CVIII. THE PHOSPHATASES OF MAMMALIAN TISSUES.

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Amongst the many phosphorus compounds which are to be found in almost every tissue of the animal body, in addition to inorganic phosphate, phosphatides and nucleoproteins, there are also present phosphoric esters of an acid-soluble nature, stable in dilute acid or alkaline solution and of relatively small molecular dimensions. The amount of phosphorus present in this ester form, in most of the tissues, is considerably in excess of that present as inorganic or phosphatide phosphorus. Enzymes capable, under certain conditions, of hydrolysing these important esters are also widely distributed in the body, and in certain tissues have been assigned specific functions which if confirmed would give these catalysts a position of very great metabolic importance.

The present paper deals with (*a*) the quantitative distribution of phosphatases in mammalian tissues, (*b*) the question as to whether a specific enzyme is required for the hydrolysis of each phosphoric ester, and (*c*) the synthetic activity of the phosphatases.

A. The quantitative distribution of phosphatases in certain mammalian tissues.

Grosser and Husler [1912], Plimmer [1913, 1], Forrai [1923] and Robison [1923] have described experiments in which ground-up tissues or tissue extracts have been shown to have differing hydrolytic activity towards glycerophosphate or other phosphoric esters, but quantitatively these results can only be considered preliminary.

The determination of the quantitative distribution of any enzyme in the tissues is, for several reasons, a problem of quite a different order from that of the distribution of a particular element or compound. In the case of an enzyme, (1) it may be found impossible to remove the enzyme from the tissues without destroying its activity, (2) extracts of different tissues may contain in addition to the enzyme greater or lesser amounts of accelerating or inhibitory substances, (3) the activity of the enzyme will vary with the c_H, giving an optimum which may be different for the different tissues, (4) the functional c_H for most intracellular enzymes is unknown, so that it is not possible to compare activities at such hydrogen ion concentrations, (5) the choice of substrate is important; the "natural" substrate may be unknown.

It is impossible to avoid all these potential sources of error; we can perform the determinations neither under strictly "natural" nor strictly artificial conditions, and a compromise is inevitable.

One fortunate preliminary finding for the phosphatase activity of all tissue extracts examined is that the optimum c_H is very nearly the same, whether the "natural" esters, separated from the tissues by precipitation of the proteins, or esters of known composition such as sodium glycerophosphate, sodium hexosediphosphate or the sodium salt of guaninenucleotide are used.

As enzyme preparations, tissue hashes have been tried; also the fluids obtained by extraction of the ground-up tissue with dilute acid and alkali, and with glycerol containing various amounts of water, of acid or of alkali. Extraction with very dilute acids, or with acid glycerol, gives poor yields. The method found to be most practicable, which gives enzyme activities on the whole greater than any other method, is simple extraction of the finely ground-up tissue with chloroform water for 24–48 hours at room temperature, followed by slow filtration through cotton wool. The turbid filtrate contains almost all of the activity of the original tissue. Extracts of gastric and intestinal mucosa are prepared as follows. In the case of the rabbit or the cat, the whole intestine is washed out well with 0·9 % NaCl, then, after draining, the first 8 inches of the small intestine are taken as duodenum, the rest of the intestine as far as the caecum is then divided into two halves, the upper half is called jejunum and the lower ileum. The mucosa is scraped off with the back of a scalpel, weighed and extracted as described. With regard to accelerating or inhibiting substances, it is found that a short dialysis (18 hours) against 20 volumes of water has little effect on the activity of either kidney or duodenal extracts, although 6 days' dialysis against repeated changes of water shows some inactivation, which in the case of kidney extracts can be partially restored by the addition of a boiled extract of kidney.

