)

The protein values also show considerable variation, particularly those for the large granule fraction. In the latter case, however, the variation might be due to the inhomogeneity previously mentioned. No correlation was found between the amounts of the various components in each fraction. The total lipid, nucleic acid and protein account for 94 % of the total solids of the large granules and for 96·6 % of the small granules. Thus, these fractions contain small amounts of other substances. Claude (1946*b*), for example, found approximately 0·5 % of inositol in the large granules. No attempt has been made in the present work to identify the unestimated components. In Table 2 the results of this investigation are compared with those of Claude (1946*b*) and Hogeboom *et al.* (1948). The values for rabbits are in good agreement with those obtained for rats and guinea pigs. The unequal distribution of nucleic acid between the large and small granules in rat livers (Hogeboom *et al.*) has not been found with rabbit liver.

Distribution of phospholipin, nucleic acid and protein between large and small granule fractions

The yield (dry weight/dry weight) of fractions from liver pulp was as follows: large granules, 3·5 ± 0·9 %; small granules, 4·9 ± 0·8 %; the ratio of the two being 0·73 ± 0·11. If it is assumed that this is also the ratio of the granules in the liver-cell cytoplasm, and if the small fractions discarded during the fractionation are neglected, then the

Table 3. *Distribution of constituents between large and small granules of rabbit liver*

	Large granules (%)	Small granules (%)
Nucleic acid	43	57
Protein	46	54
Total lipid	33	67
Phospholipin	29	71

distribution of the cytoplasmic constituents between the two types of particles may be calculated as shown in Table 3. These values must be very approximate because of the great technical difficulties of the fractionation procedure. The distribution of phospholipin, among the large granules (1), small granules (2) and supernatant (3), was similarly calculated to be as follows: (1) 26·4, (2) 64·6, (3) 9% respectively.

2. INVESTIGATIONS USING RADIOACTIVE PHOSPHORUS

The object of these experiments was to find out if there is a site in the liver cell where most of the phospholipin is synthesized. Neutral sodium phosphate containing ^{32}P was injected into rabbits and the various liver fractions prepared as described in Part 1. The specific activities (counts/min./mg.) of phospholipin phosphorus of the fractions were determined, and expressed as percentage of ^{32}P injected/kg. of body weight (counts/min./kg.).

METHODS

Administration of ^{32}P. In a few experiments of 4 hr. duration, the ^{32}P (as Na_2HPO_4 in saline) was administered to the rabbits either as a continuous intravenous infusion, or in subcutaneous injections repeated at 20 min. intervals in order to maintain a constant level of inorganic ^{32}P in the plasma, as described by Hevesy & Hahn (1940). Under these conditions, the specific activities of all P compounds—including those of the liver phospholipin—should rise until they reach the level of the specific activity of the plasma inorganic P.

In the majority of experiments, however, inorganic phosphate labelled with ^{32}P was given in a single subcutaneous injection, and the animals killed 2–72 hr. later. The total dose of ^{32}P administered was usually 200 μc./rabbit.

Analysis of blood. Blood was obtained from the rabbits as described in Part 1. It was collected in a vessel containing heparin, cooled immediately, and the plasma drawn off after centrifugation at 2°. Approximately 15 ml. of plasma were extracted with 20 vol. of ethanol-ether (3:1, v/v) and the phospholipins obtained as described previously. Approximately 5 ml. of plasma were mixed with an equal volume of 10% (w/v) trichloroacetic acid, the precipitated protein centrifuged off and the inorganic phosphate precipitated as $MgNH_4PO_4$.

Analysis of liver. A sample of liver was fractionated by differential centrifugation as described in the previous section. Liver-cell nuclei were prepared by the method of Mirsky & Pollister (1946) and dried from the frozen state. The phospholipins of the fractions were extracted and purified as already described, and were digested with 5N-H_2SO_4 and H_2O_2 ('100 vol.'). The inorganic phosphate thus obtained was precipitated as $MgNH_4PO_4$.

Immediately after removal of the liver, a small portion was blotted quickly with filter paper and dropped into a freezing mixture of acetone and solid CO_2. The frozen tissue was powdered in a steel mortar previously cooled with the freezing mixture. The crushed liver (about 5 g.) was shaken by hand with cold 5% (w/v) trichloroacetic acid (10 ml.) and the mixture filtered. Inorganic phosphate was separated from the extract as $MgNH_4PO_4$.

Assay of ^{32}P. All P samples were obtained as dry $MgNH_4PO_4$. In early experiments samples containing 50–300 μg. of P were dissolved in 0·5 ml. 0·1N-HCl. Of this, 0·3 ml. was pipetted accurately on to a small nickel disk, whilst 0·1 ml. was used for the determination of P. The deposits on the disks were dried by gentle heating, and the radioactivity was measured with a bell-shaped Geiger-Müller counter.

