Values of brain pyrophosphatase activities in the different species, both absolutely and relatively to the other phosphatases, showed wide variations. Generally, however, the levels of pyrophosphatase were higher than those of the phosphomonoesterases, except with the rabbit. A strict comparison between pyrophosphatase levels of brain tissue and of erythrocytes was not possible, but values of approximately the same order were found, except with the rabbit, where the erythrocyte enzyme was clearly present in considerably greater strength than in the brain.

DISCUSSION

The present work suggests that the pyrophosphatase of brain tissue resembles the pyrophosphatases of yeast and erythrocytes in many respects, especially in the effects of inhibitors, and to some extent in the factors of magnesium activation and optimal pH range, in spite of certain small differences. The difference between the brain and erythrocyte enzymes is, however, evident from the fact that the former can be isolated by acetone precipitation, while such treatment destroys the erythrocyte activity.

The effects of inhibitors show that the brain pyrophosphatase contains active thiol groups within its structure, and possibly active amino groups also.

It is important to consider the possible physiological role played by pyrophosphatase in brain function. No deductions regarding its relative importance may be drawn from the values given in Table 2, as these vary widely. It is conceivable, however, that the enzyme may exercise some function in connexion with phosphorylation processes. Cori (1942) has mentioned experiments in which inorganic pyrophosphate was detected in rat-liver preparations after aerobic oxidation of glutamate, pyruvate and succinate, while Cross, Taggart, Covo & Green (1949) have deduced that inorganic pyrophosphate is formed as a decomposition product in the phosphorylation reactions taking place under the influence of the cyclophorase system. It remains to be seen, however, what part is played by pyrophosphatase itself in the chain of reactions governing phosphorylation mechanisms.

SUMMARY

1. A description is given of the properties of the magnesium-activated pyrophosphatase in preparations from brain tissue.

2. The enzyme is strongly inhibited by various substances which react with thiol groups, and by calcium ions, formaldehyde and fluoride. It is also inhibited to some extent by cyanide, but is relatively unaffected by cholate and tauroglycocholate.

3. Activities of brain pyrophosphatase in several species are compared with those of the brain phosphomonoesterases and with corresponding erythrocyte pyrophosphatases.

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Are Phospholipins Transmitted through the Placenta?

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It was shown in a previous investigation (Popják, 1947), with the aid of ^{32}P, that all foetal tissues in the rat, rabbit and guinea pig synthesize phospholipins. The results suggested that most, if not all, the foetal phospholipins are obtained by synthesis within the foetus. The possibility, however, that a small amount of phospholipin preformed in the mother might pass through the placenta could not be excluded. The experiments reported here were designed to probe this question. It will be shown that, although the foetal placenta takes up large amounts of phospholipins from the maternal circulation, it does not transmit to the body of the foetus detectable amounts of phospholipins. A preliminary account of this work has been given to the Biochemical Society (Popják & Beeckmans, 1949).

METHODS

Three pregnant rabbits were used for the experiment: (1) on the eighteenth, (2) on the twenty-third, and (3) on the twenty-eighth day of pregnancy. These three dates during the rabbit's gestation mark three stages in the development of the placenta; on the eighteenth day the placenta is of the haemochorial, on the twenty-third of the haemomesothelial and on the twenty-eighth day of the haemoendothelial type. These changes mean the gradual disappearance of cell layers between the foetal and maternal circulation, until in the haemoendothelial placenta there is but one layer, the endothelium of the foetal capillaries, between the two circulations. These three dates were chosen in order to see if there is any detectable change in the permeability of the placenta in the course of its development. The exact morphological changes were not known to us at that time and we are indebted to Prof. Amoroso for drawing our attention to these. Our choice of dates thus has been more fortunate than deliberate.

The rabbits were injected with 10, 15 and 20 ml. of a serum containing phospholipins labelled with ^{32}P.