For purposes of determining the relative distribution of the enzyme in the tissues the extracts are not purified further. The method of determining the relative activity has been standardised as follows. A small portion of tissue extract (0·5 to 1 cc.), representing a known weight of original tissue and brought to the optimal c_H of the enzyme, is added to a number of tubes each containing excess of the substrate (5 cc.) in glycine-NaOH buffer (5 cc.) at a short series of c_H near to the optimum, and incubated at 37° in presence of chloroform (which is found to have a negligible effect on the activity) for exactly 2 hours. The hydrolysis is stopped by the addition of trichloroacetic acid (2 cc. of 25 %) and the amount of inorganic phosphate liberated determined by the Briggs method. Controls containing substrate + buffer, and enzyme + buffer are put up at the same time. The correction for autolysis is usually very small. The number of enzyme units per g. of original tissue is then calculated, a unit being defined as the amount of enzyme, at the optimal c_H for its activity, which liberates 1 mg. P under the above conditions. For sodium glycerophosphate, or sodium hexosephosphate or the "natural"

esters of the tissues this optimum is found to be at p_H 8·8–9·1; for the sodium salt of guaninenucleotide it is usually p_H 9·0–9·2.

In Table I are shown the average values for the tissues of the rabbit, the cat and man, with glycerophosphate as substrate. In Fig. 1 the relative activity of certain tissues of the cat towards three substrates—glycerophosphate, hexosediphosphate, nucleotide—is shown. Table II shows a comparison of two methods of extraction; the optimal c_H for phosphatase activity of extracts obtained by the two methods appears to be the same.

Table I. *Average phosphatase content of the tissues. Substrate, sodium glycerophosphate.*

Figures indicate average enzyme units per g. of original tissue (wet weight).

Tissue	Animal and number examined: Rabbit (3)	Cat (3)	Man (2)
Gastric mucosa	0·2	1·5	0·5
Duodenal mucosa	15·2	27·5	7·7
Jejunal mucosa	30·7	29·7	11·5
Ileal mucosa	16·1	23·9	13·6
Colon mucosa	5·3	10·0	3·7
Liver	3·6	1·1	0·8
Lung	3·2	7·6	1·0
Kidney	10·5	14·1	4·8
Spleen	5·6	0·9	1·0
Pancreas	—	0·7	—
Parotid gland	—	1·7	—
Suprarenal gland	—	—	1·3
Brain	1·0	0·8	0·6
Cardiac muscle	0·3	0·2	—
Skeletal muscle	0·2	0·2	—
Bone	6·0	3·0	—
Artery	—	Nil	0·1

Table II. *Two methods of extraction compared.*

Units phosphatase per g. of tissue.

Tissue	Rabbit, Tissues extracted with: Chloroform water	60 % glycerol	Man, Tissues extracted with: Chloroform water	60 % glycerol
Gastric mucosa	0·2	0·2	0·6	0·5
Duodenal mucosa	8·7	8·7	12·0	7·0
Jejunal mucosa	21·0	13·5	13·3	Lost
Ileal mucosa	11·8	12·2	19·9	16·5
Colon mucosa	5·6	6·4	—	—
Liver	3·8	3·7	0·7	0·7
Lung	2·5	4·6	0·8	0·6
Kidney	12·6	11·8	3·4	3·3
Spleen	4·5	5·1	1·3	0·8
Brain	1·4	1·5	0·3	0·4
Heart	0·3	0·3	0·2	0·2
Muscle	0·2	0·2	—	—

There is a surprising similarity between the distribution in animal tissues of glycerophosphatase (or hexosephosphatase or nucleotidase) and that of ereptase, the enzyme investigated by Vernon [1904, 1905], which hydrolyses

peptones to substances which do not give the biuret test. Two examples of this similarity are given in Tables III and IV[1].