Owing to the low radioactivity of some of the samples, and the difficulty of obtaining a uniform layer containing more than 300 μg. of P on the disk, a cylindrical, jacketed counter, described by Veall (1948) and suitable for the measurement of radioactivity of solutions, was later used. Amounts up to 5 mg. of $MgNH_4PO_4$ were dissolved in 1 ml. N-HCl and 10 ml. of water added; the radioactivity of 10 ml. of the mixed solution was then measured and samples of the solution were used for P estimations (in duplicate).

At least 5000 and usually 10,000 counts from each specimen were taken, giving a statistical accuracy of at least ±1·5%. The specific activity determinations are accurate to ±3%.

RESULTS

Table 4 contains the results of an experiment in which ^{32}P was injected subcutaneously every 20 min. over a period of 4 hr. Samples of blood (5 ml.) were taken at intervals after the first injection as indicated in Table 4, and the plasma inorganic phosphorus isolated as previously described. The final blood sample was taken and the liver removed 20 min. after the last injection.

Table 4. *Specific activity of plasma inorganic* P *and of liver-*P *fractions after subcutaneous injection of* ^{32}P

(^{32}P injected every 20 min. over a period of 4 hr.)

A. Change in plasma inorganic P with time

Time after first injection (min.)	Plasma inorganic P specific activity (counts/min./mg. P)
55	77
95	77·5
135	77
175	97
215	77
235	70

B. Specific activity of liver and plasma-P fractions 235 min. after first injection

	Specific activity (counts/min./mg. P)
Liver	
Inorganic P	39
Phospholipin P, small granules	1·30
Phospholipin P, large granules	1·14
Phospholipin P, nucleus	1·00
Phospholipin P, supernatant	0·73
Plasma	
Inorganic P	70
Phospholipin P	0·83

The specific activities of the various phosphorus fractions shown are typical. The specific activity of the inorganic phosphorus in the plasma is maintained

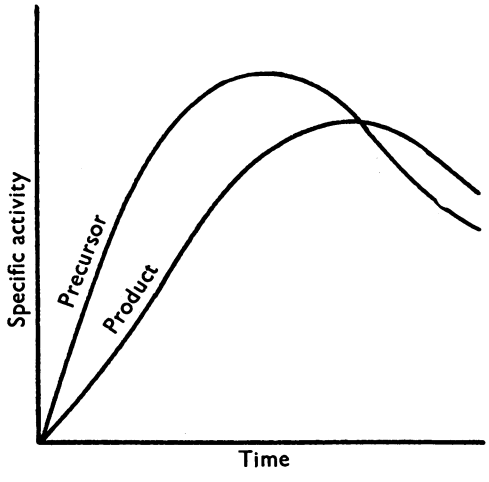

Fig. 1. Theoretical relationship between the specific radioactivity of precursor and its product.

at a reasonably constant level. Over the 4 hr. period, the specific activity of the liver inorganic phosphorus (not corrected for extracellular inorganic phosphate) rises to about half that of the plasma inorganic value, but the uptake of ^{32}P into the phospholipin fractions is much slower. The two main cytoplasmic fractions, small and large granules, are similar to each other in value, with the former always slightly higher. The specific activities of the phospholipins of the nucleus and of the supernatant are somewhat lower, the value of the latter being lower than that of the plasma phospholipin.

The result indicates that phospholipin fractions of different specific activities may exist in the liver cell under the conditions of this experiment. However, this experiment does not indicate whether the phospholipins in the fractions are synthesized independently of one another in the cell, or whether they are synthesized in one fraction—viz. the small granules, as this fraction has the highest specific activity—and are transferred from this fraction to the others.

Zilversmit, Entenman & Fishler (1943) have established mathematically the relationship between the specific activity of a labelled precursor and that of its product in experiments in which the specific activity of the precursor is a function of time. In such experiments it is necessary to give only one injection of the labelling agent (e.g. ^{32}P). This relationship is illustrated graphically in Fig. 1, from which it can be seen that the specific activity of the product at its maximum is equal to the specific activity of its precursor at the same time. Before this maximum has been reached the specific activity of the compound must be less, and afterwards it must be greater, than the specific activity of the precursor. These criteria have been employed in our experiments in which sixteen rabbits were killed at intervals of 2–72 hr. after an injection of radioactive phosphate.

