CLXXX. THE PHOSPHATASES OF MAMMALIAN TISSUES.

II. PYROPHOSPHATASE.

By HERBERT DAVENPORT KAY.

From the Medical Unit, The London Hospital.

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Neuberg and Wagner showed in 1926 that both diphenyl orthophosphate and diphenyl pyrophosphate, in aqueous solution in the form of their potassium salts, were hydrolysed practically completely, both by the phosphatase of takadiastase and by that of horse kidney, to salts of orthophosphoric acid [1926]. Neuberg and Jacobsohn [1928] have also shown that the potassium salt of di-*o*-cresol pyrophosphate is similarly hydrolysed by both of these phosphatases, and that potassium di-*m*-cresol pyrophosphate and potassium di-α-naphthol pyrophosphate are hydrolysed by takadiastase (kidney extract not mentioned). Both kidney extract and takadiastase (prepared from *Aspergillus oryzae*) contain, therefore, a pyrophosphatase. Lohmann [1928] has recently stated that pyrophosphates are present in muscle, and are hydrolysed to orthophosphates when the hashed muscle is incubated in sodium bicarbonate solution. This production of orthophosphate has hitherto been very generally ascribed to the hydrolysis of "lactacidogen," believed by Embden and others to be a hexosephosphate. If Lohmann's claim is substantiated, it follows that the greater part of the so-called "lactacidogen" phosphorus is not originally present as hexosephosphate at all. Still another factor is thus introduced into the problem of the complex rôle played by phosphate in the biochemical changes associated with the contraction and metabolism of muscle. His findings also give a new importance to the presence of pyrophosphatase in the tissues. It becomes of interest to know whether there is any relation between the enzyme which hydrolyses pyrophosphate and that phosphoric esterase, widely distributed in the tissues, which hydrolyses glycerophosphate, hexosediphosphate and nucleotide [Kay, 1928], and many other orthophosphoric esters.

Experimental.

Pure sodium pyrophosphate was prepared by heating pure disodium hydrogen phosphate to a red heat in a platinum dish. So prepared, its aqueous solution gave a white precipitate (without the slightest trace of yellow) with silver nitrate and contained, as determined by the Briggs or the Bell-Doisy

method, no orthophosphate. (A commercial sample of "pure" potassium pyrophosphate was found to contain 10 % of orthophosphate.) Although quite stable in neutral or alkaline solution, preliminary experiments indicated that the salt was very slowly hydrolysed at room temperature by the concentration of acid present in the determination of orthophosphate by the Briggs method. Under the conditions used by me the hydrolysis was at the rate of 0·016 mg. P in ½ hour, starting with 2·20 mg. pyrophosphate-P in the reaction flask, which contained the usual Briggs reagents, made up to 25 cc. Thus about 0·7 % of the original pyrophosphate was hydrolysed at room temperature by $N/2$ H_2SO_4 in ½ hour; this rate was linear for the first 2 hours at least. On this basis a correction[1] was applied where necessary. It is probable that the Briggs method, with the necessary correction, would prove useful in determining the rate of decomposition of pyrophosphate by acids, etc., under various conditions of temperature and concentration.

Both sodium and potassium pyrophosphate were found to be readily hydrolysed to orthophosphate by various tissue extracts. Pyrophosphate must be almost unique as an example of a substance stable to a bright red heat, yet capable of enzymic decomposition.

Optimum p_H *for pyrophosphatase.*

This has been determined for the pyrophosphatase present in extracts of kidney cortex, duodenal mucosa, bone and lung of the cat, the kidney of the pig, and the bones of a young rat. In all cases it lies between p_H 7·2 and 7·8 (in Palitzsch's borax-boric acid buffers), usually about 7·6 (Table I).

Table I. *Optimum* p_H *for action of pyrophosphatase.*

Palitzsch's borax-boric acid buffers used, duration of hydrolysis 2 hours at 37·5°. Figures in mg. orthophosphate-P produced in 10 cc. of reaction mixture.

