the hydrolysis: L-α-GPC + H_2O → α-glycerophosphoric acid + choline.

3. Similar extracts also attacked L-α-glycerylphosphorylethanolamine, and this diester was a competitive inhibitor of GPC hydrolysis.

4. The GPC-splitting activity of such extracts was maximal at pH 7·5 and was stimulated by the addition of Mg^{2+} (10^{-3} M). Similar concentrations of Mn^{2+} and Ca^{2+} were inhibitory and this also applied to higher concentrations of Mg^{2+}.

5. The enzyme was not sensitive to fluoride, iodoacetate, eserine or di*iso*propylphosphorofluoridate. However, ethylenediaminetetraacetic acid and Zn^{2+} were strong inhibitors.

6. Evidence has been obtained for the presence of the enzyme in other tissues of the rat, but it is virtually absent from the liver of the sheep. Activity was not observed in rat plasma, but it was found in the blood corpuscles.

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Studies on the Phosphorylcholine of Rat Liver

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The recent isolation of phosphorylcholine in considerable quantities from aqueous extracts of rat liver (Dawson, 1955*a*) has raised the question of its origin in the tissue *in vivo* and its possible role as an intermediary in lecithin synthesis. Two enzymes are known which can produce phosphorylcholine in tissues: a lecithinase which splits lecithin into phosphorylcholine and a diglyceride, and choline phosphokinase, which catalyses the direct phosphorylation of choline by adenosine triphosphate (Wittenberg & Kornberg, 1953). With regard to its possible role as a lecithin precursor, recent experiments of Rodbell & Hanahan (1955), following the work of Kornberg & Pricer (1952), have convincingly demonstrated that phosphorylcholine can be incorporated as a unit into the lecithin of guinea-pig liver mitochondrial preparations.

Experiments have therefore been performed whereby radioactive phosphate was administered to rats, and a study made of the rate of labelling of both liver phosphorylcholine and lecithin. The results indicate that phosphorylcholine is not formed in the tissue through the action of a lecithinase, and the rapid rate with which it becomes labelled with ^{32}P suggests some process such as the direct phosphorylation of choline. At the same time examination of the specific radioactivity time curves using the Zilversmit, Entenman & Fishler (1943) predictions does not indicate an immediate precursor–product relationship between liver phosphorylcholine and lecithin.

In further experiments, designed to obtain information about the metabolic relationships of phosphorylcholine with other choline-containing compounds, the level of the substance in rat liver has been compared with that of liver glyceryl-

phosphorylcholine (GPC) during various conditions such as starvation, insulin hypoglycaemia, and after choline administration.

EXPERIMENTAL

Experimental methods used in the present report have been fully described in a previous paper (Dawson, 1955*b*). Two small improvements, however, were introduced into the procedure for obtaining two-dimensional chromatograms of the water-soluble P-containing compounds of the liver. The solution was freed from cations with an Amberlite IRC 50 column, and ethanol–NH_3 was used as the second solvent in the paper chromatography. The location and identification of the phosphorylcholine spot on such chromatograms has been described previously (Dawson, 1955*a*); the ethanol–NH_3 solvent used instead of propanol–NH_3 did not alter the relative position of the spots but resulted in a greater separation of phosphorylcholine and glycerylphosphorylcholine.

Animals used for the estimation of phosphorylcholine and glycerylphosphorylcholine levels in the liver were killed by a blow on the neck, and the liver was rapidly dissected and plunged into liquid O_2 (<1 min.). The frozen tissue was then crushed and a two-dimensional chromatogram prepared of the water-soluble phosphorus-containing compounds in the tissue. The phosphorus contained in the spots was estimated in the usual way after complete oxidation of the paper with perchloric acid (Dawson, 1955*b*). Constant recoveries of approximately 75% were obtained when synthetic glycerylphosphorylcholine and phosphorylcholine were added to tissue extracts, and carried through the entire experimental procedure. Although therefore the chromatographic recoveries are not quantitative, in the absence of any other suitable methods of estimation, they do give a good indication of the relative levels of these phosphoesters in the tissue.

RESULTS

Fig. 1 compares the specific radioactivities of rat-liver phosphorylcholine and lecithin in a series of animals killed at various times after the intraperitoneal injection of a constant amount of ^{32}P as phosphate. Immediately after the injection the phosphorylcholine becomes rapidly labelled with ^{32}P, and its specific radioactivity rises well above that of the liver lecithin. For example, 100 min. after injection the phosphorylcholine has a specific radioactivity which is at least 20 times greater than that of liver lecithin. This precludes the possibility that the phosphorylcholine occurs in the tissue as an intermediary in the catabolism of lecithin. For comparison, included on Fig. 1 are data on the specific radioactivity of liver glycerylphosphorylcholine, a compound which is now known to arise in the tissue by the breakdown of lecithin (Dawson, 1955*b*). Although the specific radioactivity of liver phosphorylcholine is initially higher and subsequently lower than that of the lecithin, it does not appear to obey the Zilversmit *et al.* (1943) criteria for being the immediate phosphorus precursor of the phosphoglyceride; its specific radioactivity does not fall to equal that of the lecithin until some hours after the latter has reached its maximum specific radioactivity (Fig. 1).

