Nucleotide Metabolism in the Surviving Rat Diaphragm

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The metabolism of acid-soluble nucleotides in the isolated rat diaphragm has been studied by aid of ion exchange chromatography on Dowex-1 Resin according to the method of Hurlbert *et al.*[1]. 9 major compounds have been isolated, the predominant nucleotides being AMP, ADP, ATP and DPN. In incubation experiments the interconversion of nucleotides within the diaphragm and the liberation of nucleotides to the medium have been studied. Experiments under aerobic and anaerobic conditions have been performed. The effect of 2,4-dinitrophenol, insulin and adrenaline on nucleotide metabolism has been studied. The mechanism of glucose uptake by muscle is discussed.

1. Hurlbert, R. B., Schmitz, H., Brumm, A. F. and Potter, V. R. *J. Biol. Chem.* **209** (1954) 23.

The Interaction of Rat Muscle Hexokinase with the Mg-ATP Complex

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In experiments on partly purified rat muscle hexokinase the role of Mg^{2+} and ATP on the enzyme activity have been studied. The effects of cations, anions, inorganic pyrophosphate, ITP, UTP, GTP and CTP on the rate of the reaction have been investigated. Indication of the nature of the Mg-ATP complex with hexokinase has been obtained.

The Breakdown of Adenosine Triphosphate in Human Blood

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Using an enzymic spectrophotometric method elaborated according to Kalckar's principles, the concentrations of the adenosine mononucleotides ATP, ADP and AMP were followed in shed human blood during standing.

In freshly withdrawn blood the total concentration of these three nucleotides was about 600 μmole per liter of blood; 85 % of this was ATP. The amount of ADP was higher than AMP. During storage at 37° C the ATP decreased gradually and had disappeared in 24 hours. ADP and AMP showed an intermittent 2—5 fold increase compared to the initial concentration. During the 24 hours storage the concentration of hypoxanthine and xanthine increased about 100 fold from 5 to 500 μmole/l. The total molar sum of the adenine nucleotides, hypoxanthine and xanthine, was nearly constant during the whole period.

In mechanically haemolysed blood the ATP disappeared completely within 4 hours. In the same period the ADP and AMP increased about 3 and 10 fold, respectively, and disappeared from the blood within 8 hours. The concentration of hypoxanthine and xanthine showed a concomitant increase from 5 to 500 μmole/l. In this case also, the sum of all the measured compounds remained constant.

By adding ATP, AMP and IMP (inosinic acid) to blood these nucleotides were degraded into hypoxanthine with increasing velocity in the mentioned sequence. Adenosine and inosine were transformed with greater but uniform velocity.

The breakdown of added ATP in whole blood takes place extracellularly and consists of a stepwise dephosphorylation. From the course of the curves for ATP, ADP and AMP in the plasma of the whole blood, it is concluded that the splitting off of all three phosphate groups is catalysed by one and the same enzyme mainly localised on the blood cells.

In plasma without blood corpuscles the breakdown of ATP takes place with a much smaller velocity than in whole blood.

The breakdown of AMP and IMP is initiated by a dephosphorylation.

Hypoxanthine formation in plasma takes place with equal velocity from added AMP, IMP, adenosine and inosine. It is therefore concluded that the splitting of inosine is the velocity determining reaction.