SUBCELLULAR LOCALIZATION OF CARNITINE ACETYLTRANSFERASE IN CELLS OF VARIOUS ORGANS OF INTACT AND STARVING RATS

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Carnitine acetyltransferase (CAT) is an enzyme involved in fatty acid metabolism. According to existing views, based chiefly on biochemical data, this enzyme, like the whole process of β-oxidation, is confined to the mitochondria [5, 6, 13]. Meanwhile ultrastructural investigations have shown a definite relationship of microbodies (peroxisomes) to cell lipid metabolism. This relationship is expressed as an increase in the number of these organelles in adipocytes [6], cells of the adrenal medulla [6, 8] and corpus luteum [8], and cells of other organs producing substances of lipid nature. The number of microbodies in cells of different organs rises considerably under the influence of hypolipidemic agents [15] and also during starvation [3] and in various pathological states accompanied by excessive accumulation of lipid metabolites in the blood and tissues [4, 6]. All the authors cited above have observed close structural associations of microbodies with mitochondria and with intracellular lipid inclusions.

Participation of microbodies in the utilization of intracellular lipids became particularly clear after biochemical studies which revealed CAT in the peroxisomal fraction of mammalian liver and kidneys [13]. Nevertheless, we have so far failed to find in the accessible literature any cytochemical data giving direct evidence of the presence of CAT in microbodies. Moreover, there are only scattered pieces of morphological evidence to show that this enzyme is located in the mitochondria.

The aim of the present investigation was accordingly to look for CAT by electron-histochemical methods in cells of various organs of animals in physiological and extremal states. Starvation was chosen as the extremal state, for at certain stages of it the energy metabolism of cells is maintained principally by endogenous lipids [7], and with the assumptions mentioned above, this ought to activate the participation of the corresponding peroxisomal enzymes in their oxidation.

EXPERIMENTAL METHOD

CAT activity was determined by the uranyl ferrocyanide method [10], with unfixed tissue placed in the incubation medium. The method is based on the reducing capacity of SH groups of coenzyme A, formed by interaction between carnitine and acyl radicals, and the conversion of ferricyanide into ferrocyanide in the presence of uranyl, with the formation of an electron-dense precipitate of uranyl ferrocyanide at sites of distribution of the enzyme. The myocardium and liver of three intact noninbred male albino rats weighing 250 g and of three rats on the 5th day of food deprivation, but with water allowed ad lib., were investigated. This enzyme also was demonstrated in one intact rat in a skeletal spinal muscle, and in one starving rat in the subcutaneous areolar tissue. The reaction for catalase, a marker enzyme of microbodies [12], was used as the control. Samples of tissue, after appropriate treatment, were embedded in all cases in a mixture of equal volumes of Araldite and Epon. The sections were not stained to determine enzymes. Parallel with the histochemical analysis, the material was treated by the standard method for electron microscopy. Sections cut on an LKB-8800 Ultrotome were examined under the JEM-7A electron microscope.

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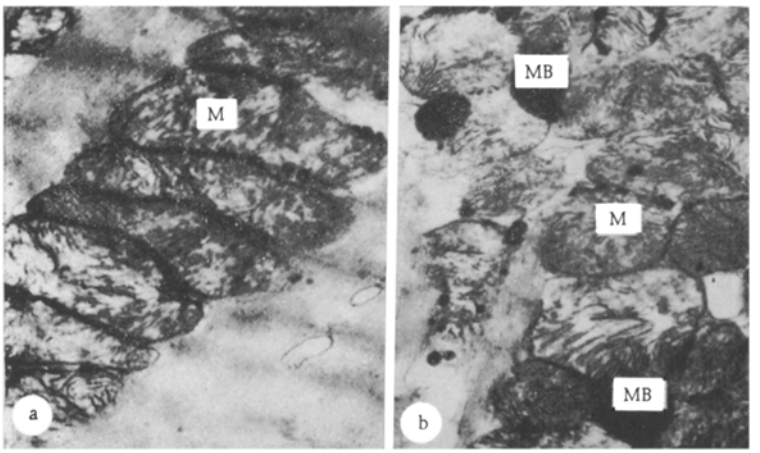

Fig. 1. Localization of CAT in cardiomyocytes of intact and starving rats. a) High CAT activity in mitochondria (M) of intact rat; b) high CAT activity in microbodies (MB) of starving rat. 25,000×.