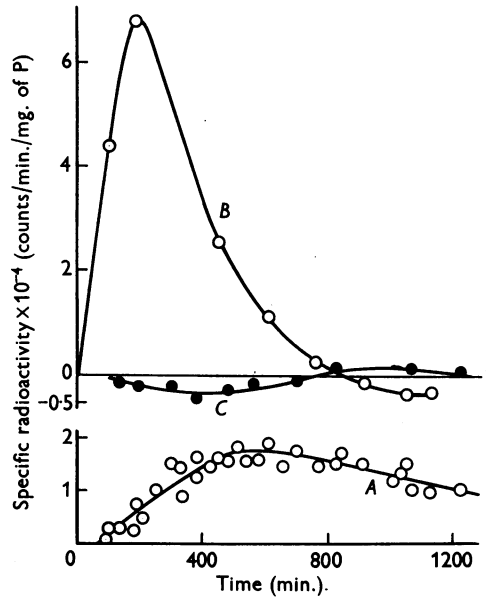

Fig. 1. Curves showing (*A*) the specific radioactivities of liver lecithin; (*B*) the specific radioactivities of liver phosphorylcholine minus that of lecithin; (*C*) the specific radioactivities of liver glycerylphosphorylcholine minus that of lecithin; at various times after the injection of labelled phosphate (2×10^6 counts/min./100 g. of body wt.).

In Table 1 are recorded the recoveries of phosphorylcholine and glycerylphosphorylcholine obtained on paper chromatography of extracts from the livers of rats killed during various physiological states. As is recorded in the experimental section, these recoveries are only approximately 75% quantitative, and they represent minimal concentration values only. Experiments were performed with series of four animals, one of which was always a control allowed free access to food and water. In these control rats the molar concentration of phosphorylcholine was usually 3–4 times greater than that of glycerylphosphorylcholine and in one animal was 8 times greater. In animals killed 45–90 min. after the intraperitoneal injection of a single dose of choline chloride (200 mg./kg.) the recovery of phosphorylcholine was always greater than that from the control animal (26–74% increase), and that of the glycerylphosphorylcholine was unaltered. On the other hand, in four rats killed after 24 hr. starvation the mean recovery of glycerylphosphorylcholine from the liver was over twice that found in the livers of control

animals, and at the same time the phosphorylcholine content of the tissue did not increase.

A further series of rats were made hypoglycaemic by the intraperitoneal injection of insulin. It was found that appreciable doses of the hormone were needed before the animals showed visible signs of the hypoglycaemia, presumably because they had not been starved before the injection.* In all these animals, the amounts of glycerylphosphorylcholine and phosphorylcholine recovered from the liver increased markedly (Table 1). Even in rats where the hypoglycaemia was not sufficient to cause the animals to have convulsions or to become comatosed, the recoveries were still well above those of the control values.

(Rodbell & Hanahan, 1955). Kennedy & Weiss (1955) have recently published preliminary evidence which suggests that phosphorylcholine is converted by liver mitochondria into 'cytidine diphosphate choline' (P^1-cytidine-5′-P^2-choline-pyrophosphate) before it participates in lecithin synthesis. However, the departure of the phosphorylcholine and lecithin radioactivity–time curves from the expected precursor–product relationship predicted by Zilversmit *et al.* (1943) cannot be explained by the assumption that phosphorylcholine is first converted into another intermediate like 'cytidine diphosphate choline', as such a compound would obey the criteria even less readily than phosphorylcholine. Thus the specific radioactivity of the

Table 1. *Chromatographic recoveries of phosphorylcholine and glycerylphosphorylcholine (GPC) from rat liver during starvation and insulin hypoglycaemia and after the injection of choline*

The concentrations expressed represent minimal values only.

