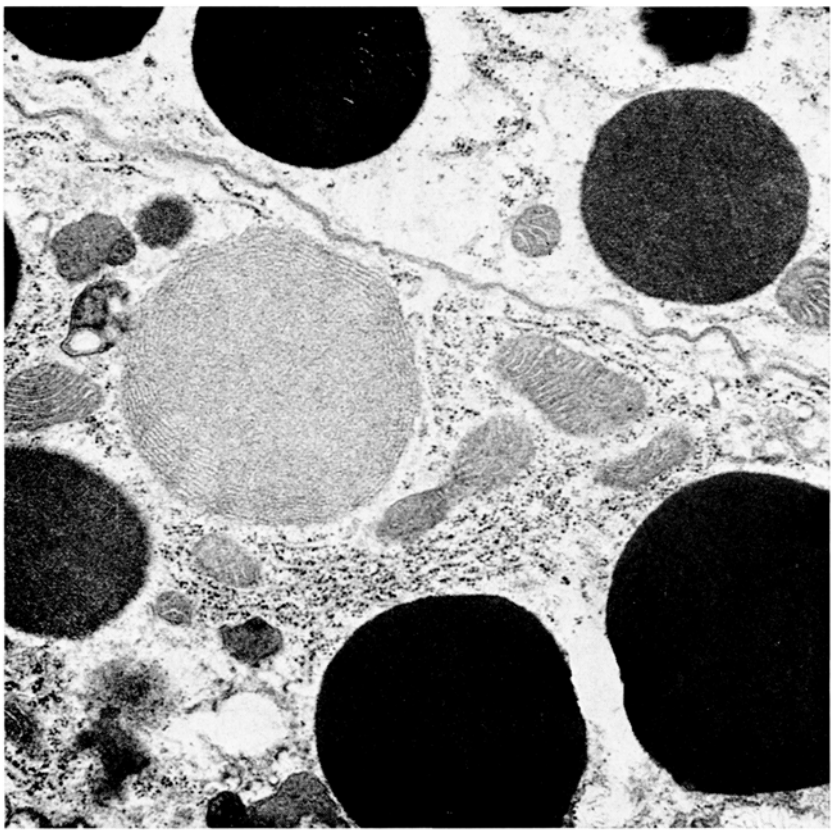

Fig. 2. Edge of salivary gland from the same animal, showing adipohaemocyte and electron-dense granules. ×20,700.

cases there was marked infiltration of the blood cells into the glandular tissue similar to the reactions shown by nerve-sectioned insects. Nymphs showed the greater haemocyte accumulation and infiltration response. The results obtained are summarized in the Table, and the extent of the reaction is roughly quantitated using the symbols − + ++ or +++.

Different types of cytoplasmic granules occurred in the haemocytes accumulated on the glands. Ultrastructural studies of the blood cells revealed irregularly-shaped mitochondria and many membrane-bound bodies containing characteristic structures (Figure 1). Granular bodies encroaching into the gland cells may represent haemocytic remnants[3] and probably parts of adipohaemocytes and spherulites[4] (Figure 2).

Discussion. The results indicate that the nymphs respond to injections of cell-free extracts or filtrates of tumourous tissues more readily and exhibit more positive reactions involving infiltration and invasion of the salivary glands than do adults, where the response, though present, is less well defined. The sensitivity of the salivary glands to such injections cannot yet be explained, but they are also the first organs to develop tumourous lesions after severance of the recurrent nerve[1,5].

Résumé. On a obtenu des tumeurs dans des blattes (*Periplaneta americana*) après la section du nerf récurrent. Des extraits ou des filtrats de ces tumeurs, exempts de cellules, ont provoqué des lésions semblables quand on les a injectés à des adultes ou des nymphes.

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A Comparison of Ultrastructural Localization of Carnitine Acetyltransferase Activity in Mouse Liver Mitochondria with that in Cardiac Muscle

This communication describes the ultrastructural localization of carnitine acetyltransferase activity in the mitochondria of mouse liver, which to our knowledge has not been previously reported, compared with the activity in the cardiac mitochondria.

