THE SYNTHESIS OF NICOTINURIC ACID BY RAT KIDNEY MITOCHONDRIA

by

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Previous workers have shown that ingested nicotinic acid is excreted partly as the glycine conjugate, nicotinuric acid, in various animals including the rat and man[1,2,3]. In the present work the *in vitro* synthesis of nicotinuric acid by rat kidney has been demonstrated. The production of nicotinuric acid was observed by the chromatographic method of KODICEK AND REDDI[4] for tertiary pyridine derivatives, using water-saturated *n*-butanol as solvent.

Nicotinuric acid formation occurred when slices of rat kidney cortex were incubated with nicotinic acid and potassium fumarate. Homogenates of rat kidney cortex prepared in 0.154 *M* KCl using the apparatus described by POTTER AND ELVEHJEM[5] also formed nicotinuric acid in a medium supporting oxidative phosphorylation and containing both nicotinic acid and glycine. Addition to the homogenising medium of versene (final concentration, 1 mg per ml) as the potassium salt was found to stimulate nicotinuric acid production. After fractionation of homogenates, prepared in 0.25 *M* sucrose containing versene, by the method of SCHNEIDER[6], the nicotinuric acid synthesising activity was found to be located in the mitochondrial fraction.

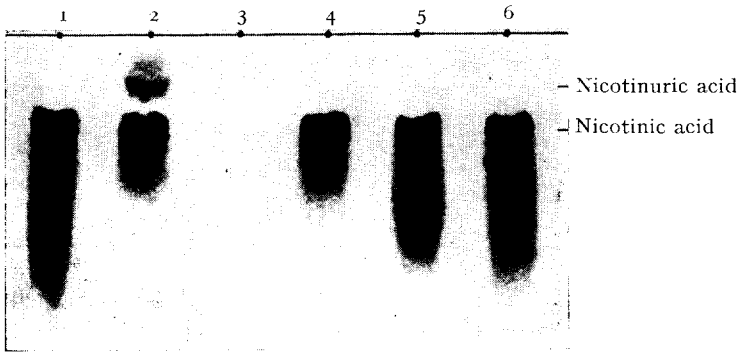

Fig. 1

Fig. 1 shows a series of experiments using a washed mitochondrial suspension incubated for 3 h under conditions supporting oxidative phosphorylation. There was no preformed nicotinuric acid present in the tissue preparation (exp. 1), but after incubation in the presence of both nicotinic acid and glycine a marked synthesis occurred (exp. 2). Omission of either nicotinic acid (exp. 3) or glycine (exp. 4) completely abolished the synthesis. The dependence of the synthesis upon oxidative phosphorylation was demonstrated in expts. 5 and 6, where no formation of nicotinuric acid occurred in the absence of a catalytic amount of ATP and in the presence of $2 \cdot 10^{-5}$ *M* dinitrophenol respectively.

It was found in further experiments that incubation under anaerobic conditions prevented formation of the conjugate. The system is fluoride sensitive, a concentration of 0.005 *M* KF being strongly inhibitory. The synthetic system appears to be stable as the amount of nicotinuric acid formed increased progressively for 6 h at 30° C.

The product of the reaction was chromatographically identified as nicotinuric acid. In two solvents (*n*-butanol-H_2O and 85% acetone-H_2O) it had the same R_F as an authentic sample of nicotinuric acid and in addition gave the same colour in the Konig reaction performed according to the method of KODICEK AND REDDI[4]. Furthermore when a sample of the compound was isolated chromatographically and hydrolysed with HCl, it was possible to identify both glycine and nicotinic acid in the hydrolysis mixture by chromatography.

The formation of nicotinuric acid has also been observed in preparations of rat liver.

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INTERACTION OF CREATINE WITH ADENOSINE PHOSPHATES

by

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Reactions involving the transfer of a phosphate group from creatine phosphate to an adenosine phosphate have been known since 1934, when LOHMANN[1] demonstrated the following reversible reaction in aqueous extracts of skeletal muscle:

$$2 \text{ creatine phosphate} + \text{AMP} \rightleftharpoons 2 \text{ creatine} + \text{ATP} \quad (1)$$

Later, it was suggested by LEHMANN[2] that the reaction takes place in two stages:

$$\text{creatine phosphate} + \text{ADP} \rightleftharpoons \text{creatine} + \text{ATP} \quad (2)$$

$$\text{creatine phosphate} + \text{AMP} \rightleftharpoons \text{creatine} + \text{ADP} \quad (3)$$

BANGA[3], assuming that two enzymes are concerned, endeavoured to isolate them. She failed to obtain an enzyme from mammalian muscle specific for reaction (3) but she isolated an enzyme promoting reaction (2), named ATP-creatine phosphopherase by her, or creatine phosphokinase by later authors. The purification and crystallisation of this enzyme has recently been reported by NODA, KUBY AND LARDY[4].

After the discovery of myokinase by COLOWICK AND KALCKAR[5], it was suggested (COLOWICK[6]) that there is no need to postulate an enzyme catalysing (3), reaction (1) being accounted for by the occurrence of reaction (2) in conjunction with the myokinase reaction:

$$2 \text{ ADP} \rightleftharpoons \text{ATP} + \text{AMP} \quad (4)$$

However, there is as yet no experimental proof of this.

In the present work evidence has been obtained for the view that reactions (2) and (4), rather than (2) and (3) are concerned in the dephosphorylation of creatine phosphate. The method used to follow such reactions employs a modification of KORNBERG'S[7] assay procedure for ATP. In the presence of glucose and a crude preparation of yeast hexokinase[8] any ATP formed gives rise to glucose-6-phosphate. This is oxidised by glucose-6-phosphate dehydrogenase present in the yeast preparation with simultaneous reduction of TPN. The rate of reduction of TPN is measured by observing the rate of change of optical density in the spectrophotometer at 340 mμ. The detection of reaction (3) depends on coupling it with a reaction by which ADP is converted to ATP, either by (2) or by (4). In Fig. 1, curve II shows that there is no reduction of TPN when creatine phosphate is added to the reaction mixture containing highly diluted rat muscle homogenate (final dilution, 1 in 20,000), AMP and the yeast enzyme system. When a small amount of ADP is added (Fig. 1, curve II) there is a rapid reduction of TPN which is mainly due to reaction (2), since a control with no added creatine phosphate shows that the myokinase reaction (4) is suppressed under these conditions (Fig. 1, curve I). If reaction (3) had taken place, the ADP formed would have been available for further phosphorylation to ATP in reaction (2) and reduction of TPN would have resulted. It follows that no phosphorylation of AMP by creatine phosphate was demonstrable.

In a second experiment (Fig. 2), the effect of high concentrations of AMP on reaction (4) was counteracted by enriching the system in myokinase. No reduction of TPN occurred when creatine phosphate was added to the reaction mixture containing AMP, added myokinase, muscle homogenate and the yeast enzyme system. The addition of a small amount of ADP produced reduction of TPN and it is therefore to be concluded that reactions leading to the formation of ATP from ADP can occur under these conditions. Thus ADP arising from reaction (3) would have been detected. This experiment supports the view that there is no phosphorylation of AMP by creatine phosphate.