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ELECTRON-CYTOCHEMICAL AND MORPHOMETRIC INVESTIGATION OF ENZYME ACTIVITY IN THYROCYTE MITOCHONDRIA DURING CARCINOGENESIS

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Activity of cytochrome oxidase and succinate dehydrogenase in the mitochondria of thyroid gland cells of rats was studied by electron-histochemical and morphometric methods during experimental carcinogenesis. The activity of these enzymes in the mitochondria was shown to vary depending on the stage of malignant transformation: In the early stages it was close to normal, but later (precancer) it fell sharply and approached the level observed in the mitochondria of cancer cells. A marked decrease in the activity of the enzymes studied in the morphologically altered mitochondria of cancer cells may be a qualitative characteristic of these cells.

KEY WORDS: *mitochondria; cytochrome oxidase; succinate dehydrogenase; malignant transformation; thyroid gland.*

A previous investigation showed marked changes in the size, shape, and ultrastructure of the mitochondria in the cells during experimental carcinogenesis [1]. It was therefore interesting to discover whether the structural changes observed in the mitochondria correlate with their cytochemical features. Almost all the enzymes are known to be located in mitochondria and some of them participate in the formation and transformation of energy. Among the most important oxidoreductases are succinate dehydrogenase (SD) and cytochrome oxidase (CO). Changes in the intensity of the reaction for SD in the mitochondria of thyroid gland cells during malignant transformation were established by the writers electron-cytochemically [2].

The object of this investigation was to study the ultrastructural localization of CO and to determine quantitative changes in the SD and CO activity in the mitochondria of thyrocytes during experimental carcinogenesis.

EXPERIMENTAL METHOD

Intact and hyperplastic thyroid glands of noninbred albino rats and tumors in them were used as the test objects. Hyperplasia and tumors were induced in the thyroid gland by prolonged daily administration of 6-methylthiouracil to the experimental animals. After 6-12 months, hyperplasia developed progressively in the thyroid gland of these animals. As a rule, tumors diagnosed as thyroid adenocarcinomas appeared after 14-20 months. The late stages of hyperplasia, preceding the appearance of a carcinoma, were regarded, as by most

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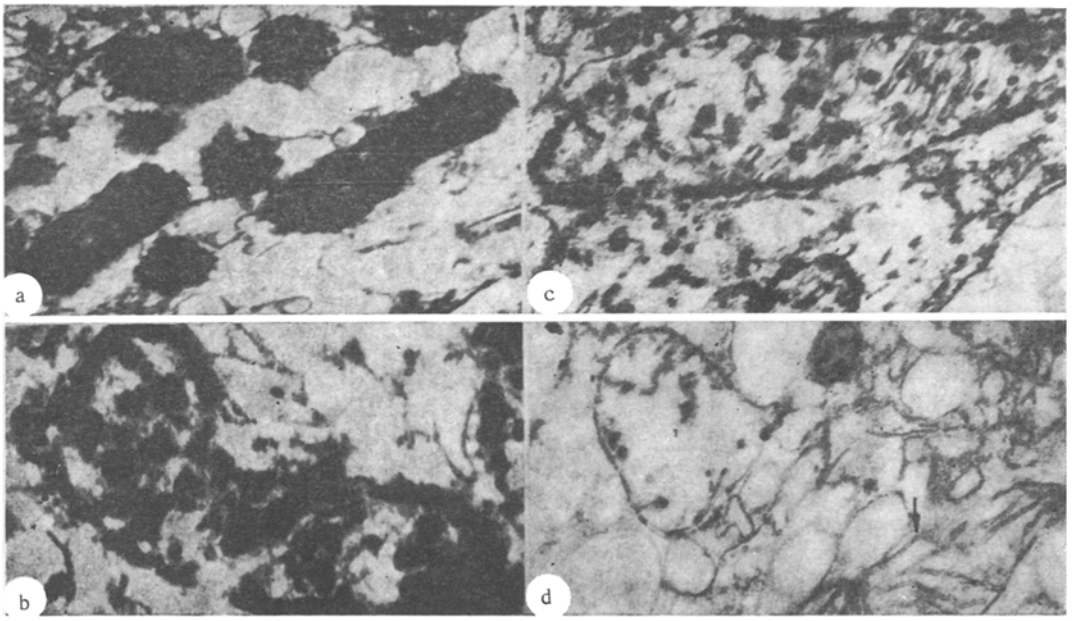

