The presence of creatine phosphate in the annelid can hardly suggest any close relationship between this phylum and the Chordata, as formerly postulated by Dohrn (1875) and Semper (1875, 1876). Developmental, embryological and anatomical evidence is against such a relationship and the occurrence of creatine is presumably due to convergent chemical evolution.

SUMMARY

1. Analysis of the phosphagen content of a number of species of annelids is reported.
2. Creatine and creatine phosphate have been shown to be present in muscles of certain annelids.
3. A preliminary investigation by chromatography of the monosubstituted guanidine phosphates is described.

Our thanks are due to the Central Research Fund of the University of London and to the Field Fund of University College, London, for grants which have permitted this work to be carried out at Roscoff. We also wish to express our thanks to the staff of the Station Biologique, Roscoff, for help and living material; also to Professor E. Baldwin for invaluable advice and encouragement and to Dr D. Walker for assistance in part of this work in 1951.

REFERENCES

- Baldwin, E. & Yudkin, W. H. (1950). *Proc. Roy. Soc. B*, **136**, 614.
- Dohrn, A. (1875). *Der Ursprung der Wirbelthiere und das Prinzip des Funktions-Wechsels*. Leipzig: Engelmann.
- Eggleton, G. P. & Eggleton, P. (1929). *J. Physiol.* **68**, 193.
- Ennor, A. H. & Stocken, L. A. (1948). *Biochem. J.* **43**, 190.
- Fiske, C. P. & Subbarow, Y. (1929). *J. biol. Chem.* **81**, 629.
- Greenwald, I. (1946). *J. biol. Chem.* **162**, 239.
- Hanes, C. S. & Isherwood, F. A. (1949). *Nature, Lond.*, **164**, 1107.
- Hobson, G. E. (1955). *Biochem. J.* **60**, viii.
- Meyerhof, O. (1928). *Arch. Sci. biol., Napoli*, **12**, 536.
- Needham, J., Needham, D. M., Baldwin, E. & Yudkin, J. (1932). *Proc. Roy. Soc. B*, **110**, 260.
- Roche, J., Felix, W., Robin, Y. & Thoai, N.-V. (1951). *C.R. Acad. Sci., Paris*, **233**, 1688.
- Semper, K. (1875). *Arb. zool. Inst., Würzburg*, **2**, 25.
- Semper, K. (1876). *Arb. zool. Inst., Würzburg*, **3**, 115.
- Thoai, N.-V. & Robin, Y. (1954). *Biochim. biophys. Acta*, **13**, 533.
- Thoai, N.-V., Roche, J., Robin, Y. & Thiem, N.-V. (1953a). *C.R. Soc. Biol., Paris*, **147**, 1241.
- Thoai, N.-V., Roche, J., Robin, Y. & Thiem, N.-V. (1953b). *Biochim. biophys. Acta*, **11**, 593.
- Walpole, G. S. (1911). *J. Physiol.* **42**, 301.

The Incorporation of Labelled Phosphate into the Phosphoglycerides of Intact Rat Liver

By R. M. C. DAWSON

Department of Biochemistry, University of Oxford, and Biochemistry Department, Institute of Animal Physiology, Babraham, Cambridge

(Received 22 April 1955)

Speculation has often occurred as to whether any of the individual phosphoglycerides present in animal tissues are formed from other phospholipids in the same tissue. Popják & Muir (1950), for example, found some evidence for the interconversion of phosphoglycerides in rabbit liver after the injection of ^{32}P -labelled phospholipid emulsions. Another method of investigating this problem is to compare the specific radioactivities of the individual liver phosphoglycerides after labelled-phosphate administration. Previous attempts to measure the specific radioactivities of the various liver phospholipids have relied upon solvent fractionation methods (e.g. Hahn & Tyrén, 1946) or adsorption of the non-choline-containing phospholipids on magnesium oxide (e.g. Zilversmit, Entenman & Chaikoff, 1948), both of which yield fractions which are extremely heterogeneous in their base distribu-

tion. It is not possible therefore to reach any reliable conclusions about phospholipid interconversion from the results of such experiments.

