CHANGES IN HISTIOCYTE ULTRASTRUCTURE IN LOOSE CONNECTIVE TISSUE IN DEHYDRATION AND STARVATION

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Changes in the parameters of the volumetric fractions of the principal cytoplasmic organelles of histiocytes in the subcutaneous connective tissue of albino rats were demonstrated during experimental dehydration and starvation. The common response of the histiocytes under these conditions was by hypertrophy of the lysosomal apparatus and infiltration of the cells with lipids. At the same time certain differences are stressed: During dehydration there is an increase in the volumetric fractions of the mitochondria and granular endoplasmic reticulum, whereas during starvation the increase affects the fraction of phagosomes. On account of dehydration the volume of the fraction of pinocytotic vacuoles is sharply reduced.

KEY WORDS: histiocytes; organelles; dehydration; starvation.

Histiocytes are generally regarded as "resting" macrophages of the loose connective tissue. Recently, however, the view has arisen that they are cells with the highest level of metabolic activity with respect to enzymes of both hydrolysis and respiration [1]. It is accepted that one of the main functions of the histiocytes of the subcutaneous connective tissue is to participate in the maintenance of osmolar homeostasis through their control over lipid oxidation. It is accordingly interesting to study the character of submicroscopic changes in histiocyte morphology during dehydration. Since laboratory animals exposed to prolonged dehydration soon refuse to eat [4], a parallel series of experiments was carried out to study the effects of starvation.

EXPERIMENTAL METHOD

Histiocytes of loose connective tissue of laboratory albino rats were studied. The animals were divided into three groups, with ten animals in each group. The animals of Group 1 were kept on dried (to 15% moisture content) food without access to water. The animals of Group 2 had free access to water but their food was restricted. Its amount was controlled in accordance with previous observations [5] on food consumption during dehydration: from 35% of the normal diet on the first day to 10% on the 5th day and total deprivation subsequently. The animals of the control group had free access to food and water.

TABLE 1. Volumetric Indices of Ultrastructure of Histiocytes of Loose Connective Tissue in Experimental Series ($X \pm m$)

Conditions	Indices						
	1	2	3	4	5	6	7
Dehydration for 10 days	19,5±1,0*	51,3±5,0*	16,7±3,6*	15,1±3,6*	7,9±2,5	9,3±2,9	2,7±0,6*
Dehydration for 5 days	20,5±0,6*	37,3±1,5	17,5±3,8*	28,2±4,3	8,0±2,7	9,0±1,6*	0,5±0,06*
Normal	22,4±0,8	36,9±3,4	26,8±3,1	23,6±3,0	5,7±1,2	7,0±1,8	0.03±0,008
Starvation for 5 days	21,8±0,7	20,3±4,0*	37,5±4,8*	27,7±4,3	9,9±2,3*	6,1±2,4	0,3±0,005*
Starvation for 10 days	17,8±0,8*	36,7±4,6	25,4±4,3	15,7±3,3*	10,6±3,1*	6,8±2,5	1,4±0,4*

Note. 1) Total volume of fractions of compartments in percent of volume of cytoplasm; 2-6) partial volume of fractions of mitochondria, vacuoles, lysosomes, phagosomes, and endoplasmic reticulum in cell organome (normalized as percentages of organome); 7) volume fraction of lipid inclusions as a percentage of cytoplasm. * Denotes indices differing significantly from normal ($P = 0.05$).

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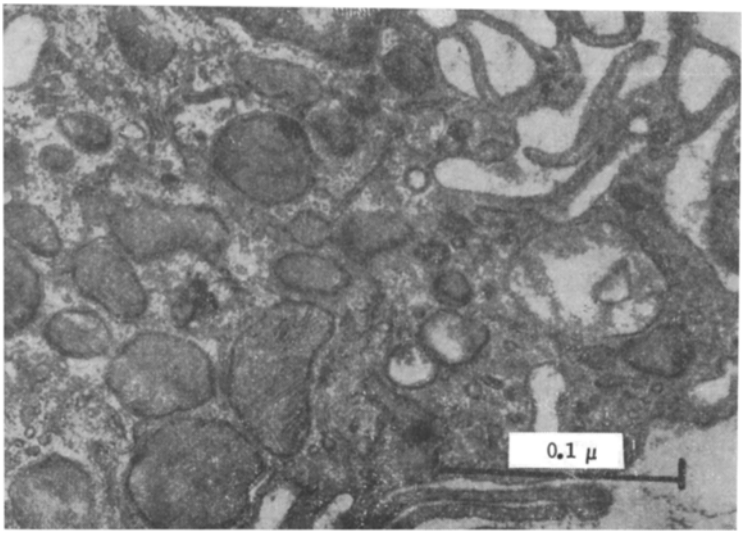

