An improved method for the isolation of barium phosphoarginine

The purpose of this communication is to outline the general principles involved in a new method for the preparation, in good yield, of barium phosphoarginine and to draw attention to the fact that the resultant compound has a structure considerably different from that hitherto described.

Phosphoarginine was first isolated from crayfish muscle by MEYERHOF AND LOHMANN[1] who used a procedure which involved precipitation of the phosphagen from the soluble Ba salts of a protein-free extract first as a sulphate and then as a barium salt. The compound isolated corresponded to the formula $(C_6H_{14}O_5N_4P)_2Ba.2H_2O$.

In connection with studies of the enzyme, arginine phosphokinase, it was necessary to prepare pure arginine phosphate, and the method of MEYERHOF AND LOHMANN[1] was modified in such a way as to avoid the large losses inherent in the older procedure.

Briefly the new method consists of the following steps; the insoluble barium salts were precipitated at pH 9.0 from a protein-free extract of crayfish muscle. The water-soluble barium salts present in the supernatant were then precipitated by the addition of 3 vols. of ethanol and collected by centrifugation. The phosphoarginine contained therein was recovered in quantitative yield as a Cu salt which was then converted into and precipitated as phosphoarginine hydrochloride after removal of the Cu as CuS. The hydrochloride which on elementary analysis corresponded to the formula $(C_6H_{15}O_5N_4P)HCl$ was then converted into a Ba salt.

The conditions under which this final precipitation was carried out were similar to those described by MEYERHOF AND LOHMANN[1] but the yield was about 30 times greater (10 g/kg fresh muscle).

It was anticipated that the compound would have the same structure as that described by MEYERHOF AND LOHMANN[1], but its elementary analysis and chemical properties corresponded to the formula $(C_6H_{13}O_5N_4P)Ba.H_2CO_3 \cdot H_2O$

The above procedure has been repeated many times and the final precipitation of the barium salt carried out at pH 7.0 and 8.0, and on each occasion a compound identical in nature to that described above was obtained. The failure to obtain a compound similar in constitution to that described by MEYERHOF AND LOHMANN[1] is difficult to explain with certainty, but the formula suggested would perhaps be anticipated havig regard to the pK of phosphoric acid moiety[2] and to basicity of the compound.

The complete details of this work will be submitted for publication elsewhere.

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Factors influencing the oxidation of dl-β-hydroxybutyrate by tumour mitochondria

In a preceding publication[1] the adenosine triphosphate (ATP) dependent oxidation of octanoate by liver mitochondria was used to collect information on the ATP-splitting activity (ATPase) of tumour mitochondria, when it was found that addition of the latter to the former mitochondria abolished the oxygen consumption of the fatty acid oxidation process completely. As a result of those and similar experiments[2] high ATPase activities could be attributed to mitochondrial preparations from a number of mouse and rat tumours; direct measurements of the amount of inorganic phosphate liberated from ATP by the tumour mitochondrial suspensions using the *tris*-KCl medium of CHAPPELL AND PERRY[3] led to the same results[4].

The ATP-splitting activities interfere with the capacity of tumour mitochondria *per se* to oxidize fatty acids. The mitochondria from five tumours, which were otherwise inactive but whose ATPase activities could be lowered sufficiently, by inclusion of versene into the medium, were found to oxidize octanoate in the presence of a "sparker" and diphosphopyridine nucleotide (DPN); the mitochondria from the other tumours studied did not show this oxidation, even in a number of cases in which the ATPases were low[2].

In search for other factors which govern the oxidative behaviour of tumour mitochondria towards fatty acids, the oxidation of *dl*-β-hydroxybutyrate (BHB) has been studied. Both *d*- and