

Using 150 mg cells/ml suspension and 5 μ mole glucose/ml only a transient change between ATP and ADP is observed. If a more diluted suspension and the same glucose concentration is used, the cells initially loose up to 60 % of their adenine nucleotides as described in the following experiment.

In a cell suspension containing 45 mg cells/ml (55 mM phosphate, Locke's solution pH 7.35) the addition of glucose (5 μ mole/ml) gives rise during the first 60 sec to a fall in ATP of about 60 % of its initial value and a corresponding rise in the ADP concentration. In the following 3 min, however, the ADP almost disappears from the cell, while the ATP concentration is not changed, resulting in a 60 % decrease in the total concentration of the adenine nucleotides.

During the disappearance of ADP a corresponding amount of decomposition products (hypoxanthine, inosine and adenosine) is observed to accumulate in the medium.

In the following 50 to 60 min the hypoxanthine, inosine and adenosine disappear from the medium and during this period the lost adenine nucleotides are resynthesized and the size of the adenine nucleotide pool restored to its initial value.

From these experiments it appears, therefore, that the amount of glucose available per cell is of great importance for evaluating its effect on the adenine nucleotide level in Ehrlich cells.

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Studies on Active Calcium Transport in Mitochondria

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Addition of calcium ions to a mitochondrial suspension results in a number of changes; activation of ATPase activity, uncoupling,

swelling, abolition of permeability barriers, and loss of mitochondrial nucleotides and other substances. By using small calcium additions, 50–200 μ A/L, and short time spans, the effect of calcium can be studied in unaltered mitochondria without the complication of secondary changes. Under these conditions similar changes are observed as after small ADP additions: a burst of respiration, a cycle of oxidation and reduction of DPN (pyridine nucleotide) and other respiratory carriers. This cycle can be repeated a few times until carriers stay oxidized, swelling sets in, and the mitochondria are completely uncoupled.

Data are presented to show that in a complete system containing an oxidizable substrate, small additions of calcium ions marked with ^{47}Ca result in a complete uptake within 20–30 sec at 20°. In the absence of a substrate the uptake is smaller. Uncoupling either by aging or by uncoupling agents like 2,4-dinitrophenol greatly diminish the uptake. Azide and cyanide also inhibit calcium uptake. Magnesium has no influence on the uptake.

In a medium containing ATP but no substrate ATPase activity after small calcium additions was followed by inorganic phosphate estimations. The ATPase activity was found to be triphasic: first a high activity during the calcium uptake, then a leveling off or cessation of activity, and finally an accelerating rate of ATPase activity. With large calcium additions the high ATPase activity in the third phase will completely overlap the preceding phases.

The calcium uptake was found to be accompanied by the building up of a pH-gradient between the mitochondria and the medium, a pH-drop being recorded in the medium. When swelling sets in, the pH-gradient disappears.

These results are interpreted as indicating the presence in mitochondria of a calcium transporting mechanism. The bearing of these results on the mechanism of calcium induced aging is discussed.

In this study rat liver mitochondria were used but a similar mechanism was found to operate in other mitochondria tested, e.g. rat kidney and pigeon heart mitochondria.

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