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ULTRASTRUCTURAL AND ULTRACYTOCHEMICAL CHANGES IN THE LUNGS IN DIABETIC RATS

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UDC 616.379-008.64-092.9-07:616.24-076.4

KEY WORDS: diabetes mellitus; ultrastructure of lungs; glycocalyx.

Experimental evidence has been obtained that lipid metabolism in the lungs is regulated by insulin, and also that the ability of the lungs to oxidize glucose and to incorporate ^{3}H-leucine into protein is inhibited in diabetes mellitus [11]. Specific receptors for insulin were identified for the first time in membrane structures of normal rat lungs in [10]. Insulin interacts quickly with these receptors, saturation being reversible and depending on time and temperature. Since lipid and protein synthesis in the lung tissue is mainly associated with type II pneumocytes [3, 7], changes are found in these cells in rats with streptozotocin-induced diabetes [13]. However, no chronic experimental investigation of the lungs has been undertaken on animals with experimental diabetes.

The aim of this investigation was to study the time course of ultrastructural changes in the lungs and the state of the glycocalyx of the pneumocytes in experimental streptozotocin-induced diabetes.

EXPERIMENTAL METHOD

Experiments were carried out on 64 male and female rats of mixed lines weighing 180-250 g. Streptozotocin was dissolved in physiological saline and given as a single intraperitoneal injection in a does of 90-100 mg/kg. Six intact rats served as the control. The onset of diabetes was recorded on the basis of glycosuria, polyuria, and polydipsia. Glucose in the urine was determined by the paper strip method (Labstix, Dextrostix).

Of the 64 rats, 39 with diabetes died in the first 2 weeks: most of them showed signs of focal pneumonia. In six rats the signs of diabetes disappeared at the end of the 2nd week and they were eliminated from the experiment. Tests were carried out on the remaining 19 rats with developed diabetes. The rats were killed after 4, 7, 15, 30, and 60 days. Under ether anesthia thoracotomy was peformed, the right bronchus was clamped, a catheter was inserted into the left bronchus, and a solution of 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.3) was injected into the lung. The right lung was used for ultracytochemical detection of the glycocalyx: a catheter was inserted into the right bronchus of the same rat and a 2.5% solution of glutaraldehyde in 0.1 M cacodylate buffer (pH 7.3), in which ruthenium red was dissolved in a concentration of 1 mg/ml, was injected into the lung. After fixation for 1 h, subsequent treatment followed Luft's formula (Geyer, 1974).

Department of Pathological Anatomy, First Faculty of Internal Medicine and Faculty of Preventive Medicine, I. M. Sechenov First Moscow Medical Institute. Central Research Laboratory, Patrice Lumumba Peoples' Friendship University, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Smol'yannikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 109, No. 1, pp. 75-78, January, 1990. Original article submitted December 6, 1988.