Preparation of serum with labelled phospholipins

Three rabbits were made slightly lipaemic by the oral administration of amorphous cholesterol suspended in water (cf. Popják, 1946*a*). Cholesterol (1 g.) was given to the animals every other day during a 10-day period. On the eighth day of the cholesterol treatment they were injected intravenously with 1·5 mc. and on the ninth day with 1 mc. of carrier-free $Na_2H^{32}PO_4$ made up in 0·9% NaCl. On the tenth day the rabbits, under nembutal-ether anaesthesia, were bled from the aorta and the sera obtained were pooled. The pooled serum was dialysed at 0° first against several changes of M/15 PO_4^{--} buffer, pH 7·4, for 3 days and then against 0·9% NaCl for 1 day. The purpose of the dialysis was to remove inorganic and other phosphates of small molecular size, which became labelled with ^{32}P. The cholesterol-lipaemia was induced before the administration of ^{32}P, because it was found that the specific activity of the plasma-phospholipin P in such lipaemic animals may be three or four times as high as in normal rabbits 24 hr. after the injection of $Na_2H^{32}PO_4$ (Popják, unpublished observation). The serum was centrifuged and warmed to body temperature before injection.

Treatment of the experimental (pregnant) animals

Blood (10–15 ml.) was withdrawn from the pregnant animals immediately before the intravenous injection of the labelled serum. Then 2 hr. after the injection they were anaesthetized with nembutal and ether and after opening the abdomen the foetuses were delivered through an incision made along the antimesometrial surface of the uterine horns. Care was taken to avoid contamination of the foetuses with maternal blood.

Preparation of tissues for extraction

In order to avoid, as well as possible, contamination of tissue phospholipins with phospholipins of blood, the tissue samples were sliced thinly and blotted between filter paper by applying pressure to the slices. The placentae were freed from the membranes and the foetal and maternal portions torn apart; only the foetal portion being used for the investigation. The tissues freed from blood were dropped into solid CO_2-acetone mixture and then ground to a fine powder in a chilled steel mortar; 2–3 g. of the tissue powder were extracted with 10% ice-cold trichloroacetic acid by shaking for 2 min. and the inorganic PO_4^{---} precipitated from the filtered extract as NH_4MgPO_4. In two experiments (see Tables 1 and 4) the acid-soluble phosphates were fractionated as described by Kaplan & Greenberg (1944) in order to obtain samples of tissue glycerophosphate for radioactive assay.

For the preparation of phospholipins 1–5 g. of tissue powder were extracted with boiling ethanol-ether (3:1, v/v) and the phospholipins separated as described before (cf. Popják, 1946*a*). The precipitated phospholipins were dissolved in moist ether and the solution, clarified by centrifugation, was transferred to digestion flasks and the solvent evaporated off. The phospholipins were then digested with 2 ml. conc. H_2SO_4 and 10 drops of HNO_3; the digestion was completed by the addition of 5–10 drops of H_2O_2 (100 vol., British Drug Houses Ltd., Microanalytical Reagent) repeatedly as required. The inorganic PO_4^{---} thus obtained was precipitated as NH_4MgPO_4.

From the 18-day-old embryos only 394 mg. of liver (from five embryos) were obtained. This small amount of tissue could not be divided for separate extraction of inorganic P and of phospholipins, therefore the tissues were first extracted with trichloroacetic acid to obtain inorganic PO_4^{---} and then the residue was further treated with ethanol-ether to obtain the phospholipins.

Assay of ^{32}P

The dried NH_4MgPO_4 samples were dissolved in 0·5 or 1·0 ml. 0·1 N-HCl and 0·2 ml. portions were pipetted on to nickel disks and dried under an infra-red lamp. Another portion was taken for the determination of P by the method of Allen (1940). A sample of phospholipin P prepared from the injected serum was used as standard. The radioactive counts were taken with a bell-shaped mica-window counter; the counts were corrected for background, and for counts lost due to the dead time of the quenching circuit used. The specific activities are expressed as counts/min./mg. P. Since the ^{32}P assays in each experiment were carried out on different days, the activities as shown in the Tables are all corrected for one particular day.