Since it is necessary to kill the rabbit in order to get sufficient liver for analysis, each animal can provide data for one point only on the time curve of liver phosphorus turnover. The metabolic idiosyncrasies of rabbits are such that it is impossible to predict beforehand how much labelled phosphate to inject in order to create exactly the same level of ^{32}P in any plasma or tissue fraction in all animals at the same time. To circumvent this difficulty a

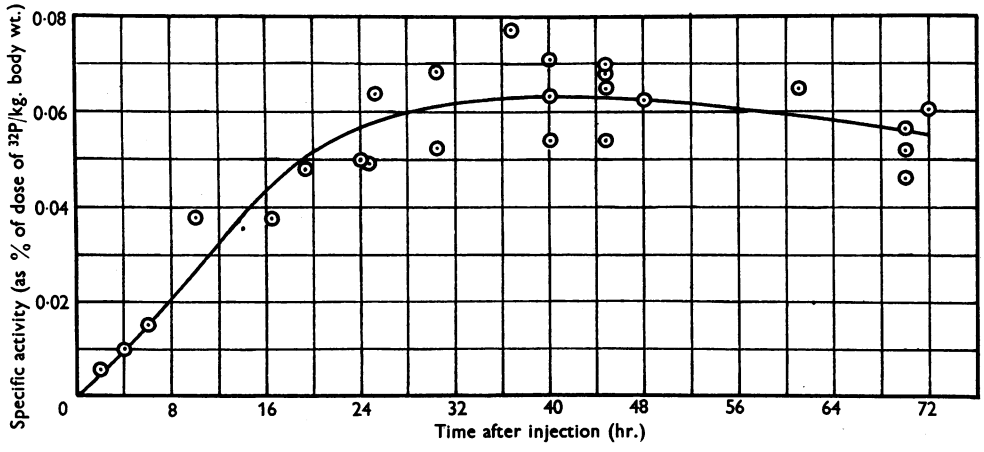

Fig. 2. Change in specific radioactivity of plasma phospholipins of rabbits injected with a single dose of inorganic ^{32}P.

specific activity-time curve of plasma phospholipins was established for an independent series of rabbits which were bled at four different intervals from 2 to 72 hr. after an injection of labelled phosphate.

This curve, shown in Fig. 2 and reproduced again in Fig. 3, was taken as the reference standard and all specific activities of the plasma-phospholipin phosphorus from the main series of experiments were adjusted to fall on this curve. Correspondingly, the specific activities of the three liver phospholipin fractions were multiplied by the same factor. Thus this correction does not alter the ratios of the specific activities of the three liver phospholipin fractions to that of the plasma phospholipins; only the absolute values are affected.

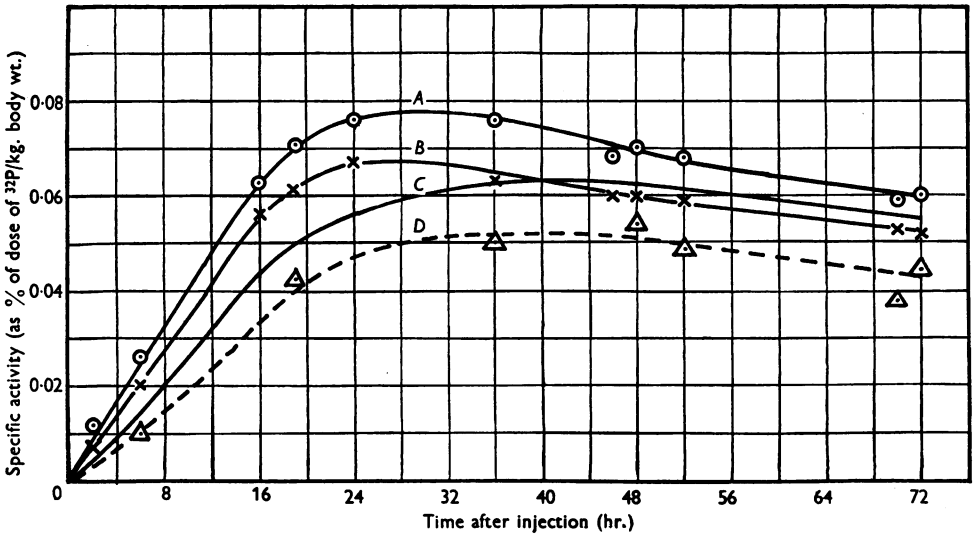

Fig. 3. Change in specific radioactivity of liver and plasma phospholipins of rabbit injected with a single dose of inorganic ^{32}P. *A*, Phospholipins extracted from small granules; *B*, phospholipins extracted from large granules; *C*, plasma phospholipins; *D*, phospholipins extracted from liver 'supernatant' after large and small granules have been removed.

From Fig. 3 it can be seen that the specific activity-time curves of the three liver fractions remain independent of each other during the experiment. That is, not one of these components behaves as a precursor with respect to any of the others, according to the conditions set down by Zilversmit *et al.* (1943).

