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The Isolation of Morphologically Intact Mitochondria from Rat Liver.*

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(Introduced by J. B. Murphy.)
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In previous communications it was reported that the respiratory enzyme systems, cytochrome oxidase and succinoxidase, were associated, probably exclusively, with the "large granule" fraction isolated by differential centrifugation either from water homogenates[1] or from saline extracts[2] of rat liver. The identification of the components making up the large granule fraction presented an important and difficult problem. Microscopical examination of the fraction prepared in isotonic saline revealed the presence of spherical bodies ranging between 0.5 to 2 μ in diameter. The corresponding preparation obtained from a water homogenate of liver contained much larger and paler spheres. It was suggested, mainly on the basis of a comparison of the size of the isolated granules with that of known cellular inclusions, that the large granule fraction consisted mostly of mitochondria.[2,3] It was realized, however, that this evidence was not conclusive proof for the hypothesis that large granules were mitochondria, since the isolated large granules did not possess the generally accepted properties of mitochondria, namely, a rod-like shape and the ability to stain vitally with Janus green.

Subsequent investigations showed that isotonic saline solutions were undesirable media for reasons other than the above cytological considerations, in that sodium chloride, as well as certain other electrolytes, caused agglutination of large granules. This agglutination was reflected in the finding that irregular and usually great amounts of succinoxidase activity were sedimented during the removal, by low-speed centrifugation, of nuclei from saline homogenates. When water was used as the medium, no agglutination was visible, and the loss of succinoxidase in the nuclear fraction was much less.

The question whether the respiratory enzymes are actually associated with mitochondria was thus resolved into the search for a medium that would preserve the morphological and cytological characteristics of mitochondria and permit their isolation in good yields. It was found initially that agglutination did not occur in homogenates prepared in isotonic sucrose (0.25 M). Further studies of the effect of sucrose at different concentrations led to the surprising observation that as the concentration in a series of homogenates was increased, an increasing proportion of the large granules were distinctly rod-like in shape, until at a concentration of 0.80-1.0 M sucrose they could not be distinguished morphologically from the mitochondria of the living cell.

The isolation of morphologically intact mitochondria was effected as follows. Rat liver was homogenized in an all-glass apparatus[4] in 0.88 M sucrose (final liver concentration was 1.0 g in 10 ml homogenate). Free nuclei and residual intact cells were first removed completely by centrifuging the homogenate 3 times at 600 $\times$ gravity for 10 minutes. Centrifugation of the supernatant for 20 minutes at 24,000 $\times$ gravity resulted in sedimentation of all the free mitochondria, together with a small number of "microsomes."[3] The latter remained in the supernatant when the mitochondria were re-

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1 Schneider, W. C., *J. Biol. Chem.*, 1946, **165**, 585.

2 Hogeboom, G. H., Claude, A., and Hotchkiss, R. D., *J. Biol. Chem.*, 1946, **165**, 615.

3 Claude, A., *J. Exp. Med.*, 1946, **84**, 51, 61.

4 Potter, V. R., and Elvehjem, C. A., *J. Biol. Chem.*, 1936, **114**, 495.

suspended in 0.88 M sucrose and resedimented at 24,000 × gravity. Resuspension of this final sediment in 0.88 M sucrose yielded a distinctly yellowish preparation that showed pronounced birefringence of flow and was made up of mitochondria that had retained their original rod-like shape. The washed mitochondria were readily stained with Janus green at a dye concentration of 1/20,000, perceptibly stained at a dye concentration of 1/40,000, and remained morphologically stable in 0.88 M sucrose over a period of several days when kept at 4°C. No extraneous elements could be seen either in preparations stained with Janus green or in preparations fixed with osmium tetroxide and examined at high magnification in the electron microscope. In several experiments, the suspensions of washed mitochondria isolated by this procedure were found to contain 70 to 80% of the succinoxidase activity of the original liver homogenate, the remainder of the enzyme activity being present in the mixture of nuclei and unbroken cells sedimented by the preliminary low-speed centrifugations.

Of some interest is the fact that results obtained with rat kidney homogenates in sucrose solutions paralleled those described above for liver. Furthermore, the morphological alteration of mitochondria within unbroken liver or kidney cells present in homogenates, a phenomenon that occurred very rapidly in isotonic saline or 0.25 M sucrose, was progressively delayed as the concentration of sucrose was increased. In 0.88 M sucrose homogenates, the unbroken cells retained a normal appearance for hours. A possible explanation for the latter finding and for the preservation by concentrated sucrose solutions of mitochondria freed by cell rupture is that the intracellular osmotic pressure at the mitochondrial membrane may be considerably higher than the blood osmotic pressure.

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Blood Picture of Adult Gold Hamster (*Cricetus auratus*) After Castration.*

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It has been shown that gonadal hormones influence the blood picture in different species of vertebrates, the males having a higher number of corpuscles and greater hemoglobin values than the females. Steinglass *et al.*[1] showed a decrease in red blood count and hemoglobin value in castrated male rats; after testosterone administration the values return to normal. Vollmer *et al.*[2] claim that androgen raises the red blood count not only in castrated rats but in hypophysectomized and in normal animals.

Stein and Carrier[3] reported an appreciable drop (25 to 30% of the normal value) in the red blood cells of the "gold hamster" after castration, a decrease of red cell volume and an increase of mean corpuscular hemoglobin content, all of which disappeared after administration of iron, liver extract and testosterone propionate in periods as short as 3 days.

These results interested both the Hematology and Endocrinology Departments of our Institute and an investigation was planned to repeat this study.

Methods. Thirty-three adult male ham-

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1 Steinglass, P., Gordon, A. S., and Charipper, H. A., PROC. SOC. EXP. BIOL. AND MED., 1941, **48**, 169.

2 Vollmer, E. P., Gordon, A. S., Levenstein, I., and Charipper, H. A., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 409.

3 Stein, K. F., and Carrier, E., PROC. SOC. EXP. BIOL. AND MED., 1945, **60**, 313.