ARGUMENT

When a pregnant rabbit is injected with labelled inorganic PO_4^{---}, the specific activity of the phospholipin P in the maternal liver is at most 5% and in the foetal tissues (foetal placenta, liver and carcass) 5–15% of the specific activity of the inorganic P in the corresponding tissues. In order to illustrate this, we record in Table 1 the results of one experiment in which a twenty-eight-day pregnant rabbit was injected with $Na_2H^{32}PO_4$. The figures show (column 5, Table 1) that 2 hr. after the injection the activities of the tissue phospholipins were in the range mentioned, although in this experiment the activity of the foetal-liver phospholipins was somewhat lower than in others not shown here.

Table 1. *Specific activities of inorganic, glycerophosphate and phospholipin phosphorus in maternal and foetal tissues after intravenous injection of* $Na_2H^{32}PO_4$ *to the mother*

(100 μc. of $Na_2H^{32}PO_4$ injected into the mother on the twenty-eighth day of pregnancy. Specific activities determined 2 hr. after injection.)

	Specific activities (radioactive counts/min./mg. P)			$\frac{III}{I} \times 100$	$\frac{III}{II} \times 100$	$\frac{II}{I} \times 100$
	Inorganic P (I)	Glycerophosphate P (II)	Phospholipin P (III)			
Maternal liver	25,400	11,500	626	2·46	5·44	45·3
Foetal placenta	17,320	11,450	2,160	12·47	18·80	66·0
Foetal liver	17,280	9,280	587	3·40	6·34	53·7
Foetal carcass	4,180	2,680	215	5·14	8·03	64·1

We argued therefore, that, if after the injection of labelled phospholipins (instead of inorganic PO_4^{---}) the specific activity of the phospholipin P were to exceed the activity of the inorganic P in any of the organs examined, definite proof would be obtained that whole phospholipin molecules had been taken up from the maternal circulation. If the specific activity of the phospholipin P in the foetal tissues were at least higher than 15 % of the specific activity of the tissue inorganic P, the result would favour the view that whole phospholipin molecules have passed the placenta.

RESULTS AND DISCUSSION

The phospholipin and ^{32}P content of the serum used for the injection of pregnant animals is shown in Table 2. Of the total ^{32}P content, 67·3 % was associated with phospholipins, 25·4 % with phosphoproteins and 7·3 % with unidentified acid-soluble P. It has been shown by Taurog, Entenman & Chaikoff (1944) that plasma phospholipins consist chiefly of lecithin and a little sphingomyelin. For all practical purposes, therefore, we are concerned with the fate of intravenously injected labelled lecithin in pregnant animals.

The essential data of the experiments are given in Table 3. The striking feature of the results is that the specific activity of phospholipin P in the maternal liver and placentae in all three experiments was greater than the specific activity of the tissue inorganic P. These two organs have, therefore, taken up phospholipins unhydrolysed from the maternal circulation. In the foetal liver and foetal carcass, however, the specific activity of the phospholipin P was 5–7·4 and 3·5–5·9 %, respectively, of the specific activities of the inorganic P. There is, therefore, no evidence to suggest that whole phospholipin molecules have been transmitted through the placenta to the body of the foetus. The radioactivity found in the phospholipins of the foetal liver and carcass were what one might expect from the radioactivity present in the inorganic PO_4^{---}. It seems a singular property of the cells of placenta to imbibe phospholipins on the maternal side and yet not to allow their passage to the foetus; a property which might be termed uni-

Table 2. *Properties of dialysed rabbit serum containing* ^{32}P*-labelled phospholipins and used for injection of pregnant rabbits*

Total phospholipin phosphorus	5·96 mg./100 ml.
	Counts/min./ml.
^{32}P activity associated with:	
(*a*) phospholipins	14,100
(*b*) acid soluble P	1,530
(*c*) phosphoprotein	5,320
Total ^{32}P content	20,950

Table 3. *Specific activity of inorganic phosphorus and phospholipin phosphorus in tissues of pregnant rabbits after the intravenous injection of serum containing phospholipins labelled with* ^{32}P

(Specific activities were determined 2 hr. after injection and are expressed as radioactive counts/min./mg. P.)