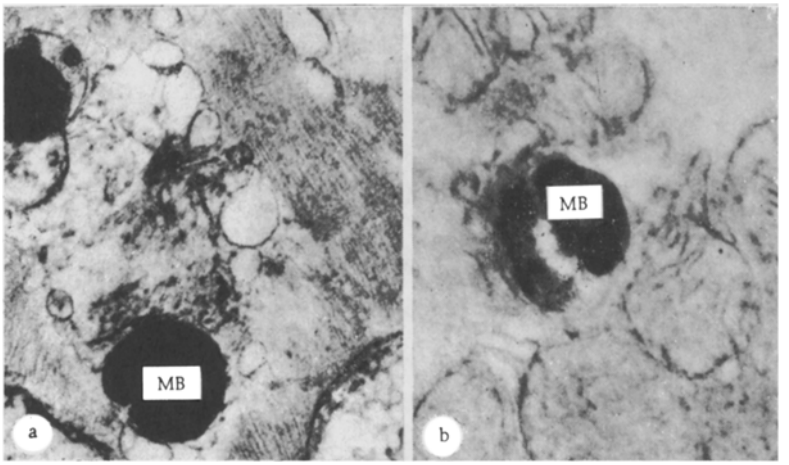

Fig. 2. Reaction of microbodies of starving rats for CAT (a) and catalase (b). 50,000×.

EXPERIMENTAL RESULTS

The electron-dense reaction product was identified clearly in mitochondria of contractile heart muscle cells of both intact and starving animals (Fig. 1).

It was located mainly along the surface of the mitochondria on the outer and inner membranes and less frequently on the cristae, a characteristic feature of incubation of unfixed tissue in the medium [10]. Frequently deposits of precipitate on the surface were bead-like. As a rule no enzyme was present in swollen mitochondria.

Besides mitochondria, an intense reaction for CAT was given in structures from 0.1 to 0.7 μ in diameter, round, oval, or irregular in shape (Fig. 1b), the smallest of which were concentrated near the surface of the mitochondria and always showed homogeneous osmiophilic staining. Larger structures also were concentrated near mitochondria, but they were found in other parts of the cell also. Electron-dense precipitate was not always uniformly distributed in them. Many of them contained one dense inclusion (nucleoid) or a somewhat granular and less electron-dense or translucent matrix. Under high power of the electron microscope a single surface membrane could be identified (Fig. 2a). These structures were microbodies, as shown by the similarity of their structure and size to structures containing catalase (Fig. 2b). Sometimes a positive reaction for the enzyme also was found in lysosome-like formations which were larger than microbodies. The electron-density of the microbodies after the histochemical reaction for CAT was fairly high even without additional con-