Table III. *Enzyme distribution in cat's tissues.*

Tissue	Enzyme: Glycerophosphatase Mean of 3	Hexosediphosphatase One individual	Ereptase (Vernon) Mean of 2
Gastric mucosa ...	1·5	2·2	3·9
Duodenal mucosa	27·5	53·3	27·7
Jejunal mucosa ...	29·5	45·7	18·2
Ileal mucosa ...	23·9	24·6	14·4
Colon mucosa ...	10·0	11·7	5·8
Liver	1·1	1·6	5·0
Lung	7·6	[3·8]*	6·9
Kidney	14·1	22·9	14·3
Spleen	0·9	2·2	7·6
Pancreas	0·7	—	6·4
Brain	0·8	[1·0]*	1·2
Cardiac muscle ...	0·2	1·5	1·6
Skeletal muscle ...	<0·2	0·4	0·8

* From a second cat.

Table IV. *Phosphatase and ereptase in the kidneys of various animals.*

	Units (average): Rabbit	Cat	Man	Guinea-pig
Ereptase (Vernon)	[8] 10·9	[8] 11·6	[2] 5·2	[7] 8·8
Glycerophosphatase	[5] 11·4	[3] 14·1	[11] 4·8	[1] 7·9

Figures in brackets show number of individuals.

Phosphatase and ereptase also appear to be extractable from the tissues in the same way, to have their optimal activity in the same region of hydrogen ion concentration, and to have similar stabilities. In addition there are also remarkable correspondences (*a*) between the amount of ereptase and phosphatase in various organs at different stages in foetal and post-natal life (compare Vernon [1905] and figure given by Kay [1926, 1]) and (*b*) between the amounts in healthy and in diseased organs (Table V).

Table V. *Enzyme activity in diseased and healthy kidneys.*

	Phosphatase units [Brain and Kay, 1927] Average	Ereptase units [Vernon, 1908] Average
Healthy kidneys	[11] 4·8	5·4
Parenchymatous degeneration	[7] 3·7	3·4
Advanced nephritis	[9] 1·6 to 0·4	2·8 to 0·86

Figures in brackets show number of individuals.

The significance of this curious correlation between the enzyme hydrolysing phosphoric esters and the enzyme hydrolysing peptones is difficult at present to evaluate, but points to some functional relationship in the cell between these apparently unconnected activities.

[1] By accident the numerical values of the units by which the activities of the two enzymes glycerophosphatase and ereptase are expressed are almost the same.

B. Possible identity of certain phosphoric esterases.

Extracts of various tissues, prepared as just described, will hydrolyse a number of naturally occurring, and of synthetic phosphoric esters. Is the agent responsible for these hydrolyses the same, or is there a specific phosphatase for each substrate?

There is a good deal of cumulative evidence in favour of the identity of the glycerophosphatase with the hexosediphosphatase (at least in the liberation of the first half of the phosphoric acid) and with the nucleotidase of mammalian tissues. This evidence is as follows.