Day of pregnancy	18	23	28
Wt. of mother (kg.)	2·5	3·16	3·08
No. of foetuses	5	9	2
Vol. of injected serum (ml.) ...	10	15	20

	Specific activity of					
	Inorganic P	Phospholipin P	Inorganic P	Phospholipin P	Inorganic P	Phospholipin P
Maternal liver	162	582	186	638	194	1060
Foetal placenta	2150	2328	1060	2185	1470	3025
Foetal liver	1000	50	1057	78	870	62
Foetal carcass	940	33	224	10·8	165	9·8

lateral 'permeability'. The liver of the adult animal is known to 'secrete' and absorb plasma phospholipins (Fishler, Entenman, Montgomery & Chaikoff, 1943; Entenman, Chaikoff & Zilversmit, 1946).

The unilateral 'permeability' of placenta to phospholipins is the parallel of what was found in an earlier investigation with cholesterol (Popják, 1946*b*) except that the cholesterol is mainly stored in the placenta, whereas phospholipins are vigorously metabolized. There does not seem to be any difference in this respect between the placentae of the eighteen-day or twenty-eight-day pregnant rabbit; even the single cell layer between the foetal and maternal circulation in the haemoendothelial placenta of the twenty-eighth-day rabbit is sufficient to withhold the phospholipin molecules from the body of the foetus. However, there is a difference between the placentae at the different dates in respect of the speed with which they degrade phospholipins.

It is impossible from the data to assess quantitatively the amounts of phospholipins taken up by the placenta from the maternal circulation, because an appreciable amount of the absorbed phospholipins had already been degraded by the end of the experiment. A measure of phospholipin uptake by the placenta relative to the maternal liver, however, may be evaluated by the following calculation.

Let $f(t)$ = the specific activity of plasma phospholipins, a function of time as stated here; A and B = the total phospholipin P content of 1 g. liver and placenta respectively; a and b = the amount of phospholipin P absorbed from the circulation by 1 g. of liver and placenta respectively. Assuming that the total phospholipin content of liver and placenta did not change significantly during the experimental period, the specific activity of the phospholipin P of liver (x) and of placenta (y) may be calculated by

$$x=\frac{af(t)}{A} \quad \text{and} \quad y=\frac{bf(t)}{B}.$$

The ratio $b/a=yB/xA$ then gives the amount of phospholipin P absorbed by the placenta relative to the maternal liver. The mean value of A is 1·3 mg./g. liver and of B, 0·6 mg./g. placenta. Taking the observed values of x and y from Table 3 we obtain for b/a 1·85 on the eighteenth day, 1·58 on the twenty-third day and 1·32 on the twenty-eighth day. Relative to the maternal liver, the placenta is more active in phospholipin uptake on the eighteenth day of pregnancy than on the twenty-eighth day, although even on the latter day it absorbs from the maternal circulation 30 % more phospholipins per g. weight than the maternal liver. The above estimates are undoubtedly the lowest limits, because the amounts of phospholipin which had been degraded in the tissues have been neglected in the calculation and the process of degradation in the placenta appears to be much faster than in the maternal liver.

The radioactivity associated with inorganic P in the maternal liver and placenta must have been obtained from (i) the decomposition of absorbed phospholipins, and (ii) of phosphoproteins. In the placenta a certain amount of inorganic ^{32}P must have also been derived from the maternal circulation; it is probable, however, that the major portion of inorganic ^{32}P in the placenta came from degraded phospholipins. Since on the eighteenth day of pregnancy the specific activity of the inorganic P in the placenta was about 90 % and on the twenty-third and twenty-eighth days only about 50 % of the specific activity of the phospholipin P, the eighteenth day placenta appears to have a greater ability to decompose phospholipins than the older ones.

It has been shown (Popják & Muir, 1949) that the P precursor and the first P-containing degradation product of lecithin and cephalin in the liver and placenta is glycerophosphate. It was, therefore, of interest to determine whether or not the glycerophosphate in the placenta obtained from the degradation of lecithin is transmitted to the foetus. We have fractionated the acid-soluble phosphates of placenta and foetal liver from the twenty-eighth-day pregnant rabbit according to the method of Kaplan & Greenberg (1944), and have compared the specific activities of phospholipin P with that of glycerophosphate and inorganic P. The results in Table 4

Table 4. *Specific activity of phospholipin, glycerophosphate and inorganic phosphate in foetal placenta and foetal liver after the mother was injected intravenously with serum containing* ^{32}P*-labelled phospholipins*

(Twenty-eighth day of pregnancy. Specific activities were determined 2 hr. after injection, and are expressed as radioactive counts/min./mg. P.)