DISCUSSION

Hevesy's (1945) original observation that the rate of renewal of phospholipin phosphorus in the liver nuclei is less than the rate in cytoplasm has been confirmed in the present series of experiments, though there does exist in the cytoplasm a small component (the supernatant) with an even slower rate of renewal.

The first set of experiments showed that phospholipin fractions of three different specific activities existed in the liver cytoplasm, and the question arose whether the phospholipins in the fractions were synthesized separately, or whether the phospholipins in one fraction, viz. the small granules, might be the precursor of the phospholipins of the others. In applying the criteria laid down by Zilversmit *et al.* (1943) for the establishment of a precursor, several assumptions, as recognized by these authors, are made. These are: (1) a steady state, in which the amount of compound present in the tissue studied must be constant during the interval over which the observations are made; (2) the rate of synthesis and degradation of the compound must be constant during the experiment; (3) the appearance and disappearance of all molecules must proceed at random, i.e. the specific activity of portions of the compound breaking down (or leaving the tissues) is equal to the specific activity of the total amount of the compound present in that tissue. In addition to these, a fourth assumption is involved in the present investigation of the components obtained by fractionation. Since they are not simple compounds, it must be shown that their properties are reproducible from experiment to experiment. The results in Table 1 show that, as far as chemical composition is concerned, this assumption may be justified.

As fully matured rabbits of 2·3–2·8 kg. body weight were chosen, the first and second assumptions

are justified. In the present investigation, the third assumption is perhaps not justified, as there is no evidence that the phosphorus of the phospholipin molecules distributed throughout a small or large granule is of uniform specific activity at any moment. If the latter assumption is made however, the curves in Fig. 3 indicate that phospholipin synthesis (or at least the step involving the incorporation of phosphorus into the phospholipin molecule) may take place in at least three sites in the liver cytoplasm.

It will be noticed from the curves in Fig. 3, however, that the large granule phospholipins appear to be the precursor of the plasma phospholipins. The criteria of Zilversmit *et al.* (1943) can only be applied in this case if it is known that the plasma phospholipins are entirely derived from the liver, i.e. that no phospholipin enters the plasma from any other source to alter significantly the specific activity of the total plasma phospholipins. As previously mentioned, the work of Fishler *et al.* (1943) and Entenman *et al.* (1946) has demonstrated that liver is the principal tissue in the body concerned with the production and removal of plasma phospholipins. More recently, Artom & Swanson (1948) have shown that phospholipins may reach the plasma from the intestine, though it appears that they do so only in very small amounts. If the assumption is made that the liver is the sole source of plasma phospholipins, then the curves in Fig. 3 indicate that plasma phospholipins are derived only from the large granule fraction. On the basis of this assumption, it can be calculated from the distribution figures previously presented that, under the conditions of these experiments, only 25–30 % of the total phospholipin formed in the liver is transferred to the plasma. Most of the phospholipins formed in the liver remain inside the cell, and presumably are used in the normal metabolism of the cell.

It has been suggested by Claude (1943) that the large granule fraction and the microsome or small granule fraction have a common origin, or that one type of granule may contribute to the constitution of the other. Claude quotes observations which appear to support the latter possibility. While the results of the present investigation do not directly confirm or deny these possibilities, it is significant that, as far as their phospholipin components are concerned, the metabolism of the two fractions is different, and it is therefore unlikely that one fraction is derived from the other.

SUMMARY

1. A modification of the method of Hogeboom *et al.* (1948) for the fractionation of liver cytoplasm by differential centrifugation has been used for the preparation of three fractions, large granules, small granules (microsomes) and supernatant, from rabbit-liver cytoplasm. The method of Mirsky & Pollister (1946) has been used to prepare rabbit-liver nuclei.

2. The microsome and large granule fractions from twelve rabbit livers have been analysed for total lipid, phospholipin, protein and nucleic acid content. Total lipid, protein and nucleic acid account for 94 % of the large granules and 96·6 % of the small granules. The total lipid (including phospholipin) content constitutes the main chemical difference between the two fractions, the large granules containing 29·6 % and the microsomes 43·4 %.

3. Approximately 26·4 % of the liver cytoplasmic phospholipins was in the large granules, 64·6 % in the small granules, and 9 % in the supernatant.

4. The metabolism of the phospholipin phosphorus of the cytoplasmic fractions and the nucleus has been studied with the aid of radioactive phosphorus (^{32}P). It is shown that at any time after the injection of radioactive phosphate into rabbits there exist in the liver cytoplasm at least three phospholipin fractions with different specific activities. The original observation of Hevesy (1945), that the rate of renewal of phospholipin phosphorus in the liver nuclei is slower than in the cytoplasm, has been confirmed.

5. The results suggest that phospholipins are being synthesized separately and metabolized independently of each other in different morphological structures of the liver cell.

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