The development of a new method of determining the specific radioactivities of the phospholipids contained in a ^{32}P -labelled tissue sample (Dawson, 1954) has presented an opportunity for gaining information about any such phospholipid interconversion. By extending observations obtained in other investigations (Dawson, 1955) it has been possible to compare the specific radioactivities of the individual liver phosphoglycerides of the rat at various times after the injection of labelled phosphate. The results, while showing (in contrast to those of many other workers) that marked differences can occur in the specific radioactivities of liver phosphoglycerides, give no evidence of any type of phospholipid interconversion.

EXPERIMENTAL

The experimental methods and animals used in the present investigation have been fully described in a previous publication (Dawson, 1955). Results were accumulated over a period of about 14 months. The specific radioactivities of the extracted liver phospholipids were determined by the method of Dawson (1954) in which the phospholipids are mildly hydrolysed in methanolic NaOH (15 min. at 37°) and the hydrolysis products resolved by two-dimensional paper chromatography. On such chromatograms of the rat-liver lipid hydrolysates there was observed a phosphorus-containing spot (I) which occurred in a similar position to that occupied by the decomposition product of diphosphoinositide on similarly prepared chromatograms from brain-phospholipid samples (Dawson, 1954). Macpherson & Lucas (1947) have described an inositol-containing lipid in the alcohol-insoluble phospholipids of rat liver, and both McKibbin (1954) and Hawthorne (1955) found evidence for the presence of an inositol-containing lipid, which is probably a monophosphoinositide, in horse and rabbit liver respectively.

Consequently a small sample of (I) was isolated from the alkaline hydrolysate of a rat-liver lipid extract in order to see whether it was derived from this lipid. The hydrolysate was applied as a starting strip on a paper chromatogram which was developed with phenol- NH_3 . The substance staying on the starting line was eluted and rechromatographed in a *tert.*-butanol-water-trichloroacetic acid (68 ml.: 32 ml.: 10 g.) solvent. The trailing phosphorus-containing compound was located by spraying marker strips to detect phosphorus and then eluted with water.

RESULTS

Confirmation of the presence of an inositol-containing phospholipid in rat liver

The unknown substance (I) isolated from an alkaline hydrolysate of rat-liver phospholipids (see Experimental section) was found to be chromatographically homogeneous in a number of solvents which have proved useful for the separation of phosphate esters (ethanol- NH_3 , pyridine- NH_3 , *tert.*-butanol-picric acid (Hanes & Isherwood, 1949). On hydrolysing for 12 min. in *N*-HCl at 100° the substance was completely decomposed, and examination of the hydrolysate by the method of Meredith & Sammons (1952) showed the presence of free inositol and glycerol. The phosphorus-containing acid-hydrolysis products could be separated into three components by paper chromatography in ethanol-ammonia (sp.gr. 0.880)-water (6:3:1, v/v). The fastest running spot was chromatographically identified as α -glycerophosphate. The other spots were not identified, but when each of them was isolated by elution and subjected to prolonged hydrolysis in 5*N*-HCl (24 hr. at 100°) it was possible to detect in both hydrolysates free inositol, glycerophosphoric acid and inorganic phosphate. It can be concluded, therefore, that the compound (I) isolated from the

Fig. 1. Curves showing the specific radioactivities of rat-liver phosphoglycerides at various times after labelled-phosphate injection (2×10^6 counts/min./100 g. body wt.). A, Phosphatidylcholine; B, phosphatidylethanolamine; C, inositol-containing phosphoglyceride; D, phosphatidylserine.

alkaline hydrolysate of rat-liver lipids contains inositol, glycerol and phosphate residues and has arisen by the removal of fatty acid(s) from an inositol-containing phosphoglyceride present in rat liver.

