XVI. SOME EFFECTS OF PYROPHOSPHATE ON THE METABOLISM OF TISSUES

BY MARGARET ELIZABETH GREIG AND MURIEL PLATT MUNRO

*From the Biochemical Research Foundation of the Franklin
Institute, Philadelphia, Pa.*

(Received 24 November 1938)

PYROPHOSPHATE has been shown by Dixon & Elliott [1929] to inhibit the respiration of minced tissue. For liver mince the inhibition was less than with cyanide, and small concentrations produced a slight acceleration. With muscle, on the other hand, pyrophosphate produced a greater inhibition than cyanide, $M/30$ pyrophosphate causing more than 80% inhibition. According to Keilin [1929] pyrophosphate has no effect on indophenol oxidase (cytochrome oxidase). Dixon & Thurlow (quoted by Dixon & Elliott [1929]) found that succinic dehydrogenase is definitely inhibited by $M/1000$ pyrophosphate while xanthine dehydrogenase is unaffected by $M/25$. More recently, Leloir & Dixon [1937] have made a systematic survey of different dehydrogenases and have found that, of the nine dehydrogenases studied, succinic is the only one which is inhibited by pyrophosphate, so that pyrophosphate probably acts as a respiratory inhibitor by specific inhibition of this enzyme.

Succinic dehydrogenase has come into prominence recently with Szent-Györgyi's theory of tissue respiration [1937] in which succinic, fumaric, malic and oxaloacetic acids, with their appropriate enzyme systems, can act as hydrogen transportases. Succinate is the last member of the chain of reactions and from this H is transferred to molecular O_2 through cytochrome and cytochrome oxidase. If the C_4 dicarboxylic system accounts for the bulk of tissue respiration one would expect respiration to fall off when succinic dehydrogenase is inhibited, the extent, of course, depending on whether or not the concentration of succinic dehydrogenase is the limiting factor. This was indeed shown to be the case by Annau [1935], Gözsy & Szent-Györgyi [1934], Greville [1936], and Stare [1936] who found that malonate inhibited the respiration of various tissues. Boyland & Boyland [1936] reported an inhibition in respiration of tumour tissue by the addition of malonate. Elliott & Greig [1937], using Ringer-Krebs medium, found that malonate inhibited the respiration of liver slices about 15% and that of kidney slices by 50–60%, presumably by specific inhibition of succinic dehydrogenase.

Johnson [1936] found that the rate of aerobic removal of Na α -glycerophosphate by normal minced brain tissue is increased by added Na pyrophosphate. He considers this effect similar to that of adenylyl pyrophosphate acting as coenzyme for yeast glycerophosphate dehydrogenase as shown by Lehmann [1934].

Pyrophosphate seems to exert a stimulating effect on certain other enzymes. Green [1936], working on isolated enzyme systems, found that pyrophosphate has no effect on lactic dehydrogenase but causes 100% acceleration of malic dehydrogenase.

Peters & Sinclair [1933, 1], using minced pigeon brain in phosphate buffer, found that Na pyrophosphate added alone had little influence upon maintenance of respiration in the surviving tissue but that, added with lactate, it increased the respiration rate considerably. Respiration was also maintained at a more constant rate over a period of 3 hr. instead of falling off rapidly as was the case with added lactate alone. They found that the improvement in respiration by pyrophosphate with added lactate was, in part, due to an increase in fluoride-sensitive respiration, and suggested that the hexosediphosphate (or triose-phosphate) system is in some way involved in the metabolism of lactate. The optimum concentration of pyrophosphate was 0.2 % which is of the same order as that possible in the cell. Peters considered that these substances (lactate, pyrophosphate and probably glycerophosphate) may be present in the tissue and cause the initial high rate of respiration, but diffuse out of the cell and gradually become ineffective.

In avitaminous brain Peters *et al.* [1933, 2; 1935] found that pyrophosphate ($M/100$) enhanced the catatorulin effect of vitamin B₁ in the metabolism of lactate and pyruvate. It may be that cocarboxylase is synthesized in this tissue from vitamin B₁ and pyrophosphate.