Fig. 1. Fragment of cytoplasm of histiocyte of subcutaneous connective tissue in intact rat, 30,000 ×.

Experimental animals weighing 180-220 g were kept in single cages and killed on the 6th and 10th days of the experiment. In the dehydration group the loss of weight on the 6th day was 23% and on the 10th day 36%, whereas in the group of starving animals the corresponding losses were 19 and 30%. Material for electron-microscopic analysis was obtained by excision of skin from the medial surface of the thigh and "testing" connective-tissue films, and it was fixed by the usual method. Sections were stained with lead citrate and examined in the IEM-100B electron microscope. Histiocytes were photographed whole and enlarged to a magnification of 25,000. The cell structure was assessed with regard to the basic rules of stereology. A regular test grid with 1-cm step was used as the instrument for morphometry. The volume of the principal cell organelles was determined (relative to the cytoplasm): mitochondria, primary lysosomes, vacuoles with phagocytosed material, vacuoles without inclusions, cisterns of the granular endoplasmic reticulum, and the volume fraction of the lipid inclusions, as follows:

$$V_{vi} \text{ of organelle} = \frac{100\ P_i\ (\text{number of test points on cross section of organelle})}{P_c\ (\text{number of test points on area of cytoplasm})}$$

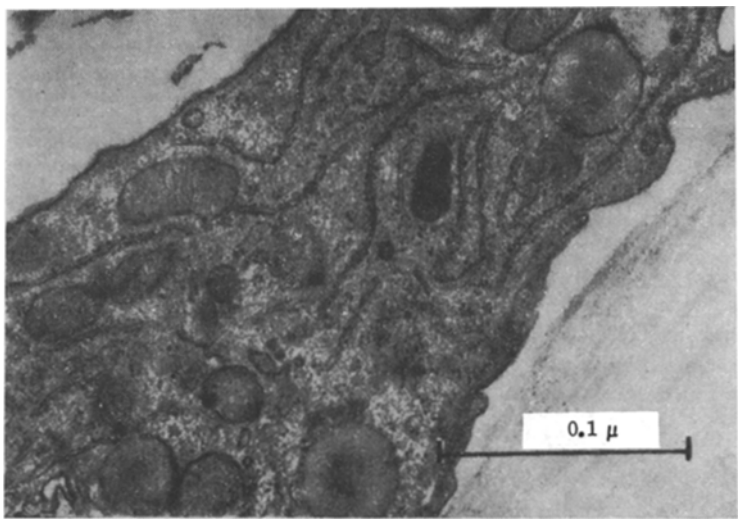

Fig. 2. Cytoplasm of histiocyte of rat after dehydration for 5 days, 30,000 ×.