Fig. 1. Mitochondria with different levels of SD activity in intact, hyperplastic, and malignant rat thyroid gland cells: a) mitochondria with high SD activity, class 1 (20,000×); b) with average SD activity, class 2 (80,000×); c) with moderate SD activity, class 3 (80,000×); d) with weak SD activity, class 4; arrow indicates a mitochondrion giving no reaction for SD (40,000×).

other workers, as precancer. The glands of the experimental rats were taken at various times after administration of the compound: 6, 8, 10, 12, 15, and 20 months. The ultrastructural localization of CO was determined by an electron-histochemical method [7]. The relative quantity of the reaction product was measured with the aid of a morphometric grid on arbitrarily chosen electron micrographs [3, 6]. Only accumulations of deposit of the substance that were clearly in focus were counted. The number of angles of the grid falling on reaction product within the contour of the mitochondria and the number of intersections of the lines of the grid with the contours of the mitochondria were counted. For quantitative assessment of CO activity the ratios between the volumes of the cristae and internal membrane occupied with reaction product and the total volume of the mitochondrion were used as indices. The results were averaged for the whole series of electron micrographs (100) studied. Confidence limits were calculated on the assumption of a normal law of distribution of the variables, using Strelkov's table [5].

To assess the SD reaction electron micrographs obtained previously for the study of the ultrastructural localization of this enzyme were used. In this case a semiquantitative classification was adopted. The population of mitochondria was divided into four conventional classes depending on the intensity and character of the reaction in them: class 1) The masses of reaction product occupied the greater part of the organelle (Fig. 1a); class 2) many large granules of the product or a wide, continuous layer in the space between the surrounding mitochondrial membranes (Fig. 1b); class 3) occasional tiny granules of the product (Fig. 1c); class 4) solitary granules of the product or its complete absence (Fig. 1d). At each experimental point (normal, hyperplasia, cancer) the number of mitochondria of these classes was counted in arbitrarily chosen photomicrographs (on the average per 1000 mitochondria). The distribution of the fractions of the classes was taken to be binomial, and its confidence limits were calculated by the usual equation [4].

EXPERIMENTAL RESULTS

Cytochrome Oxidase. Mitochondria of both intact and hyperplastic rat thyroid cells as a rule were similar, rod-like in shape, with well developed, transversely arranged cristae of moderate width and a finely granular matrix. Electron-cytochemically, the reaction product for CO in unstained ultrathin sections was located in the space between the outer and

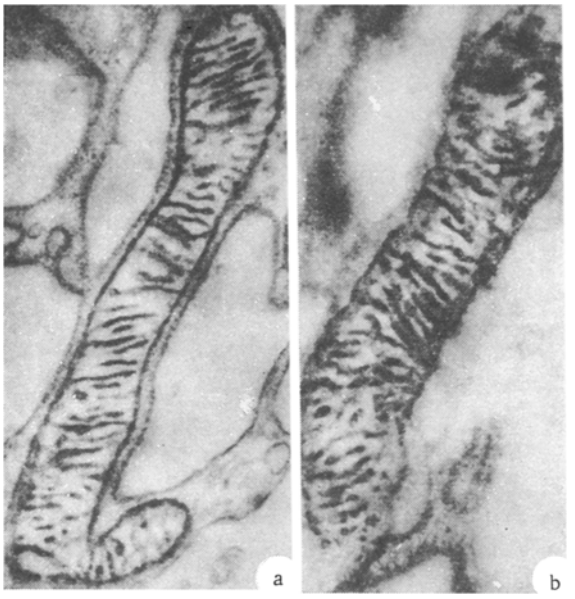

Fig. 2

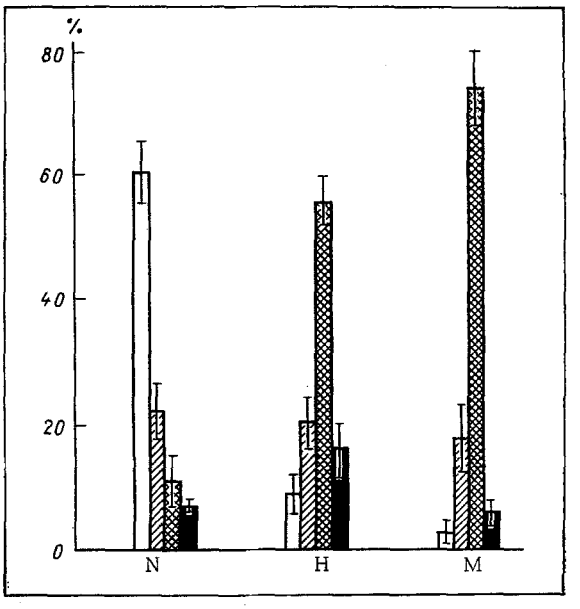

Fig. 3

Fig. 2. Cytochrome oxidase activity of mitochondria of cells from intact (a) and hyperplastic (b) rat thyroid glans (60,000×).