It thus appears that pyrophosphate has several possible effects on the respiration mechanisms of minced tissues. It can increase oxidations by a stimulating effect on malic or α -glycerophosphate dehydrogenations and it can increase lactate oxidation and the catatorulin effect of vitamin B₁, but it can also inhibit respiration through its effect on succinic dehydrogenase. The results of a survey of the effects of pyrophosphate on respiring slices of different tissues are presented here.

EXPERIMENTAL

The methods employed were those described by Elliott and co-workers [1934, 1935] for the Dixon-Keilin differential manometer. When glucose was present in the medium its concentration was 0.24 %. The concentrations of added substrates were: *dl*-lactate $M/25$; pyruvate, succinate and *l*-malate $M/50$. The concentration of pyrophosphate varied as shown in the table. The solution, freshly prepared for each experiment, was brought to pH 7.4 by addition of dilute HCl [see Leloir & Dixon, 1937]. The experimental period was 90 min. in all cases.

The rat tissues were prepared as described by Elliott *et al.* Ox eyes were obtained from the slaughter house on the morning of killing and the retina was removed as will be described in a later paper. With the rat tissues the results were calculated on the final dry wt. [see Elliott *et al.* 1937]. With retina the tissue disintegrated to such an extent, especially after the addition of acid, that final dry wts. were only approximate. The results were therefore calculated on the initial dry wts. obtained from the wet wt. of tissue taken and the dry wt./wet wt. ratio determined on separate samples.

The terms used for expressing results are those defined by Elliott *et al.* Variations of 10 % are considered to be within the experimental error and when the apparent effect of pyrophosphate is within this range the % effect is not given in Table I. When the values obtained are small, as is often the case for Q_A , the effect of pyrophosphate may cause a large % change but the values are too small for accurate experimental measurement. In these cases the % change is not given but when there is a definite acceleration or inhibition it is designated by a plus or a minus sign in Table I.