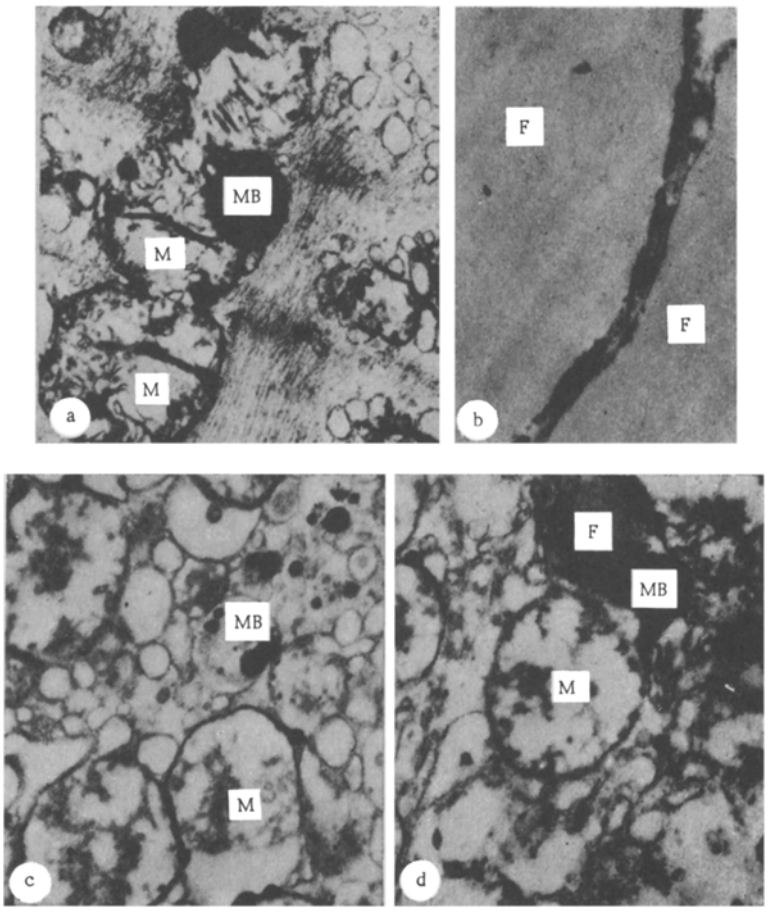

Fig. 3. Localization of CAT in skeletal muscle, fatty areolar tissue, and hepatocytes of rats. a) Fragment of skeletal muscle of an intact rat, 25,000 ×; b) fragments of adipocytes bounding fatty areolar tissue of a starving rat. F – fat. 20,000 ×; c) reaction for enzyme in mitochondria and microbodies of a starving rat; d) contact of microbody with lipid inclusion and mitochondria (starving rat). 50,000 ×.

trasting of the sections with salts of heavy metals, whereas without contrasting, and with standard treatment of the tissues with staining of the sections with lead and uranyl acetate, they could be identified with difficulty, and in uncontrasted preparations they were impossible to differentiate at all.

In cardiomyocytes of intact rats CAT was found mainly in mitochondria and adjacent small microbodies, whereas in starving animals they were found in large microbodies, the number of which was considerably increased. During starvation associations of microbodies with lipid inclusions of cardiomyocytes also could be observed more frequently than normally.

Skeletal muscle, as mentioned above, was studied for its CAT distribution only under physiological conditions. Just as in heart muscle, electron-dense precipitate was found in mitochondria and microbodies of the muscle fibers. Its topography in mitochondria of skeletal and heart muscle was similar: The dark reaction product was concentrated on the membranes and on the cristae of these organelles. In the microbodies a uniform distribution of precipitate was found over their whole area nearly always, and structures with multiple nucleoids were hardly ever found (Fig. 3a).

In the fatty areolar tissue of the adult rat adipocytes were almost entirely filled with fat and were essentially fat bodies of different sizes, surrounded by a tunica propria and cell membrane, tightly packed against each other. In sections through some of them, all the fat was preserved, whereas in others, in the course of its functional resorption, which was particularly marked in starving animals, and also in the course of solution in fixatives during treatment of the tissue, large empty vacuoles appeared. In membranes surrounding the lipid inclusions and vacuoles an intense reaction for CAT was found in all adipocytes during starvation (Fig. 3b).

In mitochondria of hepatocytes the localization of the reaction product was the same as in mitochondria of cardiomyocytes, but the quantity of enzyme present was much less, especially in starving animals. Meanwhile starvation increased the number of microbodies with an intense reaction for CAT in the hepatocytes. In their structure and size the microbodies were similar to those of cardiomyocytes, but in the liver microbodies with multiple round nucleoids were seen more often (Fig. 3c). The smallest microbodies were usually in close contact with the mitochondrial membrane and merged with it, whereas the larger ones were located near the mitochondria and in different zones of the cytoplasm. Contacts between microbodies and lipid inclusions were observed everywhere during starvation (Fig. 3d). Electron-dense material also was found in vesicles of the smooth endoplasmic reticulum, which showed signs of microvesicular transformation in the starving rats.

The results of electron-microscopic histochemical investigations thus demonstrate that CAT is located not only in mitochondria, but also in microbodies of cardiomyocytes, skeletal muscle fibers, and hepatocytes of both intact and starving rats, and also in the cell membranes of adipocytes during starvation. Since this enzyme participates in fatty acid metabolism, microbodies must therefore play a direct role in the oxidative catabolism of intracellular lipids, as the authors cited above postulated [4, 8, 9, 14] on the basis of an increase in the number of these organelles in cells with an increased lipid content, and of their interconnection with lipid inclusions in the cytoplasm, which we observed in the present investigation also.

The higher CAT activity in mitochondria of muscle tissue, especially heart muscle, compared with that in the mitochondria of hepatocytes was due not only to the high energy demands of the heart, but also, probably, to the fact that unlike in the liver, whose energy requirements under normal conditions are met primarily on account of carbohydrates, in muscle tissue energy processes are supported mainly by fatty acids [5].