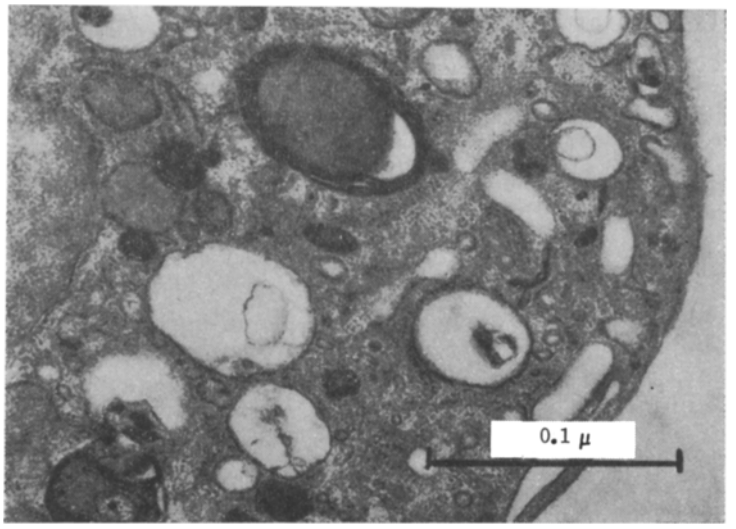

Fig. 3. Cytoplasm of histiocyte of rat after starvation for 5 days, 30,000 ×.

The differentiation profile (q_v) was estimated as the ratio of the volume fractions of the organelles to the volume of the histiocyte organome*:

$$q_{vi} = \frac{100\ V_{vi}\ (\text{relative volume of organelle})}{\sum^{n} V_{vi}\ (\text{volume of organome})}$$

Sample populations from each experimental group contained at least 100 objects, and the control population consisted of two such independent samples, i.e., of 200 cells.

EXPERIMENTAL RESULTS

Comparison of the volumetric indices of "averaged" histiocytes from the normal and experimental groups showed that, aside from certain common features of the reaction, there was also a tendency for morphological changes to diverge (Table 1). In all cases, whether during starvation or during dehydration, the histiocytes gradually accumulated lipids, which is possible evidence of the nonspecificity of this phenomenon and a reflection of a universal reaction of the organism as a whole, namely, the switching of metabolism from the carbohydrate to the lipid type [3]. A common feature was a reduction of the organome of the average histiocyte, probably due in part to the inflow of undifferentiated forms of macrophages to the periphery [1] and partly functional in origin as a result of changes in the differentiation profile. Against this background other changes of a uniform type also took place. For instance, the relative volume of phagosomes and phagolysosomes in the organome increased by 50-100%. The fraction of primary lysosomes in both experimental groups increased by 20% after 5 days and decreased (relative to normal) by 33% after 10 days.

Under normal conditions most of the histiocytes were actively resorbing cells with numerous pinocytotic vacuoles, heterophagosomes, and large primary lysosomes (Fig. 1). During fluid deprivation, histiocytes with small and relatively few vacuoles were more often seen. Usually the endoplasmic reticulum was well developed in the cytoplasm of these cells and there were many mitochondria with a moderately dense matrix and with numerous narrow cristae. Many primary lysosomes were present in such cells, but they were small (Fig. 2). For the histiocytes as a whole, during dehydration the mitochondria and the granular endoplasmic reticulum accounted for larger fractions of the cell organome as a whole, but no significant dilatation of the cisterns could be observed. In all cells the contribution of the fraction of optically empty vacuoles was reduced by almost half.

In starvation, on the other hand, cells with a well-marked resorption apparatus were predominant — cells with many cytoplasmic outgrowths and pinocytotic and phagocytic vacuoles (Fig. 3). Their cytoplasm contained many phagolysosomes and residual bodies.

*The organome is the collective term for all the organelles of the cell.

Meanwhile histiocytes of the starving animals were characterized by a considerable decrease in the volume fraction of the mitochondria and by the appearance of mitochondria with a clear matrix and of swollen mitochondria in individual cells. This phenomenon indicates intensification chiefly of the process of phagocytosis and an associated intensification of glycolysis in the starving animals [2].

The most likely cause of the convergence of the ultrastructural parameters of the histiocytes during long-term (10 days) dehydration and starvation was the animals' refusal to eat after 5 days of dry feeding [4, 5] and, consequently, the superposition of these two factors.

Analysis of the changes in the histiocytes of subcutaneous connective tissue during starvation and dehydration thus leads to the conclusion that the reaction of these cells to these conditions differs but is made up of two components: a nonspecific component — some degree of simplification of their organization, hypertrophy of the lysosomal system, and lipid infiltration of the cells; a specific component, manifested during dehydration as considerable inhibition of pinocytosis and activation of aerobic energy metabolism and protein biosynthesis, and during starvation as stimulation of pinocytotic and phagocytic activity.

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