Table I

Tissue	Addition	Final conc. of pyro- phosphate	Glucose	$-Q_{O_2}$	R.Q.	Q_A	% effect of pyrophosphate	
							Q_{O_2}	Q_A
Rat tumour Walker 256	None	—	+	9.2	1.0	+ 20.2		
	Pyrophosphate	<i>M</i> /100	+	10.5	0.78	+ 19.0		
	None	—	+	10.1	0.98	+ 18.7		
	Pyrophosphate	<i>M</i> /30	+	6.5	0.65	+ 17.7	- 35	
	None	—	—	11.0	0.68	+ 0.9		
	Pyrophosphate	<i>M</i> /30	—	10.1	0.69	+ 1.4		
	Lactate	—	—	11.9	0.79	- 0.5		
	Lactate + pyro- phosphate	<i>M</i> /50	—	10.5	0.76	+ 1.1	- 12	+
Rat testis	None	—	—	6.9	0.68	+ 1.3		
	Pyrophosphate	<i>M</i> /30	—	4.8	0.64	+ 2.2	- 30	
	None	—	+	11.5	0.94	+ 6.9		
	Pyrophosphate	<i>M</i> /30	+	9.9	0.83	+ 5.0	- 14	—
	Lactate	—	—	16.3	0.86	- 2.8		
	Lactate + pyro- phosphate	<i>M</i> /50	—	11.9	0.83	- 1.1	- 27	—
Rat liver	None	—	— *	12.8	0.85	+ 0.1		
	Pyrophosphate	<i>M</i> /30	—	11.6	0.76	+ 0.4	- 10	
	Pyrophosphate	<i>M</i> /30	+	8.8	0.81	+ 2.6	- 31	+
	Lactate	—	—	12.5	0.81	- 3.3		
	Lactate + pyro- phosphate	<i>M</i> /30	—	9.5	0.88	- 0.3	- 24	
	None	—	—	12.5	0.75	+ 1.3		
	Pyrophosphate	<i>M</i> /30	+	10.6	0.72	+ 1.8	- 16	
	Pyrophosphate	<i>M</i> /30	+	10.0	0.85	+ 1.8	- 20	
	Lactate	—	—	13.7	0.89	- 4.5		
	Lactate + pyro- phosphate	<i>M</i> /30	—	12.7	0.88	- 0.6		- 88
Rat brain	None	—	+	16.7	0.93	+ 5.8		
	Pyrophosphate	<i>M</i> /50	+	15.7	0.96	+ 7.4		
	Lactate	—	—	11.9	0.95	- 2.8		
	Lactate + pyro- phosphate	<i>M</i> /50	—	12.1	0.94	- 2.2		
Rat kidney	None	—	+	25.9	0.90	- 1.1		
	Pyrophosphate	<i>M</i> /100	+	27.1	0.79	+ 2.8		+
	None	—	—	24.4	0.75	+ 0.6		
	Pyrophosphate	<i>M</i> /50	—	22.7	0.74	+ 3.6		+
	None	—	+	27.7	0.84	+ 0.2		
	Pyrophosphate	<i>M</i> /50	+	31.4	0.82	+ 3.1	+ 13	+
	None	—	+	25.9	0.80	+ 0.4		
	Pyrophosphate	<i>M</i> /30	+	30.2	0.78	+ 3.6	+ 17	+
	Pyrophosphate	<i>M</i> /100	+	30.7	0.83	+ 2.1	+ 18	+
	Pyrophosphate	<i>M</i> /1000	+	26.8	0.89	+ 1.6		+
	None	—	+	25.1	0.85	- 0.9		
	NaCl 4 <i>M</i> /30	—	+	26.3	0.82	+ 1.6		+
	None	—	—	19.6	0.79	- 1.4		
	NaCl 4 <i>M</i> /30	—	—	17.8	0.69	+ 0.4		
	Succinate	—	—	46.7	0.74	- 18.2		
	Succinate + pyro- phosphate	<i>M</i> /50	—	40.4	0.79	- 7.1	- 12	- 61
	Malate	—	—	34.3	1.18	- 17.1		
	Malate + pyro- phosphate	<i>M</i> /50	—	29.7	0.95	- 3.7	- 13	- 78
	Pyruvate	—	—	37.8	1.23	- 20.2		
	Pyruvate + pyro- phosphate	—	—	36.2	1.08	- 7.3		- 60
	Lactate	—	—	27.2	0.81	- 6.5		
	Lactate + pyro- phosphate	<i>M</i> /50	—	27.0	0.83	- 2.3		- 65

* The presence or absence of glucose does not affect the normal respiration of liver [see e.g. Elliott *et al.* 1937].

Table I (cont.)

Tissue	Addition	Final conc. of pyro- phosphate	Glucose	$-Q_{O_2}$	R.Q.	Q_A	% effect of pyrophosphate	
							Q_{O_2}	Q_A
Ox retina	None	—	+	9.8	0.86	+ 8.4		
	Pyrophosphate	<i>M</i> /50	+	15.2	0.98	+ 9.9	+ 55	
	None	—	—	7.3	0.99	— 0.9		
	Pyrophosphate	<i>M</i> /50	—	9.3	1.00	— 0.8	+ 27	
	Lactate	—	—	11.1	0.90	— 2.3		
	Lactate + pyro- phosphate	<i>M</i> /50	—	14.5	0.97	— 3.1	+ 31	+
	None	—	—	6.4	0.93	— 1.0		
	Pyrophosphate	<i>M</i> /50	—	7.1	0.89	— 0.3	+	
	Lactate	—	—	11.1	0.85	— 2.3		
	Lactate + pyro- phosphate	<i>M</i> /50	—	16.2	0.95	— 2.9	+ 46	+
	None	—	+	11.6	0.88	+ 7.9		
	Pyrophosphate	<i>M</i> /50	+	15.2	0.91	+ 9.3	+ 31	+ 18

