viding apparatus of the cell, which is unable to satisfy completely the increased metabolic demands of a muscle which is working intensively during reheating of the body.

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MORPHOLOGICAL AND FUNCTIONAL CHARACTERISTICS OF CLARA CELLS IN ANOXIC RATS BASED ON DATA OF SCANNING AND TRANSMISSION ELECTRON MICROSCOPY

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KEY WORDS: anoxia, ultrastructure, Clara cells.

The characteristics of structural and metabolic changes taking place in the lung in various anoxic states have been dealt with sufficiently widely in the literature [1-4], whereas only isolated publications have been devoted to the ultrastructure of the Clara cells in anoxia [7-9].

The aim of this investigation was to study ultrastructural changes in Clara cells of the terminal bronchioles and to evaluate their secretory activity in chronic anoxia with the aid of transmission (TEM) and scanning (SEM) electron microscopy.

EXPERIMENTAL METHOD

Noninbred male rats weighing 150-180 g were used. Anoxia was produced in a pressure chamber in which the air pressure was reduced to 310-340 mm Hg, equivalent to an altitude of 7000 m above sea level; exposure lasted 2 h. The rats were taken from the experiments by an injection of phentobarbital 5, 15, 30, and 60 days from its beginning. For SEM the lung was fixed by intratracheal injection of a 2.5% solution of glutaraldehyde under a pressure of 10 cm water. The material was dried at the critical point in liquid CO_2. After spraying with gold the specimens were examined in a "Hitachi S-500" scanning electron microscope. Secretory activity of the Clara cells was judged from the state of the apical surface [6]. With respect to this feature all the cells were divided into four groups: I) the

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TABLE 1. Relative Percentages of Clara Cells in Different Phases of Secretory Cycle in Rats Exposed to Anoxia

Phase of secretion	Control	Duration of anoxia, days			
		5	15	30	60
I	41,45±5,19	67,77±8,92*	25,04±8,59**	21,47±6,14**	18,18±5,04*
II	54,42±5,90	22,01±8,21*	73,87±8,64**	67,34±4,64*	68,9±6,46*
III	0,39±0,87	1,27±1,61	0,26±0,57	7,05±4,00**	6,85±3,45*
IV	3,53±2,83	8,96±3,24*	0,70±1,07**	4,13±2,53***	5,76±3,16

Legend. *p < 0.05 Compared with control, **p < 0.05 compared with control and with previous time of investigation, ***p < 0.05 compared with previous time of investigation.

Fig. 1. Clara cells on 15th day of hypoxia. TEM. Increase in number of secretory granules and their redistribution beneath the cell plasmalemma. 4000 ×.

initial phase of secretion accumulation, II) the final phase of secretion accumulation, III) cells in the secretion process, IV) the postsecretory phase. The relative percentages of cells in the different phases of the secretory cycle were determined. The numerical data were subjected to statistical analysis by Student's test. Differences were considered significant at the $p < 0.05$ level.

For TEM the lungs were fixed by perfusion through the pulmonary artery with a cold mixture of 2.5% glutaraldehyde, pH 7.4. Ultrathin sections were examined in the "Tesla BS-500" electron microscope. Specimens of lungs from animals not subjected to any form of procedure served as the control.

EXPERIMENTAL RESULTS

On the 5th day of exposure to anoxia focal widening of the perinuclear space occurred in the Clara cells: the lamellar complex was concentrated in it, the number of mitochondria, membranes of the rough endoplasmic reticulum, and ribosomes was considerably increased, but the number of secretory granules did not exceed one or two per cell. In some places foci of liquifaction of the cytoplasm appeared. The number of Clara cells was increased in the initial phase of secretion accumulation, whereas the number of cells with postsecretory pores (Table 1) was increased. On the 15th day reduction of the rough and vacuolation of the smooth endoplasmic reticulum occurred. It must be emphasized that this time the number of secretory granules which had migrated into the apical zone of the cytoplasm, immediately beneath the plasmalemma, was increased (Fig. 1). The number of cells in the final phase of secretion accumulation was increased (Fig. 2) and the number of cells in the initial phase of secretion accumulation was reduced. Some investigators [5, 8, 10] have found a connection between hypersecretion of Clara cells and activation of the sympathicoadrenal system during short-term anoxia and with mast cell degranulation.

