Respiration of Tissue Slices in the Presence of β -Aminopropionitrile

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Monoamino-oxidase inhibitor ("Marsilid") aggravated the symptoms of experimental lathyrism¹. This observation prompted us to study the respiration of tissue slices in the presence of β -aminopropionitrile (β -APN).

The experiments were conducted with conventional Warburg-technique. The liver slices (dry weight 8–10 mg) of fasting Wistar rats were incubated at + 37°C in 2 ml of bicarbonate-Ringer solution containing 0.2 % glucose. Sixteen control experiments were made with no additions in the incubation medium, the mean oxygen consumption being 9.29 ± 2.94 μ l/mg/h. Decreasing concentrations of β -APN were added to the medium. The resulting oxygen uptakes were

No. of expts	β -APN	% of control
2	1 M	9
2	0.1 M	81
18	0.01 M	152
2	0.001 M	112

Similar stimulating effect of 0.01 M APN was also obtained with epiphyseal cartilage.

In eleven experiments using 0.01 M concentration of APN the disappearance of APN from the medium after 1 h incubation was studied by measuring the green colour, developed of APN by ninhydrin, at 340 $m\mu$. An average of 1.2 μ moles of APN (6 % of the APN present) had disappeared in each experiment. If detoxified to cyanoacetic acid, it would have elevated the oxygen uptake only by about half of the actual increase.

Marsilid, in 0.001 M concentration, did not have any influence on the disappearance of APN. Neither the oxygen uptake of the controls nor the stimulation of oxygen consumption by 0.01 M APN were influenced by the presence of 0.001 M Marsilid in the incubation medium, as stated in 10 experiments.

Determinations of inorganic phosphorus of the incubation fluid indicated a possible impairment of phosphorus uptake by slices incubated with 0.01 M APN.

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Histochemical Demonstration of Phosphorylase Activity in Tissue Sections

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The formation of glycogen from glucose-1-phosphate is due to phosphorylase activity. Our preliminary experiments showed that the techniques published as yet tend to be capricious and the results difficult to reproduce. All the published methods include glucose-1-phosphate as a substrate, from which phosphorylase and transglucosidase build glycogen and at the same time liberate phosphoric acid. The newly formed glycogen indicates the phosphorylase activity and can be demonstrated either by iodine solution or by the periodic acid Schiff method.

Fresh frozen sections were cut with a freezing microtome at 10–50 microns. 20 to 30 microns gave best results. The sections were transferred immediately in the incubation solution or dried on coverslips. In the incubation solution the glucose-1-phosphate concentration was varied from 0.1 % up to 5.0 %. Below 0.5 % the results were poor. The pH was varied from 4.5 to 6.0. After preliminary experiments pH 5.9 was found to be most suitable. As an inhibitor of acid phosphatase fluoride was used. Best results were obtained when the sodium fluoride concentration was about 1.7 %. Activators such as muscle adenylic acid, insulin and glycogen had little or no influence on the results. The composition of the final incubation mixture was: 250.0 mg glucose-1-phosphate, 2.0 ml saturated sodium fluoride, 1.0 ml 0.5-M acetate buffer, aq. dest. ad 5.0 ml. The sections were incubated for 2 h at 37°C.

There was a rather strong colour in those control sections which were stained with the periodic acid-Schiff technique. The best way to decrease the nonspecific colour was by ether-chloroform extraction after incubation in the substrate mixture.

Most clear-cut results were obtained with skeletal muscle. Similar or less intense reaction was also seen in heart and smooth muscle. A definitely positive reaction was also seen in heart and smooth muscle. A definitely positive reaction was also seen in the liver. As an observation not previously reported we found a uniform strongly positive reaction in the adrenal medulla.

The technique developed is by no means perfect. The coloured precipitate produced can only be a rough guide to the sites of the enzyme activity. Therefore efforts are continued to refine the technique.

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The Phosphotransferase Activity of Alkaline Phosphatases. Studies with Hydroxyamino Acids and Amines as Acceptors

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In an extensive study Morton¹ recently reported that alkaline phosphatase from calf intestinal mucosa catalysed the synthesis of phosphate esters by direct transfer of the phosphate group from phosphomonoesters to suitable hydroxy compounds. He also used serine, threonine, and ethanolamine as acceptors with some presumptive evidence of a positive result. However, he had no authentic marker compounds for comparison with the suspected phosphorylated components. Clear evidence for the formation of such compounds has now been obtained.

Radioactive glycerophosphate was used as donor. It was prepared according to Bublitz and Kennedy² using AT^{32}P and glycerokinase. A simplified method for assay of enzyme activity was used based on the observation of Ernster *et al.*³ Some radioactive inorganic phosphate formed in the reaction was separated on a Dowex-1 column.

In a series of experiments 10 mg of glycerophosphate, containing 0.46 mC ^{32}P , and 10 mg

of a preparation of alkaline bone phosphatase⁴ containing 420 Portmann⁵ units/mg N were incubated at pH 9.8 in a bicarbonate buffer for 10 min with 1 mmole of serine, threonine, glucosamine, or ethanolamine, respectively, in a total volume of 2 ml. After heating glycerophosphate was separated from the formed phosphorylated product by gradient elution on a Dowex-1 formate column⁶. The total radioactivity of the fractions measured in a L.K.B. Robot Scaler gave the following values in counts per minute:

	With enzyme	Without enzyme
Phosphorylserine	375 000	26 000
Phosphorylthreonine	26 000	460
Phosphorylglucosamine	67 000	41 000
Phosphorylethanolamine	912 000	150 000

Some interesting features are observed, the large proportion of phosphorylserine to phosphorylthreonine also found in hydrolysates of casein and animal phosphoproteins⁶. Of similar interest is the dominance of phosphorylethanolamine over phosphorylglucosamine also found in hydrolysates of the protein fraction from *E. coli*⁷. The significance of these observations with regard to possible ways for the formation of phosphoproteins is, however, unclear. The yield obtained in the enzymatic transphosphorylation was rather small, usually less than 1 μmole . The specific activity calculated as counts per minute per μg of phosphorus had about the same value for all compounds. In controls without enzyme smaller amounts of phosphorylated amino acids and amines were formed. Of interest for the present problem is the recent isolation of a free phosphorylated amino acid from extracts of *L. casei*⁸ together with some phosphopeptides.

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