bance in the microcirculatory system of various organs. In the course of acute pancreatitis the microcirculatory bed of the pancreas itself and the mesentery is first affected; later, at the stage of transition from the edematous form of pancreatic necrosis to the hemorrhagic form the hemorheologic disturbances lead to the development of microcirulatory disorders in the liver and kidneys. It can be concluded from these facts that the degree of the hemorheologic disturbances and of the microcirculatory disorders in different organs corresponds to the severity of the necrotic changes in the pancreas at different stages of the pathological process.

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ELECTRON-AUTORADIOGRAPHIC STUDY OF RNA SYNTHESIS IN DARK AND PALE ADRENAL CELLS

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Dark and pale cells are found in various organs and tissues, including the adrenals. It has been claimed that dark cells possess greater functional capacity and are adapted for long term specific activity, associated with active biosynthesis, whereas pale cells are in a state of exhaustion [1, 2, 5]. Since RNA turnover is an essential characteristic of the cell, it was decided to study this phenomenon by electron-microscopic autoradiography in different structural and functional zones of the adrenals.

EXPERIMENTAL METHOD

The test material consisted of mouse adrenal glands. The animals were given an intraperitoneal injection of the labeled RNA precursor 5-^{3}H-uridine, in a dose of 100 μCi/g. The mice were decapitated 2 and 4 h after injection of uridine under ether anesthesia, and the adrenals were fixed in 2.5% glutaraldehyde solution, washed with phosphate buffer (pH 7.4), post fixed with 1% OsO_4 solution, and embedded in a mixture of Epon and Araldite. Autoradiographs of semithin sections were prepared from each block with the aid of type M emulsion, and on the basis of the results of their analysis, zones for ultramicrotomy were selected. Electron-microscopic autoradiographs were obtained by the method described previously [3, 4]. Semithin sections were stained with toluidine blue. Ultrathin sections were examined in the JEM-100 B electron microscope. The number of grains of silver above the nucleus and cytoplasm of the dark and pale cells and the total number of grains above these cells were counted. The ratio of the number of grains of silver above the nucleus to the number above the cytoplasm was calculated. All the quantitative data were subjected to statistical analysis. The significance changes were calculated by Student's t test. Mean values were obtained from

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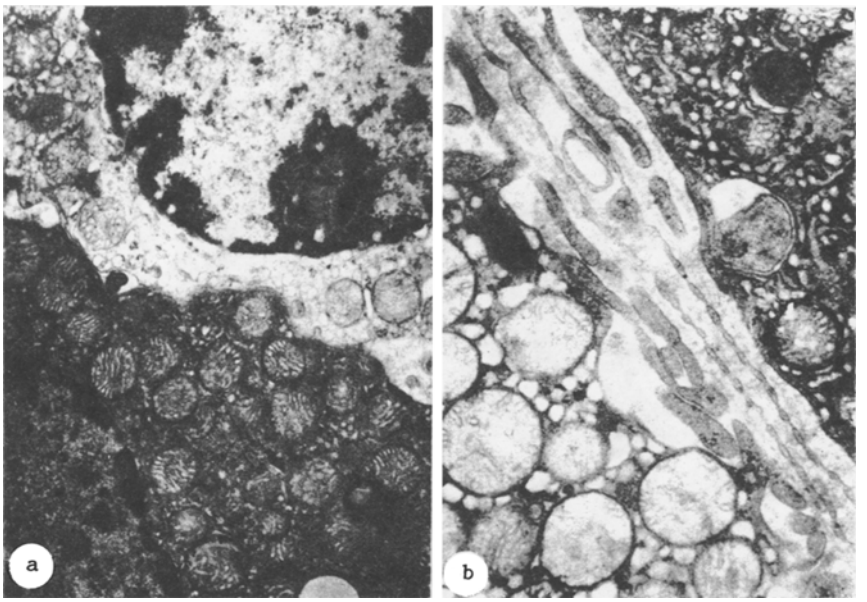