The increase in the number of microbodies during starvation in the parenchymatous cells of the heart and liver is evidently due to an increase in the supply of endogenous fatty acids, which become the principal energy substrate with the cessation of utilization of exogenous carbohydrates [7] and inhibit oxidative phosphorylation in mitochondria [1]. Furthermore, because of the deficiency of plastic material, regeneration of the mitochondria is depressed. Under these conditions microbodies containing CAT which, in the modern view, are responsible for adaptive metabolic changes [2] and for utilization of excessive quantities of high-energy compounds in the cell [6], begin to play an important role in fatty acid oxidation.

The concrete mechanism of participation of CAT of microbodies in oxidative degradation of lipids is not yet clear. We know that in mitochondria this enzyme transports acyl radicals through the outer membrane inside the organelle, and these are then broken down to their end products in the Krebs' cycle [1, 5, 10]. It has been suggested that microbodies act as a storehouse for acyl radicals and coenzyme A [13]. Incompletely oxidized fragments of fatty acids were perhaps transported with the aid of CAT from microbodies into mitochondria [6], and in all probability this explains the close structural relationship between these organelles. However, the possibility cannot be ruled out that final degradation of fatty acids also takes place in microbodies [11]. Participation of microparticles in lipid oxidation, which is accompanied by hydrogen peroxide formation, explains why they contain not only CAT, but also glutathione peroxidase and catalase, which prevent the toxic action of this compound on the cell [6].

The discovery of CAT in the membranes of lipid inclusions and in the plasmalemma of adipocytes is in agreement with the biochemical data of Markwell et al. [13], who discovered this enzyme not only in mitochondria and microbodies, but also in the lipid-rich membrane fraction of liver and kidneys. It can be tentatively suggested that the role of CAT in fatty areolar tissue is to transport acyl radicals, formed during resorption of fat, through the adipocyte membrane into intracellular spaces to adjacent capillaries.

The positive reaction for CAT in different subcellular structures of various organs and tissues is evidence that oxidation of intracellular lipids with the aid of this enzyme is a widespread reaction in the body, and that its intensity increases in extremal situations.

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EFFECT OF SOME PESTICIDES CONTAINING CHLORINE ON HEMOLYTIC RESISTANCE AND ACETYLCHOLINESTERASE ACTIVITY OF ERYTHROCYTES

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Pesticides can affect the structure and biological activity of blood cells [1, 3, 7, 8, 10]. Erythrocytes are a convenient object with which to study the harmful action of pesticides on cells and their membranes, for destruction of the cell membranes leads to readily recordable hemolysis of these cells [1, 8]. Dependence of the hemolytic activity of some pesticides and herbicides on the conditions of initial activity of intracellular enzymes [8, 12] and on the ability of the substance to reduce the "flowability" of the membrane [12] has been studied. However, the kinetic characteristics of action of pesticides containing chlorine on hemolysis of erythrocytes and their resistance to mechanical action, which may characterize certain principles governing the physiological activity of these compounds, has not been studied.

The aim of this investigation was to study the kinetics of action of pesticides containing chlorine on hemolysis and mechanical resistance of erythrocytes to the action of ultrasound and on membrane-bound acetylcholinesterase (AChE) activity and also to obtain quantitative criteria with which to compare the effectiveness of action of pesticides.

EXPERIMENTAL METHOD

Suspensions of erythrocytes isolated from human blood were studied; specific AChE activity was determined by a potentiometric method with automatic recording [3]. The kinetics of erythrocyte hemolysis in isotonic medium under the influence of pesticides was studied by a photocolorimetric method based on the increase in light transmission of a suspension (10^7-10^8 cells/ml) during cell destruction [2]. The comparison cuvette contained a suspension of erythrocytes hemolyzed in distilled water. The pesticides containing chlorine which were used included herbicides: the sodium salt of trichloroacetic acid (TCA) and pentachlorophenolate (PCP-Na); the insecticide chlorophos; the fungicide pentachloronitrobenzene (PCNB), and the sulfur-containing pesticide rogor (Table 1). Resistance of erythrocytes treated with the pesticides to mechanical hemolysis through the action of ultrasound (frequency 1 MHz, intensity 0.4 W/cm^2) was investigated by automatic recording of the kinetics of erythrocyte destruction in a spectrophotometer cuvette [2]. The parameters determined from experimental kinetic curves of ultrasonic hemolysis characterize the mechanical resistance of erythrocytes and its change as a result of treatment of the erythrocytes by the chemical compounds chosen for testing.

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