This enzyme catalyses the reaction; Acetyl-CoA + Carnitine $\rightleftarrows$ Acetylcarnitine + CoA. The cytochemical method of HIGGINS and BARRNETT[1] utilizes the reducing property of the released SH group of free CoA to reduce potassium ferricyanide to potassium ferrocyanide, which

in the presence of uranyl ions, forms an electron-dense precipitate of uranyl ferrocyanide.

Another method utilizes the mercaptide-forming property of the free SH group of CoA, which forms a precipitate with cadmium ions. The first method was employed in the present investigation. It has been reported that the carnitine acetyltransferase activity in mitochondria from various tissues is highest in heart muscle and lowest in liver[2].

In an attempt to compare the cytochemical localization of the mitochondrial enzymes[3] in various tissues from the normal and the muscular dystrophic mouse, we found similar distribution of carnitine acetyltransferase between cardiac mitochondria and liver mitochondria.

Unfixed slices of cardiac muscle and liver from the mouse were incubated in the following medium: potassium ferricyanide, 5 mg, uranyl acetate 2.5 mg, acetyl CoA 2.0 mg, carnitine 4.0 mg, dextrose 100 mg, dissolved in a total of 2.5 ml of 0.05 M maleate buffer (pH 7.0).

Controls were preincubated for 5 min in 4×10^{-4} M mercuric chloride which has been reported to be an inhibitor[1,4]. Also the omission of either the acceptor or donor substrate from the incubation medium was employed as further controls. Some slices were briefly fixed in the glutaraldehyde (1%)-formaldehyde (4%) solution in 0.1 M cacodylate buffer (pH 7.4).

The optimal results were obtained in fresh slices incubated for 30 min at room temperature (22 °C). After incubation tissues were washed briefly with cold buffer and fixed in 4% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 30 min followed by post-fixation in 2% osmium tetroxide for an additional 60 min. Most mitochondria in mouse liver as well as those in cardiac muscle showed reaction product without marked heterogeneity amongst the mitochondria (Figure 1). Reaction product between the inner and outer mitochondrial membrane was more stable than in the intracristal space. Activity in the latter site was found only in the fresh tissue and not in the controls fixed in the glutaraldehyde-paraformaldehyde solution. Thin needle-like reaction product, uranyl-ferrocyanide, was located mainly along the outer surface of the inner membrane both in cardiac muscle (Figure 2) and in liver (Figure 3).

In the liver cell, the deposits of uranyl-ferrocyanide were occasionally found on the surface of the bile canaliculi and the endoplasmic reticulum. The mitochondria in the darker cell, adjacent to the lighter cell, were usually devoid of the reaction product (Figure 4). In the sections preincubated in mercuric chloride no activity

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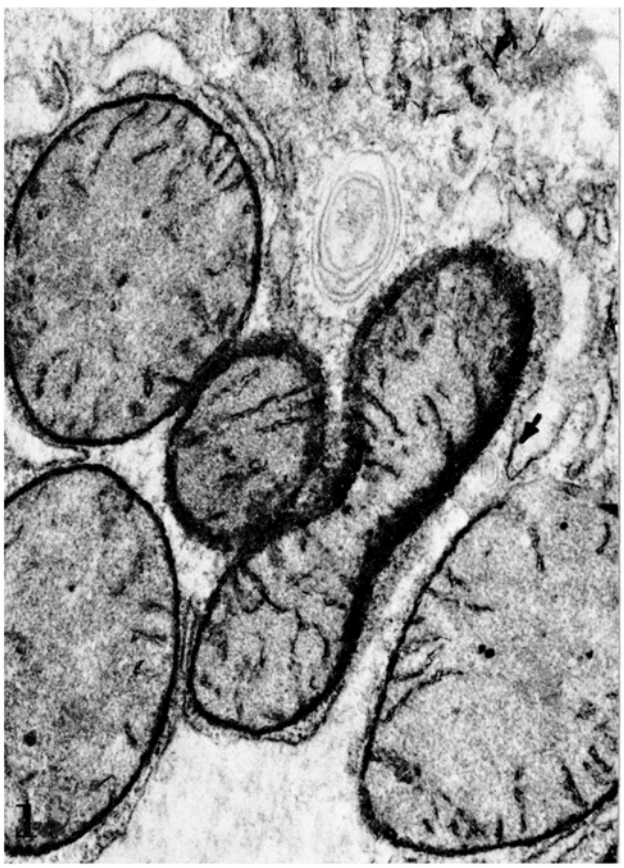