Fig. 1. Dark and pale cells of the adrenal glands: a) zona glomerulosa: on right) dark cell with abundant organelles – mitochrondria, polysomes, rough endoplasmic reticulum; on left) pale cells with fewer organelles, mitochondrial matrix is translucent. 15,000×; b) zona fasciculata: above – dark cell, mitochondria with electron-dense matrix and numerous cristae, abundant polysomes, elements of rough endoplasmic reticulum; below – pale cell with large mitochondria, few cristae not reaching the central part of the organelle, matrix translucent, cisterns of endoplasmic reticulum widened and translucent. Here and in Figs. 2 and 3: magnification 20,000×.

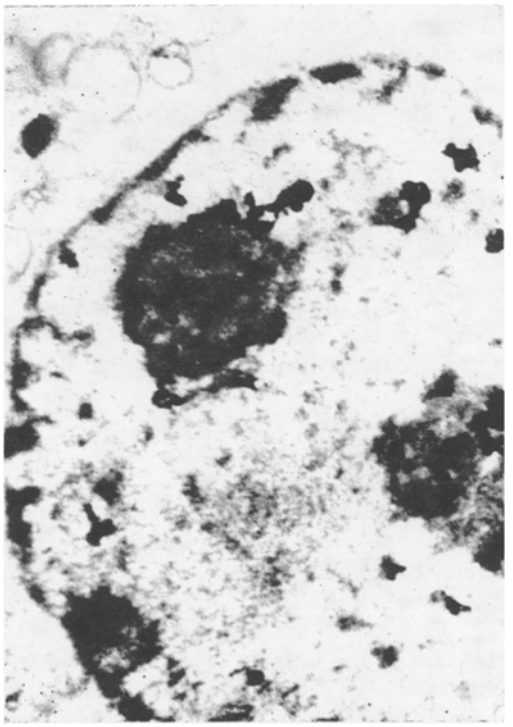

Fig. 2. RNA synthesis in cell of zona glomerulosa of adrenal. Distribution of grains of silver mainly above nucleolus of cell 2 h after injection of ^{3}H-uridine into animal.

