This suggests the correlation of this enzyme to the testosterone.

Cytochemical studies on the effect of testosterone[6] on the distribution of this enzyme, and a comparison of the activity in mitochondria in the skeletal muscle of mice normal and dystrophic will follow[7].

*Résumé.* En microscopie électronique l'etuee de la carnitine acétyltransférase a montré une même localisation dans les mitochondries du foie et du muscle cardiaque de souris. La grande différence d'activité que décèlent les méthodes biochimiques entre ces deux types de mitochondries peut donc être attribuée simplement à la présence 'und nombre beaucoup plus grand de crêtes dans les mitochondries du muscle cardiaque.

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## Ultrastructural Localization of Arylsulfatase B in Mitochondria of Epithelial Cells of the Proximal Convoluted Tubules of the Rat Kidney

This communication describes the previously unpublished fine structural localization of arylsulfatase activity in the mitochondrion. The physiological functions of arylsulfatase are unknown at present[1] but arylsulfatase is regarded as a lysosomal enzyme. It is similar to acid phosphatase in several aspects, such as activation by osmotic pressure, sodium chloride, mechanical disruption, freezing and thawing or by thermal activation[2]. At the electron microscope level, arylsulfatase has been reported only in the lysosome or in the lysosomal granule in the thyroid follicular cell[3], intestinal mucosal cell[4], pulmonary alveolar cell[5], metrial gland cell[6] and the epithelial cell of kidney convoluted tubules[7–11].

Biochemical analysis of the intracellular distribution of sulfatase, however, suggests that the mitochondrial fraction contains as high as 50% of the total arylsulfatase activity in the rat liver cell[12]. In an attempt to obtain the mitochondrial localization of arylsulfatase, tissue was dissected from rats perfused with lactated Ringer's solution (Abbott) for a few min and 2% glutaraldehyde in 0.1 *M* sodium cacodylate buffer (pH 7.4) containing 5% dimethyl sulfoxide (DMSO) for several min. DMSO increases the osmolarity of the fixative and accelerates its penetration into the tissue[13,14]. After additional perfusion of the lactated Ringer's solution to wash out the fixative, unfrozen sections, about 40 µm in thickness, were cut on a Sorvall tissue sectioner. They were then incubated for 5 to 30 min at either 4 °C, 22 °C or 37 °C in the Goldfisher's medium[7] as modified by HOPSU et al.[9], which contains *p*-nitrocatechol sulfate (Sigma) as substrate (23 m*M*) and lead nitrate (48 m*M*) as capturing reagent in 0.1 *M* veronal acetate buffer (pH 5.5). HOPSU et al.[9] recommended barium chloride instead of lead nitrate as the capturing reagent but we employed lead nitrate to avoid the treatment with ammonium sulfide for visualization[10]. Incubated sections were washed thoroughly in the same buffer until the yellowish stain from the incubation medium was practically removed. This was followed by refixation in the buffered glutaraldehyde for an additional 30 min. After post-fixation in buffered 2% osmium tetroxide for 40 to 60 min, the sections were dehydrated through a graded series of alcohol for a total of 60 min. Sections were embedded in Epon 812 without staining and then re-examined after staining with lead hydroxide (13 min) and uranyl acetate (1 min) to enhance the contrast[15]. A number of sections inactivated by heating for 3 min or incubated in substrate-free medium were examined as controls. The optimal results were obtained with sections incubated for 30 min at 22 °C in a medium of pH 5.5.