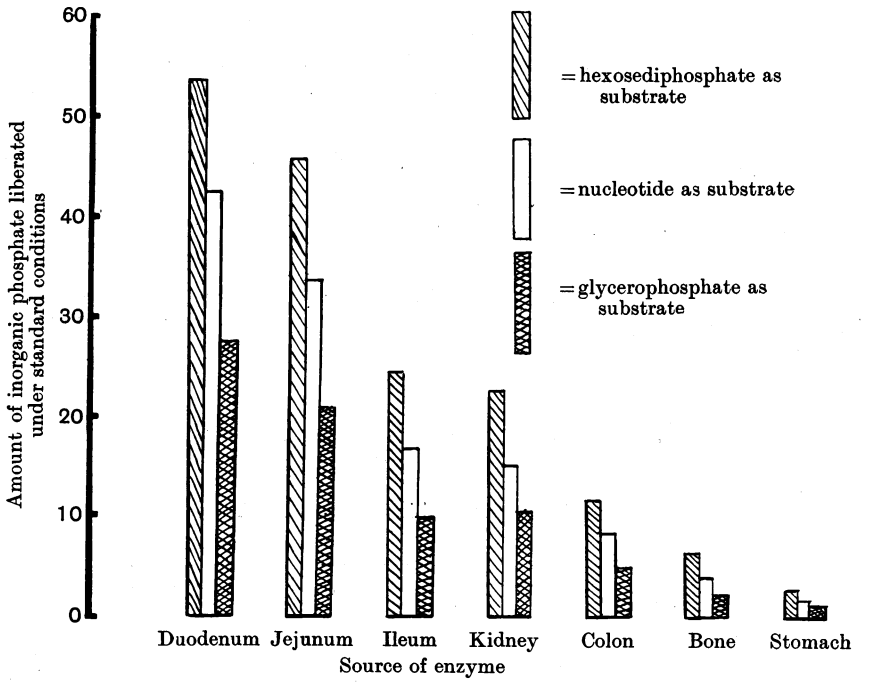

Fig. 1. Possible identity of glycerophosphatase, hexosediphosphatase and nucleotidase.

(*a*) *Optimal* c_H *for hydrolysis.* If a tissue preparation attacks different substrates at a different optimum c_H, it does not necessarily follow that different enzymes are concerned. But if a tissue preparation attacks closely related substrates at the same optimum c_H, there is a strong likelihood that the same enzyme is responsible. It has already been shown [Kay, 1926, 2] that the hydrolysis of both sodium hexosediphosphate and sodium glycerophosphate by kidney phosphatase has the same optimum c_H. The same optimum c_H has since been found for the action of the various intestinal extracts and of lung, liver and bone phosphatase on these two substrates, though usually the optimum c_H for glycerophosphate hydrolysis by these

extracts is at about p_H 9·0, and for hexosephosphate at about p_H 9·1. With the same extracts guaninenucleotide (pure specimen prepared by the method of Buell and Perkins [1927] from yeast nucleic acid) is hydrolysed at an optimum p_H of about 9·2. These optima are thus sufficiently near to one another to arouse the suspicion that the enzymes concerned are not very dissimilar.

(*b*) Using extracts from various tissues to hydrolyse the same three substrates under the same conditions (of organic phosphorus concentration per cc., time, temperature, quantity of enzyme) it is found that there is a constant ratio between the amounts of inorganic phosphate liberated from the three substrates by any one of these extracts. This is sufficiently well indicated by Fig. 1 to require few further remarks. A similar ratio holds for human and rabbit tissues as for those of the cat (from which the values shown in Fig. 1 are derived), and this same ratio holds good within a small experimental error whether the tissue extracts are made with chloroform water or with 60 % glycerol.

(*c*) If two of the above substrates in the same solution are together exposed to the action of *small quantities* of such tissue extracts, the total liberation of inorganic phosphate is considerably less than what would be expected if two independent enzyme systems were present. It is, of course, essential that only small quantities of the hydrolysing agent be used, in order to keep the enzyme working at maximal activity during the period of the experiment. In Table VI are given the results of a few such experiments.

Table VI. *Identity of glycerophosphatase, hexosediphosphatase and nucleotidase.*

P in mg. liberated in 2 hours at 37·5°.

	Enzyme used, extract of			
Substrate used*	Cat's duodenum	Rat's kidney	Rat's bone	Cat's duodenum
Glycerophosphate	0·064	0·058	0·169	0·304
Hexosediphosphate	0·136	0·120	0·246	—
Nucleotide	—	—	—	0·299
Two together:				
(*a*) found	0·132	0·089	0·180	0·301
(*b*) calculated if two independent enzymes present	0·200	0·178	0·415	0·603

* The organic P concentration of the substrates is not necessarily the same.

(*d*) If sodium fluoride is added to the reaction mixture containing a phosphatase and either glycerophosphate or hexosediphosphate, there is less hydrolysis during a given time than in the absence of this salt. Sodium glycerophosphate and sodium hexosediphosphate of the same original concentration of organic phosphorus have been hydrolysed by the same tissue extract in presence of increasing amounts of sodium fluoride, and it has been found that the percentage inhibition runs approximately parallel in both cases. With both substrates the effect of sodium fluoride may be just detected in as

low a concentration as $M/100$, the inhibition is 25–35 % in $M/25$ and 60–70 % in $M/6$ fluoride. The fact that an inhibiting agent interferes with the hydrolysis of both phosphoric esters at about the same rate is another small piece of evidence in favour of the identity of the two enzymes concerned. Sodium fluoride also inhibits the hydrolysis of nucleotide, but comparative quantitative experiments have not been made.