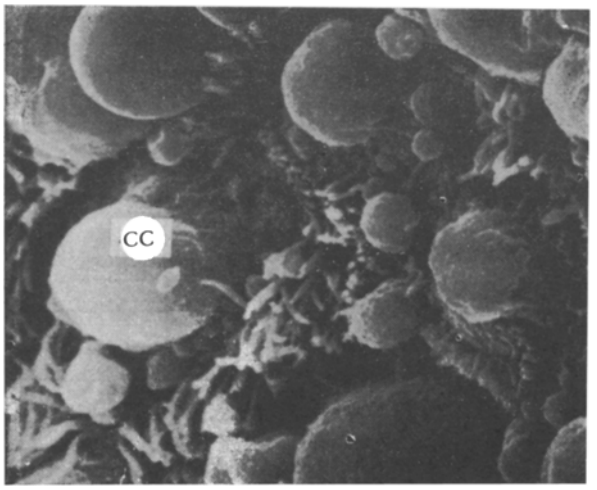

Fig. 2. Clara cells (CC) in final phase of secretion accumulation. SEM. 15th Day of anoxia. 10,000 ×.

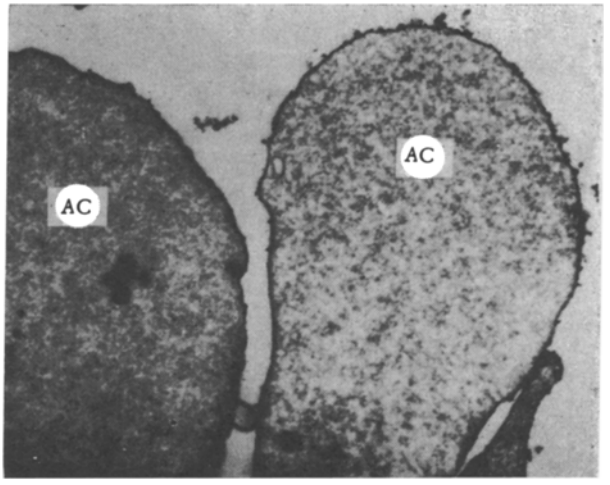

Fig. 3. Apical cap (AC) of Clara cell due to edema of cytoplasm. TEM. 30th Day of anoxia. 8000 ×.

On the 30th day redistribution of the organelles into the peripheral zones of the cytoplasm was clearly defined, with the formation of a large apical "cap" (Fig. 3). There was an increase in the number of cells on whose surface small secretory granules were visible; the number of cells in the initial phase of secretion accumulation was reduced even more.

On the 60th day of anoxia the ultrastructure of the Clara cells showed a mosaic pattern, with destruction of a small proportion of the mitochondria. Many dense secretory granules were located beneath the plasmalemma, which they sometimes perforated to emerge into the lumen of the bronchioles, by a process resembling merocrine secretion [6]. On SEM, a characteristic finding was of small round holes at the sites of escape of the secretion, and a tendency toward a decrease in the number of cells in the initial phase of secretion accumulation and an increase in the number of cells in the final phase of secretion accumulation was preserved; cells in the secretion process were more numerous than in the control. Compared with the previous time, however, differences between all the above parameters were not significant.

The experiments thus showed that Clara cells respond to respiratory anoxia by modification of their secretory activity. In short-term anoxia increased output of secretion is observed, with the formation of spherical structures on the apical part of the cells.

In chronic anoxia secretion accumulation is stimulated and this process is accompanied by hyperplasia and hypertrophy of the synthetic apparatus of the Clara cells.

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PATHOMORPHOLOGICAL CRITERIA OF THE EXPERIMENTAL ANTIATHEROSCLEROTIC ACTION OF SAPONINS OF PLANT ORIGIN

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Regression of atherosclerosis is observed during its treatment by preparations such as clofibrate, lipostabil, Adriamycin, diethylhexylphthalate, and so on [10, 12-14]. However, their long-term administration runs the risk of toxico-allergic side effects. In recent years attention has been drawn to preparations of plant origin, with little or no toxicity, containing steroid glycosides and saponins, such as polysponin, diasponin, etc. [5, 7]. The action of these preparations has been tested on animals on a chronic high-cholesterol diet. In 20% of the animals spontaneous atherosclerosis was observed, to which rats are less prone [8, 9, 15]. Atherosclerosis is characterized by successive changes, occurring in stages, in the heart, blood vessels, and other organs. Special attention must be paid to the early stages of atherogenesis with intravasation of serum proteins and lipids into the wall of blood vessels, de-endothelization of their intima, platelet aggregation, accumulation of sulfur-containing glycosaminoglycans, transformation of smooth-muscle cells into foam cells, and fibroelastosis [1, 2, 4, 6, 11]. These changes lead to the formation of lipid streaks and stains, atheromatous plaques, and complicated lesions, reflected in an atherosclerotic index of the I to the IV degree, together with scar changes in the internal organs [1].

The aim of this investigation was a pathomorphological evaluation of the action of furostanol glycosides and saponins of plant origin on experimental atherosclerosis.

EXPERIMENTAL METHOD

The experimental animals were 700 noninbred male albino rats aged 1 year, of which 100 were controls. The other 600 animals were kept for 9 months on a special high-cholesterol

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