In addition to localization within the lysosomes reaction product was found in the space between the inner and outer membranes and the intracristal space of the mitochondria. Every mitochondria of the cell showed similar reactions without the marked heterogeneity (Figure 1) that is seen with the activity of succinic dehydrogenase[16]. At higher magnification (Figure 2) the deposit of lead precipitate in the submitochondrial space was not uniform. An irregular distribution pattern of reaction product in the intra cristal space is in common with that of other enzymes in mitochondria. It may reflect the functional heterogeneity of the substructure but is more probably due to such factors as the irregular penetration of the incubation medium. The relation of the reaction sites to the membrane structure

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was not evident because the reaction product was diffused in the space between the mitochondrial membranes. The matrix of the mitochondria was devoid of reaction product. In some mitochondria the reaction product, which appeared to accumulate at the outer surface of the mitochondria, may migrate to the mitochondria from the neighboring ruptured lysosomes.

The reaction in the intra cristal space could not be identified in some cells (Figure 3) nor in the mitochondria of the majority of liver cells of the animal. The reaction both in the mitochondria and the lysosomes was not present in the control sections. The nuclear envelope and endoplasmic reticulum also contained lead precipitate (Figure 3) while the Golgi complex, microbodies (peroxisomes) were devoid of reaction product. In respect to this observation, it is interesting that acid phosphatase, the typical lysosomal enzyme, has been demonstrated in part of the Golgi complex. The endoplasmic reticulum of the microsomal fraction is one of the presumed locations of the arylsulfatases. POKROVSKII et al.[17] reported that arylsulfatase is localized in the nuclear membrane.

According to ROY[1] the intracellular distribution of sulfatase in rat liver cells is 15% in the nuclei, 50% in the mitochondria, 22% in the microsomes and 14% in the soluble fraction, which indicates that the majority of the sulfatases are of mitochondrial origin. However others have reported that as much as 70% of the total activity is present in the microsomes. ROY attributes this discrepancy to his use of a different substrate *p*-nitrocatechol sulphate (2-hydroxy-5-nitrophenyl-sulphate) which was employed in the present study instead of potassium *β*-acetylphenyl sulphate.

In general the liver is the organ richest in arylsulfatases, but considerable amounts also occur in the kidney, pancreas and adrenals[1]. Mitochondrial arylsulfatase, however, was more evident in the kidney rather than in the liver. This probably could be attributed to different affinities[18] of various mitochondrial components of the liver and kidney in precipitating the lead rather than a reflection of the amount of total activity.

Previously published electron micrographs of arylsulfatase in the convoluted tubules of the kidney demon-