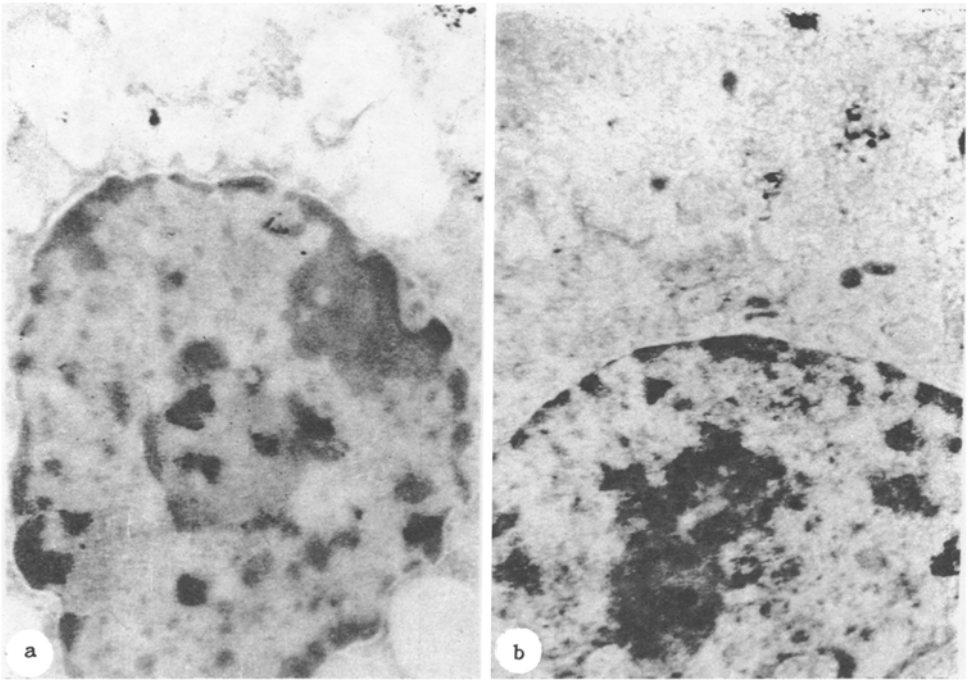

Fig. 3. RNA synthesis in cells of zona glomerulosa (a) and zona fasciculata (b) of adrenal. Number of grains of silver above nucleus is reduced, whereas number above cytoplasm is increased 4 h after injection of ^{3}H-uridine into animal.

the results of counting 2000 cells in each zone of the cortex and medulla of the adrenal glands from each animal.

EXPERIMENTAL RESULTS

In all zones of the adrenal cortex and medulla dark and pale cells were discovered. Dark cells in the cortex were most numerous in the zona glomerulosa (76.03%), and their number gradually decreased in the zone fasciculata (58.22%) and zona reticularis (41.97%). The cytoplasm of the dark cells had higher electron density because of the abundance of organelles (mitochondria, polysomes, rough endoplasmic reticulum), and the mitochondria had more numerous and more extensive cristae, and a matrix with higher electron density than the pale cells. Dilatation and translucency of the cisterns of the endoplasmic reticulum were observed in the pale cells, their mitochondria were larger and swollen, and their cristae were less regularly arranged and did not reach the central part of the organelles; the intracristal space was widened and translucent (Fig. 1). Pale cells predominated in the medulla (58.03%).

In preparations fixed 2 h after injection of ^{3}H-uridine the grains of silver were distributed mainly above the nucleus of the adrenal cells. The concentration of grains was greatest above the nucleolus (Fig. 2). Comparison of the dark cells located in different zones of the cortex shows that the total number of grains above the nucleus and cytoplasm of the dark cells was the greatest in the zona glomerulosa (16.20 ± 0.88) and it gradually decreased in the zona fasciculata (14.76 ± 0.79) and zona reticularis (9.78 ± 0.61). Above the nuclei of the dark cells therewere 1.36-1.65 more grains of silver than above the nuclei of pale cells in the corresponding zones of the adrenal gland. The mean number of grains of silver above the nucleus of the dark cells was greatest in the zona glomerulosa (12.18 ± 0.83), it was smaller in the zona fasciculata (10.19 ± 0.65), and least of all in the zona reticularis (7.33 ± 0.55). The total number of grains above the pale cells was less than above the dark cells, namely 12.05 ± 0.73 in the zona glomerulosa, 11.33 ± 0.72 in the zona fasciculata, and 8.21 ± 0.58 in the zona reticularis. Nuclear labeling of the pale cells was maximal in the zona glomerulosa (7.33 ± 0.53), less in the zona fasciculata (6.25 ± 0.44), and less still in the zona reticularis (5.13 ± 0.38). The number of grains above the cytoplasm of the dark and pale cells in each group of the adrenal cortex was not significantly different.

The total density of the label above the cortical cells 4 h after injection of ^{3}H-uridine showedno significant change compared with the previous time of investigation. Intensi-

fication of labeling was observed above the cytoplasm, with a simultaneous decrease in intensity above the nucleus of the cells (Fig. 3). In the dark cells, located in different zones of the cortex, the number of grains of silver above the nucleus was reduced by 1.25-1.47 times whereas their number above the cytoplasm was increased by 1.43-1.97. Incidentally, the decrease in density of the label above the nucleus was connected with a decrease in the number of grains of silver above cell nucleolus (by 1.82 times), since the number of grains above the nonnucleolar zone of the nucleus did not change significantly, and amounted to 7.83 ± 0.91 and 7.01 ± 0.87 grains of silver respectively after 2 and 4 h. The ratio of the number of grains of silver above the nucleus to their number above the cytoplasm after 4 h in the dark cells was reduced by 2.92 times in the zona glomerulosa, by 2.07 times in the zona fasciculata, and by 1.49 times in the zona reticularis. This ratio did not differ significantly in the dark and pale cells after 4 h, for in the zona glomerulosa it was 1.57 ± 0.15 and 1.58 ± 0.14, in the zona fasciculata 1.32 ± 0.12 and 1.31 ± 0.15, in the zona reticularis 1.24 ± 0.11 and 1.21 ± 0.12, and in the medulla 1.69 ± 0.40 and 1.67 ± 0.43 above the dark and pale cells respectively.

