A PHOSPHORYLATED OXIDATION PRODUCT OF PYRUVIC ACID

Sirs:

It has been shown that pyruvic acid oxidation is dependent on the presence of inorganic phosphate,[1] but so far it had not been possible to demonstrate the formation of a phosphorylated intermediate. Since pyruvic acid was found to promote adenylic acid phosphorylation,[2] any such intermediate must contain an energy-rich phosphate bond. Phosphopyruvic acid had been excluded by earlier experiments, but recent evidence suggested that acetyl phosphate might be the intermediate.[3] It was found that the phosphate of synthetic acetyl phosphate could be transferred to adenylic acid by an enzyme present in *Bacterium delbrückii*. Tentatively the oxidation was formulated as follows: $CH_3 \cdot CO \cdot COOH + H_3PO_4 + O_2 = CH_3 \cdot COOPO_3H_2 + CO_2 + H_2O_2$.

In order to obtain a method for determining acetyl phosphate, a closer study of its stability was undertaken. It developed that at room temperature acetyl phosphate is rapidly broken down above pH 8.5 or below pH 2, but is fairly stable between pH 5 and 7. Hence, the magnesia mixture used for the determination of acid-unstable creatine phosphate is too alkaline for this purpose. It has now been found, however, that inorganic phosphate is completely precipitated at *neutral* reaction with calcium chloride in 30 per cent ethyl alcohol, whereas the calcium salt of acetyl phosphate is soluble under these conditions. Moreover, cold trichloroacetic acid can be used for deproteinization, causing prac-

[1] Lipmann, F., *Enzymologia*, **4**, 65 (1937). Banga, I., Ochoa, S., and Peters, R. A., *Biochem. J.*, **33**, 1980 (1939).

[2] Lipmann, F., *Nature*, **143**, 281 (1939). Colowick, S. P., Welch, M. S., and Cori, C. F., *J. Biol. Chem.*, **133**, 641 (1940).

[3] Lipmann, F., *Nature*, **144**, 381 (1939); in Cold Spring Harbor symposia on quantitative biology, Cold Spring Harbor, **7**, 248 (1939).

tically no decomposition. Thus the calcium precipitation method can be used to determine acetyl phosphate by difference.

Pyruvate	Fluoride	O_2 consumed	Ca ppt., inorganic P (I)	Direct estimation, inorganic + acid-unstable P (II)	Mg ppt., inorganic + alkali-unstable P (III)	Acetyl phosphate P (II − I)		$\frac{\text{Acetyl P}}{\text{Excess } O_2}$
		c.mm.	*mg.*	*mg.*	*mg.*	*mg.*	*c.mm.*	
−	−	116	1.30	1.32				
+	−	796	0.57	1.30		0.72	486	0.7
−	+	114	1.29	1.31	1.32			
+	+	734	0.52	1.32	1.31	0.77	556	0.9

Experiments were carried out with enzyme solutions obtained from *Bacterium delbrückii*. After pyruvic acid oxidation for about 1 hour, the cooled solution was deproteinized with 2 per cent trichloroacetic acid, the filtrate rapidly neutralized, and the true inorganic phosphate precipitated with $CaCl_2$. As shown by the data presented in the table, large amounts of inorganic phosphate disappear either in the absence or presence of fluoride, and are nearly equivalent to the extra oxygen consumed, in accordance with the equation, due allowance being made for the imperfect stability of the product. The organic phosphate so formed behaves like acetyl phosphate; *i.e.*, it is split readily and completely by the acid required to determine phosphate directly (II in the table) and (in contrast to creatine phosphate) by alkaline magnesia mixture (III). Colorimetric determination of phosphate was carried out according to Lohmann and Jendrassik.[4]

The demonstration here of a phosphate compound of such limited stability, which is formed metabolically, might well necessitate some revision as to what has usually been regarded as inorganic phosphate in cells and tissues.

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[4] Lohmann, K., and Jendrassik, L., *Biochem. Z.*, **178**, 419 (1926).