György [1925] has shown that phosphatolysis in all the tissues he examined (blood, liver, kidney, brain, heart muscle) appears to have a similar mechanism. It is stimulated by hydroxyl, by a low concentration of lactate or by potassium ions, checked by hydrogen, calcium and (a high concentration of) lactate ions.

It is submitted that although each of the foregoing points is, by itself, far from sufficient proof of the identity of the three enzymes, yet taken together they make a strong case in favour of such identity.

C. Synthetic activity of the phosphatases.

To the short list of enzymes proved to be capable of synthetic activity must be added tissue phosphatase. Previous workers (Bodnár [1925], Martland and Robison [1927]) have shown that under certain conditions inorganic phosphate disappears in presence of tissue extracts derived either from the plant or the animal. Embden and Zimmermann [1924] have isolated hexose-diphosphoric acid from the reaction mixture after allowing muscle press juice to act at low temperature (10°) on glycogen plus inorganic phosphate in presence of sodium fluoride. The reaction is evidently a complex one. So far this is the only case on record in which a phosphoric ester has been isolated following the synthetic enzymic activity of a tissue extract.

Using tissue extracts prepared as described earlier in the present paper, the enzymic synthesis of certain phosphoric esters can be accomplished with great ease at 37° and at p_H 8–9, provided the alcohol concerned is present in sufficiently large excess in the reaction mixture. The balanced reaction

$$\text{alcohol} + \text{acid} \rightleftharpoons \text{ester} + \text{water}$$

is thus pushed over toward the right hand side (the alcohol concentration being high and constant and the effective water concentration low). Synthesis goes on until an equilibrium is reached, the position of which depends in practice mainly on the amount of alcohol present.

Preliminary experiments have been carried out by mixing sodium phosphate solutions at p_H about 8·4 with duodenal or kidney extracts, and adding various alcohols or strong aqueous solutions of alcohols to the mixture. A sample of the reaction mixture is then taken at once, the proteins precipitated and inorganic and total phosphorus determined in an aliquot portion of the filtrate. The reaction mixture is raised to 37·5° and at intervals samples are taken and their content of inorganic and total phosphorus determined. (Controls using boiled enzyme are invariably used, and in no case show appreciable change in the concentration of inorganic phosphate.)

By this means the following facts have been established:

(*a*) Both kidney and intestinal extracts are capable of esterifying phosphoric acid in presence of high concentration of methyl or ethyl alcohol, ethylene glycol or glycerol.

(*b*) With propyl alcohol and dextrose, although definite synthesis has been observed, the amounts so synthesised have been very small.

(*c*) The products formed from glycerol (and glycol) were stable to 1 % sodium hydroxide at 100° for 4 hours at least. This is characteristic of glycerophosphates (Plimmer [1913, 2]).

(*d*) By simply diluting a portion of the reaction mixture with water, partial hydrolysis of the synthesised ester was usually observed.

(*e*) Sodium fluoride diminishes the *rate* of synthesis as of hydrolysis but probably does not affect the position of equilibrium. A typical experiment is shown in Table VII.

Table VII. *Effect of sodium fluoride on synthetic activity of phosphatase.*

Tube *A*. 1 cc. Na_2HPO_4 solution at p_H 8·4; 2 cc. cat's duodenal extract; 5 cc. glycerol, 2 cc. water.
Tube *B*. As *A*, but 1 cc. 0·8 *M* NaF (at p_H 8·4) and 1 cc. water in place of 2 cc. water.
Tube *C*. As *A*, but 2 cc. 0·8 *M* NaF in place of 2 cc. water.
Tube *D*. As *A*, but enzyme first boiled.
Tubes *E*, *F*, *G*, *H*, as tubes *A*, *B*, *C*, *D*, but using kidney extract from same cat.