Fig. 3. Histogram of distribution of classes of mitochondria differing in SD activity: N) intact thyrocytes (normal); H) hyperplastic; M) malignant. Mitochondria of class 1) unshaded, class 2) obliquely shaded, class 3) cross-hatched, class 4) shaded black. Vertical lines denote 95% confidence limits. Ordinate, frequency of occurrence of various classes of mitochondria (in %).

inner mitochondrial membranes and also within the intercristal space (Fig. 2). The reaction product was diffusely distributed as homogeneous or finely granular, dark-colored material. Mitochondria with a very strong reaction had clearly outlined contours and cristae. Electron-cytochemically it was impossible to detect any difference in the intensity of the reaction or the distribution of its product visually in the mitochondria in the early stages of hyperplasia (6-8 months of administration of the compound) compared with the mitochondria of intact cells. In the cells in the later stages of hyperplasia (12 months) the character of distribution of the reaction product in the mitochondria varied a little: from more homogeneous to finely granular. In some mitochondria reaction product was absent in the intercristal space. However, no clear difference in the CO content could be found visually in these cells compared with normal.

The morphometric analysis revealed a small but significant difference: a decrease in CO activity in the mitochondria of the cells in the late stages of hyperplasia, corresponding to the initial stages of malignant change, compared with intact thyroid cells (3.26 ± 0.21 and 3.93 ± 0.24, respectively; level of significance 0.95).

Succinate Dehydrogenase. Electron-cytochemical investigation revealed heterogeneity of the mitochondria based on the level of SD activity in the same cell of the intact, hyperplastic, and cancerous gland [2]. A strong reaction for SD was found in mitochondria of the cells of the intact and hyperplastic glands and a decrease in the intensity of the reaction was found in the mitochondria of the cells of a malignant thyroid tumor. In the cancer cells there were also sharp changes in the size, shape, and ultrastructure of the mitochondria. The size and shape of the granules of the final reaction product also were changed in these mitochondria: from large and irregular in shape to small, round, relatively uniform granules (Fig. 1c).

Morphometric analysis showed a significant decrease in the fraction of mitochondria giving a strong reaction for SD (class 1) in cells in the late stages of hyperplasia (precancer) and, in particular, in the cancer cells compared with intact (9.0 ± 3.0, 3.0 ± 2.0, and 60.0 ± 5.0%, respectively; Fig. 3). Under these circumstances there was a significant

increase in the fraction of mitochondria giving a weak reaction (class 3) in the hyperplastic (precancer) and cancer cells (55.0 ± 4.0 and 73.0 ± 6.0%, respectively, compared with a normal value of 11.0 ± 4.0%).

This electron-cytochemical investigation and quantitative analysis, based on cytochemical reaction products, of the activity of two of the most important oxidoreductases — cytochrome oxidase and succinate dehydrogenase — showed a significant decrease in the quantity and, consequently, in the activity of these enzymes in the mitochondria of thyrocytes in the course of malignant change. For instance, activity of CO and SD in the mitochondria of the intact rat thyroid gland was relatively high. In the early stage of hyperplasia, indicative of functional stress of the organ and cells, the activity of these enzymes was still relatively high, but in the late stage of hyperplasia preceding the appearance of a tumor and interpreted as the initial stage of malignant transformation a decrease in the activity of the enzymes in the mitochondria was observed. Their activity fell even more in the mitochondria of the cancer cells.

Changes in the activity of oxidoreductases (CO and SD) in the mitochondria were thus established quantitatively in the mitochondria in relation to the stage of malignant change: In the early stages it was close to normal; later (precancer) it fell sharply and approximated to the level of activity observed in the mitochondria of cancer cells, although the ultrastructure of the mitochondria under these circumstances still remained relatively unchanged. The marked decrease in enzyme activity in the morphologically changed mitochondria of the cancer cells could be not simply a functional, but also a qualitative characteristic.

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