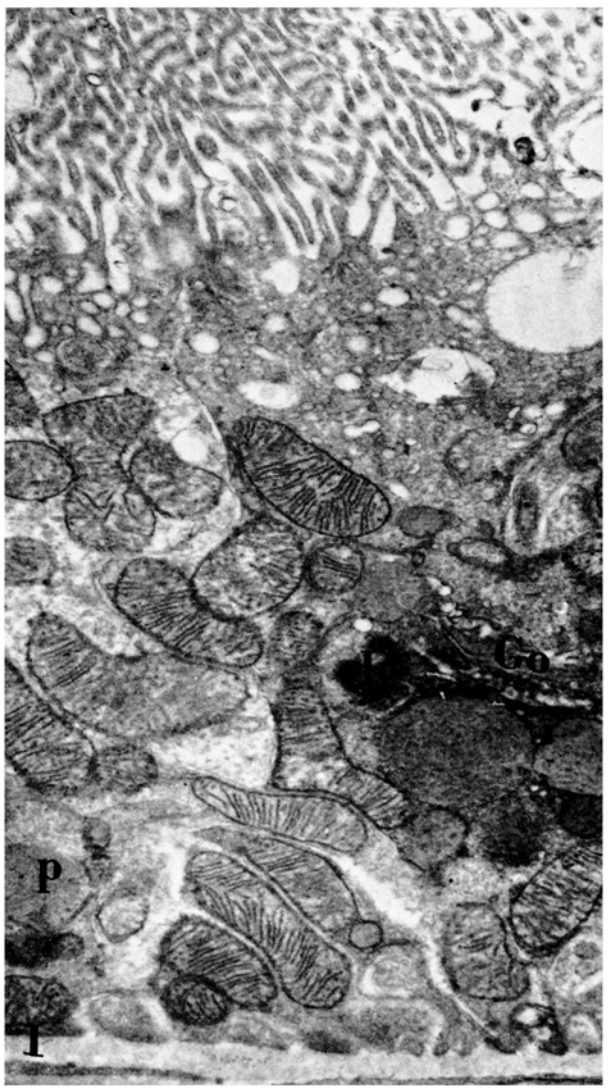


Fig. 1. Arylsulfatase B in the mitochondrion of the epithelium cell of convoluted tubules of the rat kidney. Lysosomes (ly) and mitochondria have reaction product while the Golgi (Go) complex and peroxisomes (P) are devoid of activity. ×17,000.

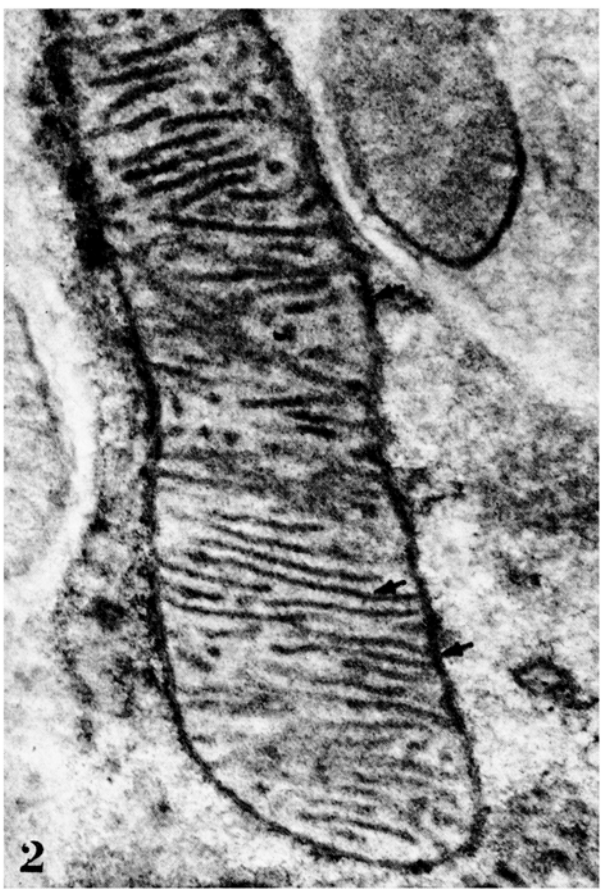


Fig. 2. A higher magnification of the mitochondrion. The final product of arylsulfatase is in the space between the inner and outer membrane and the intracristal space (arrows). ×66,500.

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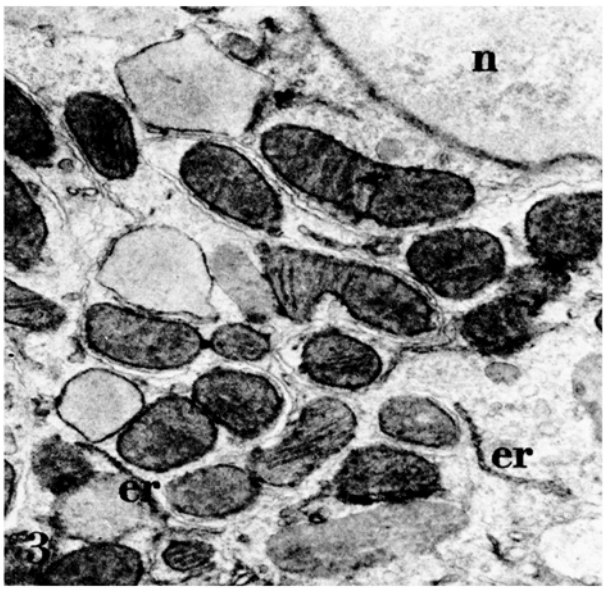


Fig. 3. The nuclear (N) envelope and the sacs of the endoplasmic reticulum (er) also contain reaction product in some cells. The reaction in the intracristal space of mitochondria (arrow) is not visible in some cells. ×20,000.

strate only lysosomal location of reaction product[7-11]. The reason for this discrepancy is not yet obvious but it may be due, in part, to the use of a minimal perfusion time for fixation and the addition of DMSO to the fixative. HOPSU et al.[9] report that prolonged fixation cut down about 22% of the activity in the kidney, 12% in the liver, 18% in the spleen and 8% in the brain.