	Percentage synthesis							
	Duodenal extract				Kidney extract			
Time (days after start)	*A*	*B*	*C*	*D*	*E*	*F*	*G*	*H*
1	17	16	11	0	5	5	3	0
4	26	23	15	0	20	18	10	0
13	26	24	18	0	24	23	14	0
37	25	23	21	0	24	22	18	0

The above table also shows that the same position of equilibrium is reached with the extracts derived from two quite different tissues as catalysts.

The time relationships of the balanced reaction.

$$Na_2HPO_4 + C_3H_5(OH)_3 \overset{\text{phosphatase}}{\rightleftharpoons} C_3H_5(OH)_2PO_4Na_2 + H_2O$$

have been studied. Two solutions are made up having the same amount of total P per cc.; in one, however, all the phosphorus is present as inorganic phosphate, in the other, all as sodium β-glycerophosphate, the p_H of each being 8·9. To each is added an equal volume of duodenal extract at p_H 8·9 (previously dialysed for 18 hours) and twice the volume of neutral glycerol. Controls with inactivated enzyme are also made up.

The results are shown in Fig. 2, curves *A*, *A*. Curves *B*, *B* represent the results of a similar experiment in which the reaction has taken place in 75 % glycerol (by volume). In both experiments a further determination (not shown in the figure) has been made on the reaction mixtures after 6 days without showing significant change in the state of equilibrium.

From Fig. 2 it is clear that the same equilibrium position is reached whether inorganic sodium phosphate or sodium glycerophosphate is present to begin with, provided, of course, that water and glycerol are present in

amounts which are large relative to the amount of phosphorus (the concentration of P was only 22 mg. per 100 cc. of the reaction mixture). The position of the equilibrium, and the speed at which it is reached, are also seen to be dependent on the relative concentrations of glycerol and water.

Curves similar to those of Fig. 2 have also been obtained using kidney phosphatase as the catalyst.

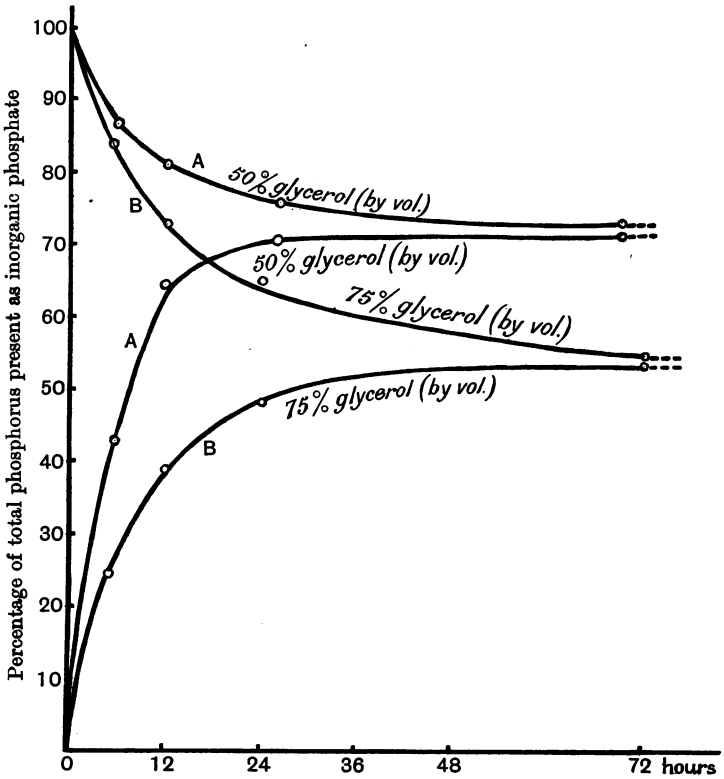

Fig. 2. Synthesis and hydrolysis of sodium glycerophosphate by duodenal phosphatase.