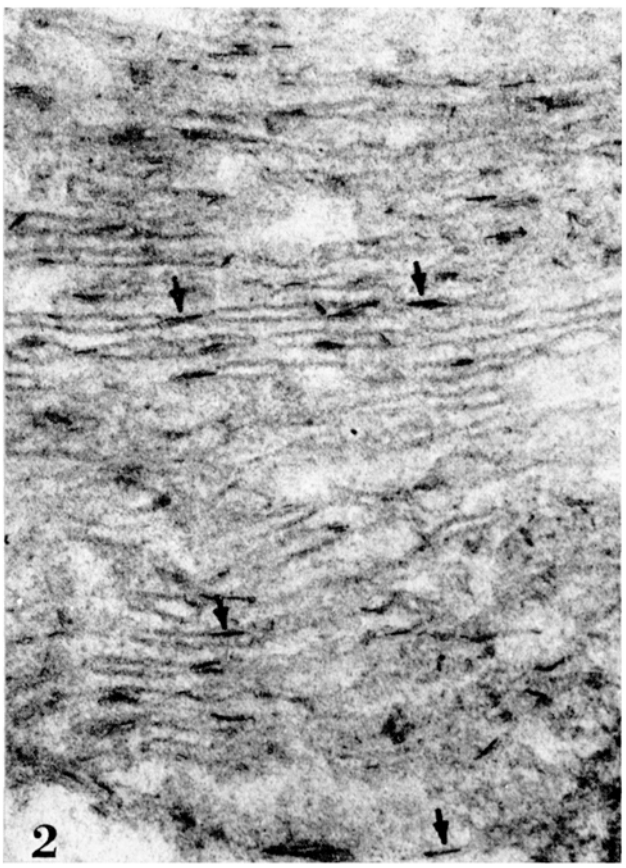


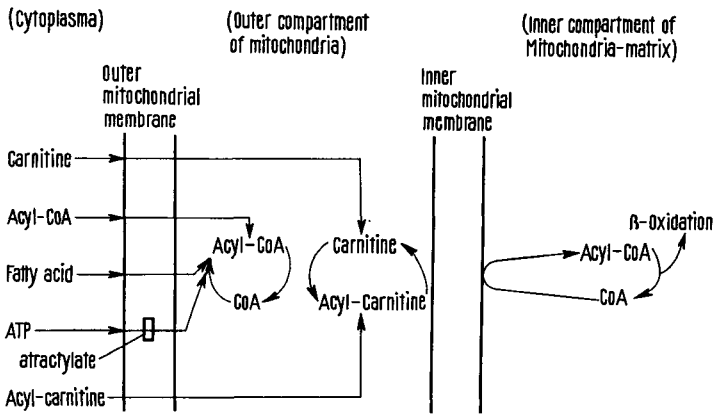
Fig. 1. The reaction product of the carnitine acetyltransferase, uranyl-ferrycianide, is distributed in all mitochondria in the mouse liver cell. Occasionally the reaction product is found in the surface of microvilli (arrow) and the endoplasmic reticulum (arrow).
Fig. 2. The cardiac mitochondrion which has more crests than the liver mitochondrion, has carnitine acetyltransferase activity along the outer surface of the inner membrane as well as the inner surface of the crests (arrows).

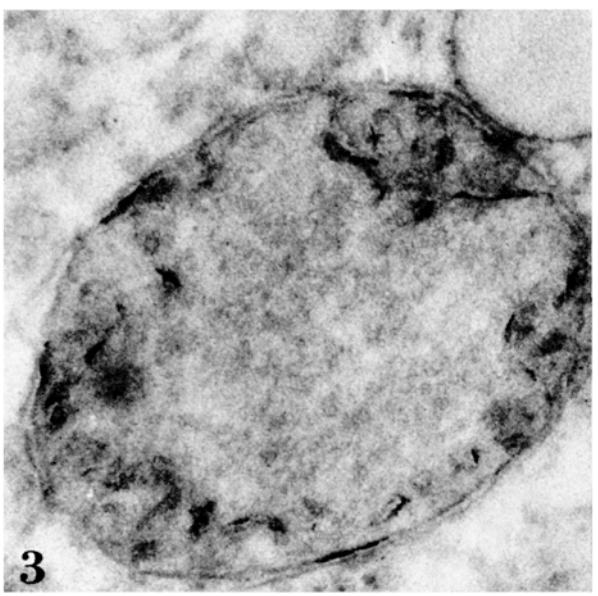
was found, nor in the sections incubated in the medium without carnitine and acetyl CoA. When carnitine alone was omitted only the intracristal reaction remained.

