corresponded to an uptake of water by the mitochondria.

This swelling in hypotonic solutions could be reversed by the addition of low concentrations of ATP (maximum rate with 0·004 *M* ATP), as is illustrated in Fig. 1. The marked increase in the optical density indicates a reversal of swelling, and simultaneous estimations showed that in such experiments the water content of the sedimented mitochondrial pellet had fallen from 90 to 80 per cent. Increasing the potassium chloride concentration also caused some reversal of swelling in hypotonic conditions, but in this respect potassium chloride was much less efficient than ATP in solutions showing an identical depression of freezing point. The ATP effect appeared to be specific for the following reasons.

(1) Adenosine diphosphate (ADP) in concentrations similar to those of ATP which gave a marked reversal of swelling produced only a slow reversal (Fig. 1), as could be expected by its conversion into ATP by the action of the small amounts of myokinase in the mitochondrial preparation.

(2) In the presence of ADP and creatine phosphate slow reversal was obtained, but as Fig. 1 shows the effect was much increased by the addition of an active creatine phosphokinase preparation.

Inosine triphosphate reversed the swelling at concentrations similar to those at which ATP was effective.

The inhibition of the swelling of liver mitochondria, but not its reversal, has recently been independently reported by Raaflaub[12] using ATP, and by Macfarlane and Spencer[6], who achieved inhibition by active metabolism in the presence of adenylic acid.

Preliminary studies indicate that there is a correlation between the effects of DNP on the activation of the mitochondrial ATPase and its effect on the reversal of swelling induced by ATP. For example, 1×10^{-4} *M* DNP fully activates the ATPase and completely inhibits the reversal of swelling induced by 0·0016 *M* ATP. At lower DNP concentrations the activation of the mitochondrial ATPase and the inhibition of the ATP-induced reversal of swelling are proportionally lower.

These results support the view that ATP may have a special role in maintaining the water balance of the mitochondrion.

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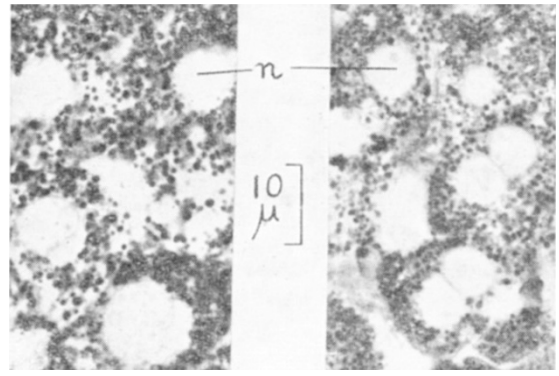
Cytological Fixation of Mitochondria by Acids

Certain acids have been found surprisingly effective for the cytological fixation of mitochondria in various tissues from mice. In some cases, 0·1 *N* hydrochloric acid has proved almost as satisfactory as Altmann's or Helly's fluid (see Fig. 1). Usually, small pieces of tissue are fixed for 18–20 hr. in the acid, washed for two or more hours in water, dehydrated, cleared and embedded in paraffin. The washing does not appear to be essential. Tissues may be dehydrated directly from the acid, starting with 50 per cent ethanol. Sections cut at 3 μ are stained by either Hirschler's hæmatoxylin or Metzner's acid fuchsin technique. With the latter, differentiation is so rapid that it is preferable to use a reagent containing half the usual amount of picric acid. With the hæmatoxylin method, staining is not always uniform. After hydrochloric acid fixation, the mitochondria may be demonstrated in the cells of the liver, intestinal epithelium, renal tubules and pancreatic acini. Other tissues have not yet been studied. The best results have been obtained with liver, the least satisfactory with pancreas. Nitric or sulphuric acid may be used in place of hydrochloric, but the fixation is not quite so good. Chromic, phosphoric, formic, acetic, oxalic and citric acids, potassium dihydrogen phosphate and potassium hydrogen phthalate are unsatisfactory. After acid fixation, there appears to be no advantage in post-chroming the above tissues except, possibly, the pancreas. In the case of hydrochloric acid at least, the results are not improved by making the solution isotonic.



(*A*) (*B*)

Fig. 1. Photomicrographs of mouse livers fixed in 0·1 *N* hydrochloric acid (*A*) and in Altmann's fluid (*B*). The mitochondria have been stained with acid fuchsin by Metzner's method (*n*, nuclei)

It is not suggested that hydrochloric acid will replace the familiar mitochondrial fixatives; but it is thought that the resistance of the mitochondria in some cells to certain acids should be made known. These observations also further illustrate the importance of the anion in the fixation of mitochondria, as noted by Zirkle[1].

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