Inhibitory effect of free phosphate.

Free phosphate, even in relatively low concentrations is found to have a marked inhibitory effect on the hydrolysis of glycerophosphate by kidney or duodenal phosphatase (Table VIII).

Table VIII. *Inhibition of phosphatase by inorganic phosphate.*

Concentration of glycerophosphate at start	Concentration of inorganic phosphate at start	% inhibition after 2 hours' hydrolysis
M/40	Nil	0
,,	*M*/400	4·6
,,	*M*/200	12
,,	*M*/133	30
,,	*M*/100	62

Although low concentrations of phosphate thus markedly inhibit hydrolysis, free glycerol in much larger concentrations (up to about $M/2$) has no such effect. Since the phosphatase is clearly specific, not for the alcohol portion, but for the phosphoric acid portion of the molecule (which is the only part common to all the esters) one would expect the phosphate configuration to be in close relationship with the structure of the enzyme molecule, and the finding that the activity of the enzyme is very sensitive to the presence of inorganic phosphate is not surprising.

Isolation of products of synthesis by phosphatase.

Sodium glycerophosphate has been isolated from the reaction fluid after allowing duodenal extracts to act on a mixture of sodium phosphate and glycerol for a week.

Method. 10 g. of Na_2HPO_4, $12H_2O$ were dissolved in 100 cc. of extract of cat's duodenal mucosa (1 in 20) which had stood at room temperature in presence of chloroform for a week, and contained only traces of organic phosphorus. To this solution were added 160 cc. of pure glycerol. A sample of the liquid was taken at once for free and total P determination, and further samples at intervals afterward.

Time (days)	Free P (mg. per cc. liquid)	Total P	Organic P
0	3·60	3·61	0·01
4	2·91	—	0·70
7	2·39	—	1·22
10	2·36	3·66	1·30

After 10 days the reaction mixture was heated to 100° for 10 minutes to inactivate the enzyme, then diluted with an equal volume of water, and $Ca(NO_3)_2$ added in slight excess followed by ammonia till no more precipitate was obtained. The mixture stood for a short time and was then filtered. The filtrate (500 cc.) contained no free phosphate, but 200 mg. organic phosphorus. It was boiled to remove ammonia, neutralised to litmus, and excess of lead acetate (25 %) added. After standing overnight the mixture was centrifuged. The supernatant liquid now contained no free phosphorus, but 14 mg. of organic phosphorus. After washing the precipitate twice with water it was resuspended in warm water and the lead removed by H_2S. The filtrate from lead sulphide now contained 6 mg. free P and 180 mg. organic P. 5 g. barium acetate were dissolved in it, and baryta was added until the reaction was alkaline to phenolphthalein. After filtering off the barium phosphate, barium glycerophosphate was precipitated by adding 4 volumes of alcohol. This process was repeated twice. The resulting product was dried *in vacuo* at 100° over phosphorus pentoxide. It weighed 1·60 g.

Found:	Ba, 44·5 % 44·6 %	P, 9·9 %
Calculated for $C_3H_7O_6PBa$	Ba, 44·7 %	P, 10·1 %.

A portion dissolved in water was precipitated by the exact quantity of sodium sulphate, and the filtrate found to contain organic phosphorus readily hydrolysed by kidney phosphatase but quite stable to 1 % NaOH at 100° for 6 hours. A portion of the barium salt gave the acrolein test for glycerol. The isolated salt was therefore the Ba salt of glycerophosphoric acid. Both the free acid and the barium salt in aqueous solution were optically inactive.