Similar changes were observed in pale cells of the zona glomerulosa, but migration of the label from the nucleus into cytoplasm in these cells took place 1.77 times more slowly than in the dark cells. Only a tendency for such changes to develop was discovered in the zona fasciculata and zona reticularis (they did not reach the level of significance).

Compared with the previous time of investigation, after 4 h migration of RNA from nucleus into cytoplasm in both types of cells was increased to the maximum in the zona glomerulosa, where the number of grains above the cytoplasm was increased by 1.73 times. Migration of newly synthesized RNA took place more slowly in cells of the zona fasciculata and zona reticularis, where the number of grains above the cytoplasm of all cells was increased by 1.42 and 1.34 times respectively.

In the medulla 4 h after injection of ^{3}H-uridine the number of grains of silver above the nuclei of both dark and pale cells was reduced (by 2.87 and 2.44 times respectively). However, migration of the label from the nucleus into cytoplasm of the dark cells took place 1.3 times faster than in the pale cells. After 4 h a certain tendency was observed for the number of grains of silver above the cytoplasm of both dark (by 9%) and pale (by 12%) cells to decrease.

The study of the kinetics of RNA turnover after two stages of observation led to the conclusion that migration of RNA from nucleus into cytoplasm in cells of the adrenal cortex is accomplished quite quickly, for the density of autoradiographs above the cytoplasm was appreciably increased 4 h after injection of the label compared with the denstiy found after 2 h. Reduction of the number of grains of silver above the nucleolus and an increase in the number above the cytoplasm 4 h after injection of ^{3}H-uridine are in harmony with the view that RNA, synthesized in the nucleolus, then becomes a component of the cytoplasmic RNA.

Cells of the medulla have a higher rate of RNA migration from nucleus into cytoplasm, because after 4 h the quantity of label above the nucleus in cells of the medulla fell 2.5 and 2 times faster (above dark and pale cells respectively) than in the cortex. At the same time, a faster rate of RNA degradation was observed in the cytoplasm of the medullary cells, for the intensity of label above the cytoplasm after 4 h was not significantly changed compared with the previous time of investigation. Meanwhile there was a stable increase in the number of grains of silver above the cytoplasm of the cortical cells 4 h after injection of the labeled RNA precursor. Since the lower density of grains of silver above cells of the medulla could be the result not only of more rapid degradation, but also of accidental causes, such as a lower intensity of RNA synthesis in the animals observed after 4 h of the experiment, we compared the concentration of label in the medulla with that in the cortex of the same animals. It was found that after 4 h the concentration of grains in the cortex remained the same as after 2 h, whereas in medullary cells it was reduced by 1.97 times. This result can be explained only by more rapid degradation of labeled RNA in cells of the medulla.

Dark cells of the adrenal glands thus differ from pale cells in their higher rate of uptake of labeled RNA precursor and migration of newly synthesized RNA from nucleus into cytoplasm. Synthesis and migration of RNA into the cytoplasm take place most intensively in the adrenal cortex in the dark and pale cells of the zona glomerulosa. The intensity of synthesis and rate of migration of RNA are reduced in the zona fasciculata and, in particular, in the zona reticularis. In dark and pale cells of the medulla, compared with the adrenal

cortex, newly synthesized RNA migrates more rapidly into the cytoplasm and RNA degradation is accelerated.

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