DISCUSSION

The effect of pyrophosphate on tissue slices varies with the concentration of pyrophosphate, the tissue used and the substrate being metabolized. With rat tumour (Walker 256), dilute pyrophosphate (*M*/100) in the presence of glucose increases the respiration slightly without affecting glycolysis. A more concentrated solution (*M*/30) in the presence of glucose inhibits respiration 35 %. In both cases the R.Q. is considerably decreased. In the absence of glucose, *M*/30 pyrophosphate has practically no effect on respiration and glycolysis. It also has no effect on lactate metabolism. Elliott *et al.* [1935] and Elliott & Greig [1937] showed an extremely low succinoxidase content in Walker 256 tumour so that either respiration does not go through succinate or succinoxidase is likely to be the limiting factor. The definite effects of pyrophosphate, particularly on the R.Q., show that it does penetrate cells to a certain extent. If we can assume that it penetrates considerably we must expect succinoxidase inhibition; the absence of more marked effects on respiration then means that succinate is not an essential intermediate of respiration in this tissue.

With testis, which is also low in succinoxidase activity, pyrophosphate, in a concentration of *M*/30, inhibits O_2 uptake to some extent both in the presence and absence of glucose. Oxidation of lactate is inhibited by 27 % by pyrophosphate indicating that removal of this acid in testis is probably to some extent dependent on the succinate system.

Pyrophosphate has little effect on the normal respiration of liver slices, but in the presence of glucose respiration is inhibited by 31 % and acid formation is increased. With added lactate O_2 uptake is inhibited by about 24 % and acid removal is practically completely inhibited. This indicates that the metabolism of liver, especially with added lactate, is, like that of testis, at least in part dependent on the succinoxidase system.

Pyrophosphate has no effect on the respiration of brain slices in the presence of glucose or on its metabolism of lactate. The fact that our experimental procedure and the duration of our experiment were quite different from those of Peters may explain the absence in our experiment of the stimulating effect of pyrophosphate on lactate metabolism. Since pyrophosphate does not inhibit

lactate oxidation in brain slices, this would seem to indicate that the main path of oxidation is not through succinate. However, the absence of any effect whatever might indicate that pyrophosphate does not penetrate the cells of sliced brain.

With kidney the effects are different from those found with the above tissues. In the absence of glucose pyrophosphate causes a slight inhibition in O_2 uptake and some accumulation of acid; in the presence of glucose there is increased respiration and increased glycolysis. These results may indicate that there is an alternative path of oxidation to that proceeding by way of succinate, or it may be that succinoxidase is not completely inhibited and perhaps other systems such as the malic and lactic systems are accelerated so that the net result is increased oxidation in the presence of glucose.

That the effect of pyrophosphate on glucose oxidation is not a salt effect was shown by adding NaCl approximately isotonic with the pyrophosphate used. This had no effect on respiration but it caused a small increase in acid formation.

With added succinate the effect of pyrophosphate is similar to that of malonate [Elliott & Greig, 1937]. The removal of acid groups is inhibited 60% and O_2 uptake is inhibited about 12%, but respiration is still greater than that in the absence of substrate or inhibitor, indicating that some succinate is still being oxidized. This is to be expected in view of the extremely high succinoxidase content of kidney [see Elliott and Greig, 1937]. In the presence of pyruvate, pyrophosphate inhibits removal of acid groups about 60%, i.e. about the same as for succinate, but the O_2 uptake remains the same as without inhibitor. Pyrophosphate decreases acid disappearance in the presence of malate by about 78% and respiration is decreased to about the same extent as with added succinate, although it is still higher than in the absence of substrate. These results are similar to those of Elliott and Greig with malonate and indicate that the succinoxidase system is concerned with the complete oxidation of malate to CO_2 and H_2O . With added lactate, pyrophosphate has no effect on O_2 uptake but decreases acid removal by 65% which also indicates the participation of the succinoxidase system in the complete oxidation of this substrate.