There is a distinction to be drawn between true phosphatases and other enzymes which are phosphatases incidentally. The true phosphatases, whose point of attack is the phosphoric acid portion of the ester molecule, are relatively stable enzymes. Tissue extracts kept at 0–4° in presence of chloroform retain their ability to hydrolyse phosphoric esters for a long time. One specimen of pig's kidney extract, which had an initial content of 0·31 unit per cc., had after 8 months a content of 0·26 unit, and other specimens have been kept for even longer periods without serious loss of activity. Dried tissues keep their phosphatase activity almost indefinitely. In muscle extracts prepared by the method described above there is very little of the true phosphatase. The activity towards glycerophosphate is practically nil, although there is distinctly more activity if hexosediphosphate is used as substrate. The difference is more marked with hashed muscle which hydrolyses hexosephosphate fairly readily but hardly attacks glycerophosphate. In the case of muscle extracts therefore the ratio between P liberated from hexosephosphate and from glycerophosphate by the same extract is anomalous and very different from the figure obtained under the same conditions using kidney, bone or intestinal extracts. Meyerhof [1926] found that an unstable enzyme could be separated from frog's or rabbit's muscle which hydrolysed glycogen and other polysaccharides to lactic acid, and also hydrolysed hexosephosphate, even after warming to 38°. Lactic acid being produced in the latter case also, the point of attack of this enzyme on the hexosephosphate molecule is clearly the alcohol radicle. It is probable that this explains the anomaly, the muscle extracts prepared by the ordinary extraction method still containing small quantities of the unstable *pseudo*-phosphatase. The true phosphatase appears not to produce lactic acid from naturally occurring phosphoric esters. The present author in conjunction with Dr J. T. Irving has recently found that kidney phosphatase will not produce lactic acid from the acid-soluble phosphoric esters of the kidney tissue, nor apparently from sodium hexosediphosphate[1].

Meyerhof [1927] believes that the formation of a phosphoric ester is necessary before lactic acid can be formed from polysaccharides. Possibly one function of the true phosphatases *in vivo* is the synthesis of such esters. This possibility is supported by the experiments described in section C. There is no reason to believe that such synthetic activity would be limited to simple

[1] Duodenal phosphatase also hydrolyses fermentation hexosediphosphate without production of lactic acid. An extract of cat's duodenal mucosa hydrolysed 90 % of a solution of hexosediphosphate in 6 hours, producing 0·82 mg. inorganic P per cc., without producing any detectable quantity of lactic acid (*i.e.* less than 0·01 mg. per cc.).

esters. Since the phosphatases hydrolyse nucleotides (*in vitro*) with great ease, it is possible that under controlled conditions in the cell they are able to catalyse the synthesis of these phosphoric esters from nucleosides. This has not yet been attempted *in vitro*. Tissue phosphatases may similarly enter into the early stages in the formation of phosphatides. Further experiments on the synthetic activity of these enzymes are in progress.

It is nevertheless well established that *in vivo* the phosphatases may act hydrolytically. If sodium hexosephosphate or glycerophosphate be administered to an animal either intravenously or by mouth, the inorganic phosphate content of the blood and of the urine rises rapidly.

Summary.

1. The phosphatases of mammalian tissues (*i.e.* the relatively stable enzymes present in extracts from such tissues which hydrolyse acid-soluble phosphoric esters) all appear to have an optimum p_H between 8·8 and 9·3. The character of the substrate influences this optimum to a slight extent. The distribution of phosphatase in the tissues of the rabbit, of the cat and of man has been quantitatively determined. This distribution is remarkably akin to that found for ereptase by Vernon. Other correspondences between these functionally widely dissimilar enzymes are described.

2. It is probable that the same enzyme is responsible for the hydrolysis of glycerophosphate, of hexosediphosphate and of nucleotide.

3. In presence of excess of the alcohol concerned, inorganic phosphate can be esterified, using phosphatases derived from various tissues as catalysts. Glycerophosphoric acid (isolated as the barium salt) has been synthesised in this way from glycerol, and several other alcohols have been shown to combine with inorganic phosphate. The equilibrium

$$\text{glycerol} + \text{phosphate} \rightleftharpoons \text{glycerophosphate} + \text{water}$$

has been studied, using phosphatase as catalyst.

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