The addition of DMSO has been reported to activate the acid phosphatase[19]. The solution of DMSO-glutaraldehyde, which was employed here, might result in either acceleration of the flow of medium into the mitochondria or activation of the enzyme. Denaturation of the membrane of the mitochondria as well as the limiting membrane of the lysosome by DMSO might play a role in these effects.

The cytochemical finding of arylsulfatase activity in mitochondria encourages study on the similar localization of acid phosphatase, the intracellular location of which has been restricted to lysosomes and related structures such as part of the Golgi complex. The different location of arylsulfatases in lysosomes and the dual incubations[11] for arylsulfatase and acid phosphatase indicate the heterogeneity of lysosomes in regard to their enzyme content[7]. Such heterogeneous distribution of arylsulfatase in the lysosome is in contrast to that of the mitochondrion.

Arylsulfatases A and B (type II) in the mammalian liver cells can be distinguished from C (type I) by their substrate specificity, by strong phosphate and sulfate inhibition, and by their optimal pH. Arylsulfatase A, B and C have their optimal pH range about 4.2, 5.5 and 8.0 respectively[1].

At the electron microscope level, there was no noticeable difference between arylsulfatase of A and B over a wide range of pH (4.5–7.4)[9]. As for arylsulfatase C, which is presumed to be located mainly in the microsomal fraction in contrast to arylsulfatase A and B which are located both in the microsomal and the mitochondrial fractions, an attempt was made to replace lead nitrate with lead citrate as the capturing reagent, which resulted in a clear incubation medium in the higher pH range for alkaline phosphatase[20]. This is still in progress in our laboratory but so far we need further study on the substrate. However the occasional localization of arylsulfatase in the sacs of the endoplasmic reticulum is noticed[21-23].

*Résumé.* Des tissus de rats fixés par perfusion avec une solution de glutaraldéhyde et de DMSO ont permis de localiser le produit de réaction de l'arylsulfatase B dans les mitochondries ainsi que dans d'autres organites. Dans les mitochondries, l'arylsulfatase a été décelée entre les membranes externe et interne de même qu'à l'intérieur des crêtes. La matrice mitochondriale n'a donné aucune réaction.

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## Determination of the Sensitive Phase for Bristle Organ Modifications upon Injection of Mitomycin C into Larvae and Pupae of *Drosophila melanogaster*

The injection of Mitomycin C into the body cavity of third instar larvae of *Drosophila melanogaster* during cellular differentiation results in the formation of bristles without sockets and bracts on the head, thorax, wing, leg, and external genital organ of the adult fly[1]. However, no altered bristle organs could be found on the abdomen. Furthermore, it has been shown[2] that the treatment of foreleg imaginal disks of *D. melanogaster* with Mitomycin C in vitro and subsequent implantation into larval hosts results not only in the formation of bristles without sockets, but considerably reduces the number of bristle organs that differentiate. Therefore, the questions arose 1. whether or not the abdominal bristle organs have a different sensitive phase for the Mitomycin C effect than do all other bristle organs, and 2. whether the sensitive phase for reducing the number of bristle organs and for the loss of sockets on the integument are the same. The easily distinguishable large and small bristles of the thorax and head facilitated comparison between the sensitive phase for macrochaetae and microchaetae formation. The investigation of thousands of bristles after Mitomycin C injection also revealed structural modifica-