	Phosphorylcholine (μmoles/g.)	GPC (μmoles/g.)
Controls		
(6 rats) Means ± S.E.	1·52 ± 0·14	0·35 ± 0·04
Range	(1·15–1·91)	(0·22–0·53)
Choline chloride injection (200 mg./kg. body wt.)		
(5 rats) Means ± S.E.	2·14 ± 0·15	0·39 ± 0·04
Range	(1·90–2·70)	(0·29–0·55)
	$P < 0·02$	—
Starvation (24 hr.)		
(4 rats) Means ± S.E.	1·34 ± 0·14	0·82 ± 0·14
Range	(1·02–1·76)	(0·48–1·12)
	—	$P < 0·01$
Insulin hypoglycaemia		
(5 rats) Means ± S.E.	2·81 ± 0·14	1·46 ± 0·17
	$P < 0·01$	$P < 0·01$
Rat injected with 40 units of insulin at 150, 60 and 30 min. before killing. Activity slightly depressed	2·46	1·17
Rat injected with 26 units of insulin at 145 and 30 min. before killing. Activity depressed	2·85	1·64
Rat injected with 80 units of insulin at 150 and 30 min. before killing. Semi-comatose	2·60	1·66
Rat injected with 90 units of insulin at 270, 180 and 150 min. before killing. In convulsions	3·24	1·04
Rat injected with 80 units of insulin at 145 and 20 min. before killing. In convulsions	2·96	1·76

DISCUSSION

It must be emphasized that the present isotopic results merely suggest that phosphorylcholine is not the sole immediate phosphorus precursor of liver lecithin, and not that the ester plays no part in lecithin synthesis *in vivo*. Indeed such participation seems highly likely in view of the recent demonstration of its incorporation *in vitro* as a unit into the liver lecithin of mitochondrial preparations

* Subsequent work has shown that rats allowed food *ad lib.* can tolerate 1000 units of insulin/kg. body wt. injected intraperitoneally.

choline P in such an intermediary would fall to equal that of the liver lecithin at a time later than for phosphorylcholine. The failure to obey the Zilversmit *et al.* criteria may be connected with the recent demonstration that another pathway of lecithin synthesis, independent of phosphorylcholine as an intermediary, exists in liver mitochondria (Kennedy, 1954; Kennedy & Weiss, 1955). The breakdown of the Zilversmit *et al.* (1943) criteria in a metabolic system in which a compound is synthesized from more than one precursor has recently been emphasized in a theoretical paper by Reiner (1953).

As the present results support the view that liver choline can be rapidly phosphorylated *in vivo*, it seems likely that the level of phosphorylcholine will to some extent be a reflexion of the relative rates of formation and further metabolism of free choline. On the other hand, the GPC content can be expected to depend on the rate of lecithin breakdown occurring in the tissue, as this compound has now been identified as an intermediate in the catabolism of liver lecithin (Dawson, 1955*b*). Any GPC liberated from liver lecithin would be subsequently hydrolysed into free choline and glycerophosphoric acid under the action of the enzyme GPC diesterase present in liver tissue (Dawson, 1956).

Thus in the present investigation the injection of choline into the rat was associated with an expected increase in the phosphorylcholine level without alteration of the GPC content. Starvation, which in the mammal produces a rapid loss of phosphoglycerides from the liver (Kosterlitz, 1947; Hodge *et al.* 1948), causes a marked rise in the GPC content, presumably because of the extra lecithin catabolism, and it might therefore be expected that the phosphorylcholine level would also rise. That such an increase does not occur during starvation may be related to the absence of dietary choline and methionine intake, and the requirements of the animal for these substances for transmethylation reactions. In insulin hypoglycaemia the level of GPC in the tissue is markedly increased, and this, as is to be expected, is associated with an elevation of the phosphorylcholine content.

SUMMARY

1. Specific radioactivities of liver phosphorylcholine and lecithin have been measured at various times after the injection of radioactive phosphate into the rat.

2. The results indicate that phosphorylcholine is not formed in the liver by the breakdown of lecithin, nor does it appear to obey the Zilversmit *et al.* (1943) radioactive criteria for the sole immediate phosphorus precursor of liver lecithin.

3. Chromatographic recovery of phosphorylcholine in the liver is increased after the injection of choline but not after starvation.

4. Conversely, the recovery of liver glycerylphosphorylcholine is increased after starvation but is unaltered by choline administration.

5. Chromatographic recoveries of both liver phosphorylcholine and glycerylphosphorylcholine are markedly increased during insulin hypoglycaemia.

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Enzymic Properties of Microsomes and Mitochondria from Silver Beet

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Extensive studies on cells of animal origin from a wide variety of tissues have already established the localization of enzymic activity in cytoplasmic particles. The enzymic activities of mitochondria from animal tissues indicate that these structural units are sites of respiratory activity (see Schneider, 1953). A more limited series of studies have shown that the mitochondria from plant cells have a similar function (Millerd & Bonner, 1953; Goddard & Stafford, 1954). However, in neither plant nor animal cells is there as yet any clear indication of the metabolic function of the submicroscopic cytoplasmic particles (so-called 'microsomes'), although a number of enzymic activities appear to