Ox retina differs from the other tissues studied in its reaction to pyrophosphate. Like testis it has a low succinoxidase content but added pyrophosphate, instead of inhibiting respiration, causes a large increase in O_2 uptake and a small increase in glycolysis in the presence of glucose. Without added substrate there is usually some increase in respiration. In all cases pyrophosphate raises the R.Q. With lactate as substrate pyrophosphate also causes a considerable increase in O_2 uptake and a small increase in acid disappearance.

It will be shown in a forthcoming paper that the rate of succinate oxidation by ox retina is not great enough for it to be a link in the chain of reactions for the oxidation of lactate. That pyrophosphate does not inhibit but indeed accelerates lactate and acid removal is further evidence that this reaction does not go through succinate.

From these results one might assume that in liver, testis and kidney succinate is an intermediate in carbohydrate metabolism, and pyrophosphate, in inhibiting succinoxidase, reduces respiration. Perhaps in kidney there is an alternative path for glucose oxidation. However, in brain and retina the succinate-fumarate system does not seem to play a necessary role. In the case of these tissues inhibition of the succinoxidase system does not inhibit respiration or acid disappearance. Pyrophosphate seems to act catalytically in retina in the oxidation of glucose or lactate, perhaps by formation of cocarboxylase or other coenzyme or by formation of some phosphorylated intermediate.

SUMMARY

Using the Dixon-Keilin manometric technique, it was found that pyrophosphate exerts various effects on the metabolism of tissue slices depending both on the tissue and the substrate employed.

1. It has no effect on the metabolism of brain slices.
2. The respiration of testis is inhibited in the presence or absence of added substrate.
3. Pyrophosphate has no effect on the normal respiration of tumour and, in low concentrations, has no effect on glucose oxidation, while in higher concentrations both glucose and lactate oxidations are inhibited.
4. It has little effect on the normal respiration of liver but inhibits respiration in the presence of glucose and lactate.
5. Pyrophosphate enhances the rate of glucose metabolism of kidney, has no effect on normal respiration and inhibits the complete oxidation of succinate, malate, pyruvate and lactate.
6. It has practically no effect on the normal respiration of retina but it considerably increases the rate of oxidation of glucose and lactate.

The authors wish to express their appreciation to Dr Ellice McDonald, Director, for his support and interest throughout the work, and to Dr K. A. C. Elliott for much helpful advice and criticism.

REFERENCES

- Annau (1935). *Hoppe-Seyl. Z.* **236**, 58.
Boyland & Boyland (1936). *Biochem. J.* **30**, 224.
Dixon & Elliott (1929). *Biochem. J.* **23**, 812.
Elliott, Benoy & Baker (1935). *Biochem. J.* **29**, 1937.
— & Greig (1937). *Biochem. J.* **31**, 1021.
— — & Benoy (1937). *Biochem. J.* **31**, 1003.
— & Schroeder (1934). *Biochem. J.* **28**, 1920.
Göszy & Szent-Györgyi (1934). *Hoppe-Seyl. Z.* **224**, 1.
Green (1936). *Biochem. J.* **30**, 2095.
Greville (1936). *Biochem. J.* **30**, 877.
Johnson (1936). *Biochem. J.* **30**, 33.
Keilin (1929). *Proc. roy. Soc. B*, **104**, 206.
Lehmann (1934). *Biochem. Z.* **272**, 95.
Leloir & Dixon (1937). *Enzymologia*, **11**, 81.
Peters, Rydin & Thompson (1935). *Biochem. J.* **29**, 53.
— & Sinclair (1933, 1). *Biochem. J.* **27**, 1677.
— — (1933, 2). *Biochem. J.* **27**, 1910.
Stare (1936). *Biochem. J.* **30**, 2257.
Szent-Györgyi (1937). Studies on Biological Oxidation and some of its Catalysts. Budapest: Renyi.