	Source of enzyme			
p_H	Cat's kidney extract, fresh, 1/20	Pig's kidney extract, 1 year old, 1/20	Cat's duodenal extract, 1/160	Young rat's bone extract, fresh, 1/20
6·8	0·092	0·089	0·074	0·245
7·1	0·104	0·111	0·094	0·303
7·36	0·114	0·114	**0·117**	0·366
7·6	**0·126**	**0·117**	0·086	**0·403**
7·9	0·123	0·111	0·059	0·387
8·2	0·104	0·088	0·034	0·341
8·5	—	0·061	—	0·245

It will be noticed that the optimal activity of pyrophosphatase at about p_H 7·6 is in a much more physiological range than that of the orthophosphoric esterase (p_H 8·8–9·3).

[1] In view of this slow hydrolysis of pyrophosphate by the Briggs reagents, and Lohmann's finding of relatively large quantities of pyrophosphate in fresh muscle, it may be necessary to apply a correction, varying in magnitude with the exact conditions, to the quantitative figures obtained for "phosphagen" in muscles by various workers, who have estimated it by the difference between inorganic phosphate determined by magnesia precipitation (or some similar method) in alkaline solution and by the Briggs colorimetric method (or some similar method) in acid solution.

Distribution in the tissues.

Extracts of the following tissues (arranged in order of their activity) were found to be hydrolytically active on solutions of pure sodium pyrophosphate in borate buffers: duodenal mucosa[1] (most active), growing bone, kidney, lung, liver, adult bone (least active). Muscle extract, blood-plasma and extract of gastric mucosa also contained a weak pyrophosphatase. The extracts were made as previously described [Kay, 1928]. Boiled extracts showed no hydrolytic activity. The active extracts, tested against glycerophosphate solutions of the same concentration of P per cc. as in the pyrophosphate solution, gave the usual optimum for the former substrate of p_H 8·8–9·3. The order of hydrolytic activity displayed by the various tissue extracts was the same for glycerophosphate at p_H 8·9 as for pyrophosphate at p_H 7·6. (Such extracts hydrolysed pyrophosphate at p_H 8·9, and glycerophosphate at p_H 7·6, but only very slowly.) Usually with the more active extracts the amount of orthophosphate-P produced at the optimal p_H of 7·6 from pyrophosphate solutions was about half of that produced from glycerophosphate solutions at p_H 8·9, *i.e.* the ratio

$$\frac{\text{amount orthophosphate-P produced from excess glycerophosphate at } p_H\ 8{\cdot}9}{\text{amount orthophosphate-P produced from excess pyrophosphate at } p_H\ 7{\cdot}6}$$

was usually found to be approximately constant from one tissue to another.

The hydrolysis of pyrophosphate by tissue extracts is brought about, therefore, by an enzyme whose distribution in the tissues appears to be similar to that of glycerophosphatase (= hexosediphosphatase = guanine nucleotidase), and it seems likely that the same phosphoric esterase is responsible. The reverse reaction of enzymic synthesis of phosphoric esters by tissue extracts I have shown to take place in the case of glycerophosphate [1928] and glycophosphate (hydroxyethyl phosphate) by actually isolating the synthesised esters as the pure barium salts, but several attempts to accomplish the synthesis of pyrophosphate along similar lines from solutions of orthophosphates have so far been without success; the strong salt solutions used flocculate and inactivate the enzyme.

SUMMARY.

Pyrophosphatase is widely distributed in mammalian tissues; its distribution is similar to that of the orthophosphoric esterase previously described, but its zone of optimal activity is between p_H 7·2 and 7·8, as against p_H 8·8–9·3 for the latter enzyme.

REFERENCES.

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[1] The great activity of intestinal extracts would suggest that pyrophosphates taken by mouth are probably hydrolysed to orthophosphates before absorption from the gut.