*Specific radioactivities of rat-liver
phosphoglycerides*

The specific radioactivities of the individual rat-liver phosphoglycerides at various times up to 21 hr. after the injection of labelled phosphate are presented in Fig. 1. The curves *A*, *B*, *C* and *D* were drawn by calculating the mean specific radioactivities of successive groups of observations (observations occurring in every 200 min. interval) and inserting these values on the graph at the

Fig. 2. The specific radioactivity/time curves of Fig. 1 superimposed and the points omitted for clarity. *A*, Phosphatidylcholine; *B*, phosphatidylethanolamine; *C*, inositol-containing phosphoglyceride; *D*, phosphatidylserine.

appropriate mean time for the group. The curve was then drawn to pass through these mean points. Considering the many variable biological factors which can affect the determination and the extended experimental period (14 months), the results show satisfactory consistency. The determination of the specific radioactivity of phosphatidylserine was made especially difficult because of the low concentration of this lipid in liver tissue (Swanson & Artom, 1950). After all exchange times up to 21 hr. the specific radioactivity of the phosphatidylethanolamine was found to be higher than that of the other phosphoglycerides, whereas that of phosphatidylserine was always lower than the others. In no instance do any two curves (Fig. 2) obey the theoretical precursor-product criteria as deduced by Zilversmit, Entenman & Fishler (1943).

DISCUSSION

Although the isotopic results obtained in this investigation have been examined for precursor-product relationships by the criteria theoretically deduced by Zilversmit *et al.* (1943), it is well to stress that the assumptions used by these authors in their derivation may not apply in practice in biological systems. The results do not, for example, exclude the possibility that phospholipid interconversion may represent a minor pathway for the formation of certain phosphoglycerides.

The observations of Stetten (1941, 1942) showed that, in the intact rat, labelled serine could be decarboxylated to form ethanolamine, and that this base could then be methylated to form choline. The present results indicate that a similar process cannot occur to any appreciable extent while these bases are combined in phospholipid molecules. The specific radioactivity of phosphatidylserine remains much lower than that of phosphatidylethanolamine, thus excluding the possibility of appreciable decarboxylation of the former occurring *in vivo*. This agrees with the results of Sloane-Stanley (1953), who was unable to detect any appreciable CO_2 evolution from phosphatidylserine when it was incubated anaerobically with guinea-pig-brain homogenates. The specific radioactivity of liver phosphatidylethanolamine does not fall below that of phosphatidylcholine even after the latter has reached its maximum specific radioactivity, and it thus does not satisfy the criteria for the precursor of phosphatidylcholine. The recent *in vitro* experiments of Kennedy (1954) have indicated that liver mitochondria are able to incorporate choline directly into their phospholipid fraction.

The immediate phosphorus-containing precursors of liver phosphoglycerides are at present unknown, so that attempts to calculate their turnover rates are misleading. If phosphoglycerides are synthesized from a common precursor, e.g. phosphatidic acid, the present results combined with the levels of phospholipid bases in rat liver given by Swanson & Artom (1950) would indicate that the amount of lecithin synthesized per gram of liver tissue is approximately equal to that of phosphatidylethanolamine, and that there is a very much slower synthesis of phosphatidylserine (< 10 %).

SUMMARY

1. The specific radioactivities of the liver phosphoglycerides have been measured at various times after the injection of labelled phosphate into the rat.

2. The specific radioactivity/time curves give no evidence of any interconversion of the phosphoglycerides.

3. The presence of an inositol-containing phosphoglyceride in rat liver has been confirmed.

The author is indebted to Dr R. B. Fisher for his advice on the drawing of the specific radioactivity/time curves, and to Dr J. N. Hawthorne for a sample of ethanol-insoluble ox-liver phospholipid.