The activity of individual mitochondria appeared to be similar both in liver and cardiac muscle. The deposits were uniformly distributed along the membrane both on the inner surface of the cristae and on the outer surface of the inner mitochondrial membrane. The carnitine acetyltransferase plays a role in the transport of active acetate across the mitochondrial membrane[1,5].

The difference between the reaction product located in the intracristal space and the space between the outer- and the inner-mitochondrial membrane[1] suggests that the intracristal enzyme plays a role in Acetyl-CoA → CoA reaction. There is remarkable difference in the extent of activity amongst mitochondria from various tissues. Activity in mitochondria (μmoles/min/g protein) of cardiac muscle, skeletal muscle, kidney, brain, liver was reported to be 440, 410, 109, 12.3 and 5.1 respectively[2].

However we found cytochemical reaction of liver mitochondria to be similar to that of cardiac mitochondria. The difference in overall activity between liver mitochondria and heart mitochondria appears to be attributed to a marked difference in the number of crests between the two types of mitochondria. Enzyme activity in several tissues has been reported to be correlated with the amount of acetylcarnitine and carnitine except in the testis in which the enzyme activity is higher than the amount of carnitine and acetylcarnitine.





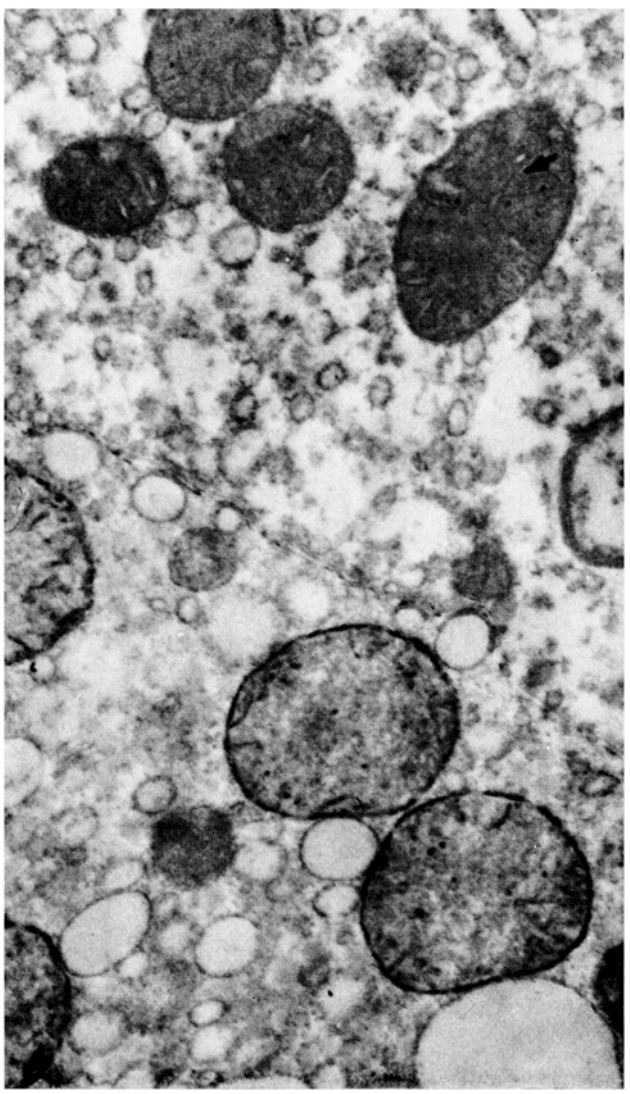


Fig. 3. A higher magnification of the liver mitochondrion shows distribution of enzyme activity similar to the cardiac mitochondria. Fig. 4. The mitochondria in the darker cells (arrow) are usually devoid of enzyme activity while those in the adjacent cell have reaction product.

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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