	Specific activity of		
	Phospholipin P	Glycerophosphate P	Inorganic P
Foetal placenta	3025	2200	1470
Foetal liver	62	409	870

show that the order of events in the placenta must be phospholipin $^{32}P \rightarrow$ glycerophosphate $^{32}P \rightarrow$ inorganic ^{32}P and in the foetal liver the reverse. The radioactivity associated with glycerophosphate (as also with phospholipin) in the foetal liver is of the order of magnitude observed after the injection of inorganic $^{32}PO_4^{---}$ into the mother (cf. Table 1), and therefore we have to infer that even glycerophosphate does not pass through the placenta unhydrolysed. We have no evidence yet as to the fate of the other degradation products of phospholipins in the placenta, i.e. fatty acids, glycerol and choline. We shall present, however, in another communication, data strongly suggesting that a certain amount of

fatty acids pass the rabbit placenta from mother to foetus and that these are stored in the foetal liver. This of course raises the problem of the mechanism of fatty acid transfer across the placenta.

The results of our present investigation, taken together with the earlier findings, lead to the inevitable conclusion that all the phospholipins in the foetus (excluding placenta of foetal origin) are synthesized *in situ* from the necessary components and are not derived from the preformed maternal compounds. Nielson (1941–2) has injected pregnant rats with emulsions of ^{32}P-labelled phospholipins and found much less radioactivity in the foetuses than after the injection of inorganic $^{32}PO_4^{---}$. It was not, however, ascertained whether or not the injected phospholipins were first degraded outside the foetus. Another criticism of Nielson's experiments has been given previously (Popják, 1947).

A further point which emerges from the data shown in Table 3 concerns generally the permeability of foetal cells to PO_4^{---} at different stages of development. On the eighteenth day the specific activity of the inorganic P of the foetal carcass (i.e. the whole foetus without its liver) was nearly the same as that found in the foetal liver, whereas on the twenty-third and twenty-eighth days the former was only about one-fifth of the latter. This difference means that in the younger embryo all cells are uniformly permeable to PO_4^{---}; with advancing differentiation, however, the extrahepatic tissues become less permeable to PO_4^{---}, a condition approaching more closely that found in the adult animal, in which liver is the tissue most permeable to PO_4^{---}, and muscle and brain the least permeable.

SUMMARY

1. Three rabbits, on the eighteenth, twenty-third and twenty-eighth day of pregnancy, were injected with serum containing phospholipins labelled with ^{32}P, and the radioactivity of phospholipins was compared with that of inorganic phosphorus in the maternal liver, foetal placenta, foetal liver and carcass.

2. The foetal placenta absorbs more phospholipin from the circulation than the maternal liver, but it does not transmit unhydrolysed phospholipin molecules to the foetus. Glycerophosphate, which is the first phosphorus-containing degradation product of lecithin in tissues, does not pass unhydrolysed through the placenta either.

3. The placenta of the eighteenth-day pregnant rabbit is more active than that of the twenty-eighth day pregnant animal in the absorption and degradation of phospholipin.

4. The cells of the 18-day-old rabbit embryo are uniformly permeable to phosphate ions, whereas in the 23- and 28-day-old foetus a condition more approaching that in adult animals is found.

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In Search of a Phospholipin Precursor

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It is well known that soon after the injection of inorganic $^{32}PO_4^{---}$ to an animal both tissue and plasma phospholipins become labelled with ^{32}P. The biochemical reactions leading to the incorporation of inorganic PO_4^{---} into phospholipin molecules, however, are not known. The various possibilities and researches on this problem have been reviewed by Chaikoff (1942). This investigation aimed at finding the phosphorus precursor of phospholipins (i.e. of lecithin and kephalin). It is considered that glycerophosphate or a substance with very similar properties is now identified as such a precursor which enters into a reversible reaction in the synthesis of lecithin and kephalin.

In studies of turnover of phospholipins under various experimental conditions with ^{32}P it is not