REFERENCES

- Dawson, R. M. C. (1954). *Biochim. biophys. Acta*, **14**, 374.
 Dawson, R. M. C. (1955). *Biochem. J.* **59**, 5.
 Hahn, L. & Tyrén, H. (1946). *Ark. Kemi Min. Geol.* **21**, 1.
 Hanes, C. S. & Isherwood, F. A. (1949). *Nature, Lond.*, **164**, 1107.
 Hawthorne, J. N. (1955). *Biochem. J.* **59**, ii.
 Kennedy, E. P. (1954). *J. biol. Chem.* **209**, 525.
 Macpherson, L. B. & Lucas, C. C. (1947). *Fed. Proc.* **6**, 273.
 McKibbin, J. M. (1954). *Fed. Proc.* **13**, 262.
 Meredith, P. & Sammons, H. G. (1952). *Analyst*, **77**, 416.
 Popják, G. & Muir, H. (1950). *Biochem. J.* **46**, 103.
 Sloane-Stanley, G. H. (1953). *Biochem. J.* **53**, 613.
 Stetten, D. (1941). *J. biol. Chem.* **140**, 143.
 Stetten, D. (1942). *J. biol. Chem.* **144**, 501.
 Swanson, M. A. & Artom, C. (1950). *J. biol. Chem.* **187**, 281.
 Zilversmit, D. B., Entenman, C. & Chaikoff, I. L. (1948). *J. biol. Chem.* **176**, 193.
 Zilversmit, D. B., Entenman, C. & Fishler, M. C. (1943). *J. gen. Physiol.* **26**, 325.

The Effect of Nucleotides on the Formation of Phosphopyruvate

By W. BARTLEY

Medical Research Council Unit for Research in Cell Metabolism, Department of Biochemistry, University of Oxford

(Received 10 May 1955)

Utter & Kurahashi (1954*a, b*) have isolated from chicken liver an enzyme that catalyses the reactions

where ATP, ITP, ADP and IDP represent adenosine triphosphate, inosine triphosphate, adenosine diphosphate and inosine diphosphate respectively. ITP was found to accelerate the decarboxylation more effectively than ATP. In studies on sheep-kidney cyclophorase Bartley (1954) showed that the phosphate of phosphopyruvate was derived from ATP. In this paper a comparison has been made between ATP, ITP and inorganic phosphate as a source of the phosphate in phosphopyruvate. It was found that when oxaloacetate or malate is oxidized phosphopyruvate can be formed without participation of either nucleotide.

EXPERIMENTAL

Tissue preparations. Twice-washed particles (cyclophorase) of pigeon-liver homogenates, prepared according to Bartley & Davies (1954), were used in most experiments. The homogenates were made in a stainless-steel homogenizer of the Potter-Elvehjem type. In a few experiments washed mitochondria were used.

Media. In most experiments 2 ml. of the suspension (about 70 mg. dry wt.) were added to 2.5 ml. of a solution containing K phosphate buffer, pH 7.4 (0.008M), MgCl₂ (0.0016M), aminotrihydroxymethylmethane (tris) buffer pH 7.4 (0.04M), substrate (0.02M) and the nucleotide under test; the amounts of the nucleotide are given in the description of the individual experiments.

Special chemicals. ITP was prepared by deamination of ATP by the method of Kleinzeller (1942); inosine monophosphate (IMP), adenosine monophosphate (AMP) and coenzyme I (DPN) were commercial products. The other chemicals were those used by Bartley & Avi-Dor (1955).

Analytical methods. The procedures described by Bartley & Avi-Dor (1955) were used.

RESULTS

Effect of ATP or ITP on malate or oxaloacetate metabolism

Table 1 gives the results of preliminary experiments, with malate or oxaloacetate as substrate, in which phosphopyruvate formation by pigeon-liver cyclophorase was measured in the presence or absence of ATP or ITP. ATP increased oxygen-uptake, malate consumption and also, in most experiments, phosphopyruvate formation. The increase in phosphopyruvate production was smaller when the amount of ATP added was large (Expts. 1 and 2). The stimulation by ATP of oxygen and malate consumption was less when the tissue concentration was high (Expts. 2 and 4). ITP stimulated respiration in the presence of malate (Expt. 4), but did not influence malate utilization or phosphopyruvate formation. When oxaloacetate was substrate, ATP but not ITP stimulated respiration (Expt. 5), but neither nucleotide in the concentration used affected phosphopyruvate formation. At higher concentrations of nucleotide (Expt. 6) the yield of phosphopyruvate was greater in the presence of ITP than of ATP. The addition of ATP and ITP