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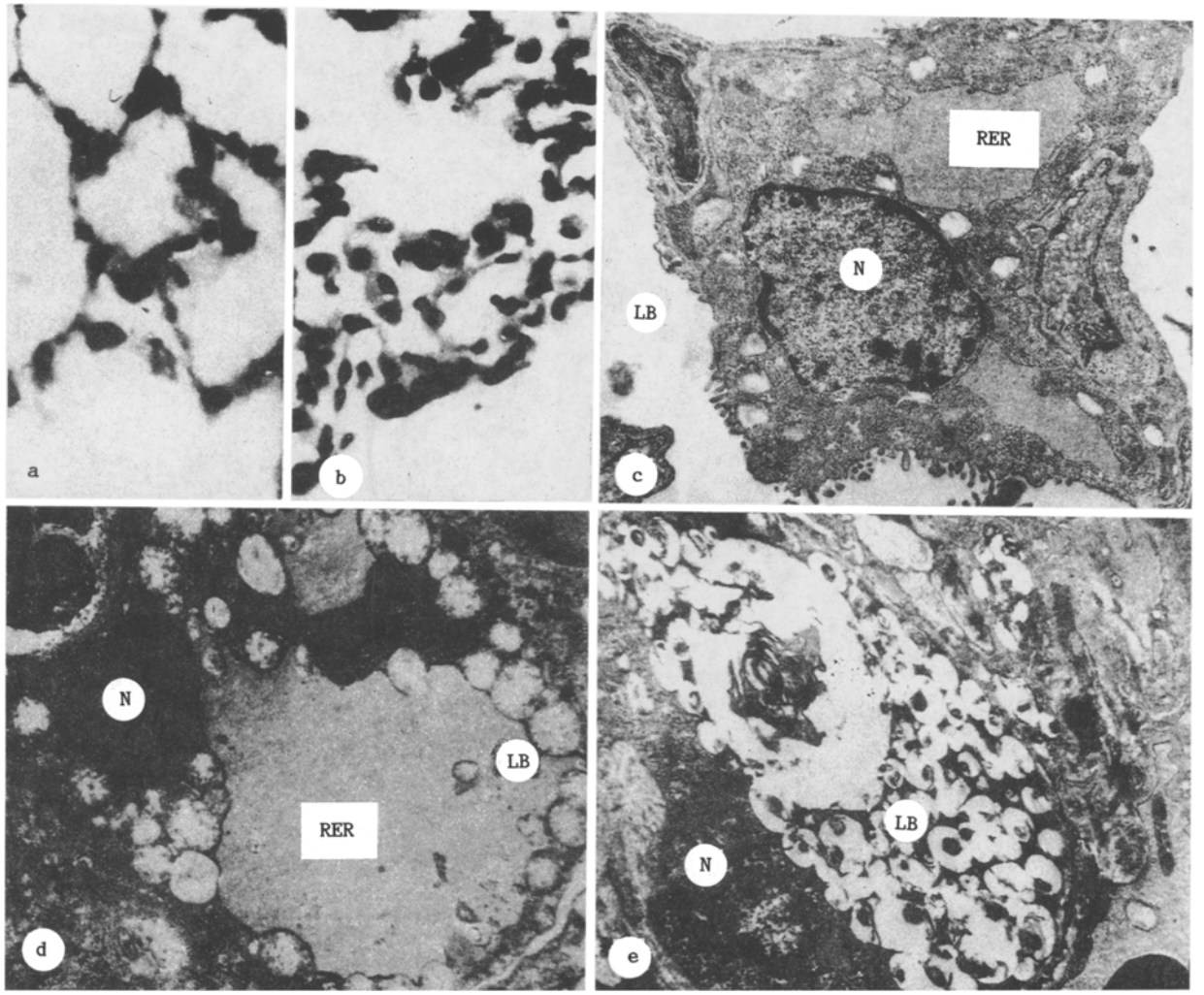

Fig. 1. Changes in the lungs of diabetic rats. a) Intact lung: single type II pneumocytes can be identified. Hematoxylin and eosin. 500 ×. b) 4th day of experiment: alveolar walls lined with many type II pneumocytes. Hematoxylin and eosin. 500 ×. c) 7th day of experiment: marked dilatation of tubules of RER of type II pneumocytes. 6000 ×. d) 15th day of experiment: marked dilatation of tubules of RER with displacement of organelles to periphery of cytoplasm of type II pneumocyte. 8000 ×. e) 15th day of experiment; considerable accumulation of lamellar bodies can be seen in cytoplasm of type II pneumocyte, with formation of large conglomerate (in center). 6000 ×. Here and in Fig. 2: N) nucleus, AL) alveolar lumen, LB) lamellar bodies.

EXPERIMENTAL RESULTS

The general state of the animals (n = 7) 4 days after injection of streptozotocin became serious: they frequently drank large amounts of water, became wasted, lost their hair, and their movements were restricted. Glucose was detected in the urine (05-1.0 g%). On histological examination an increase in the number of type II pneumocytes was found in some areas, in certain alveoli they had displaced the type I pneumocytes (Fig. 1a, b). Electron-microscopic examination showed that changes affected mainly the type II pneumocytes, and were expressed as moderate dilation of tubules of the rough endoplasmic reticulum (RER) and some degree for shortening and reduced density of the microvilli. In alveoli lined exclusively with type II pneumocytes, these cells were at different stages of maturation, and many lysosomes could ibe identified by the cytoplasm of some of them, which was not observed in the control.

In intact animals the reation with ruthenium red was found only on the luminal side of the type I and II pneumocytes, in the form of a uniform electron-dense layer of equal thickness for the two types of cells (Fig. 2b). The glycocalyx of the endothelium, the

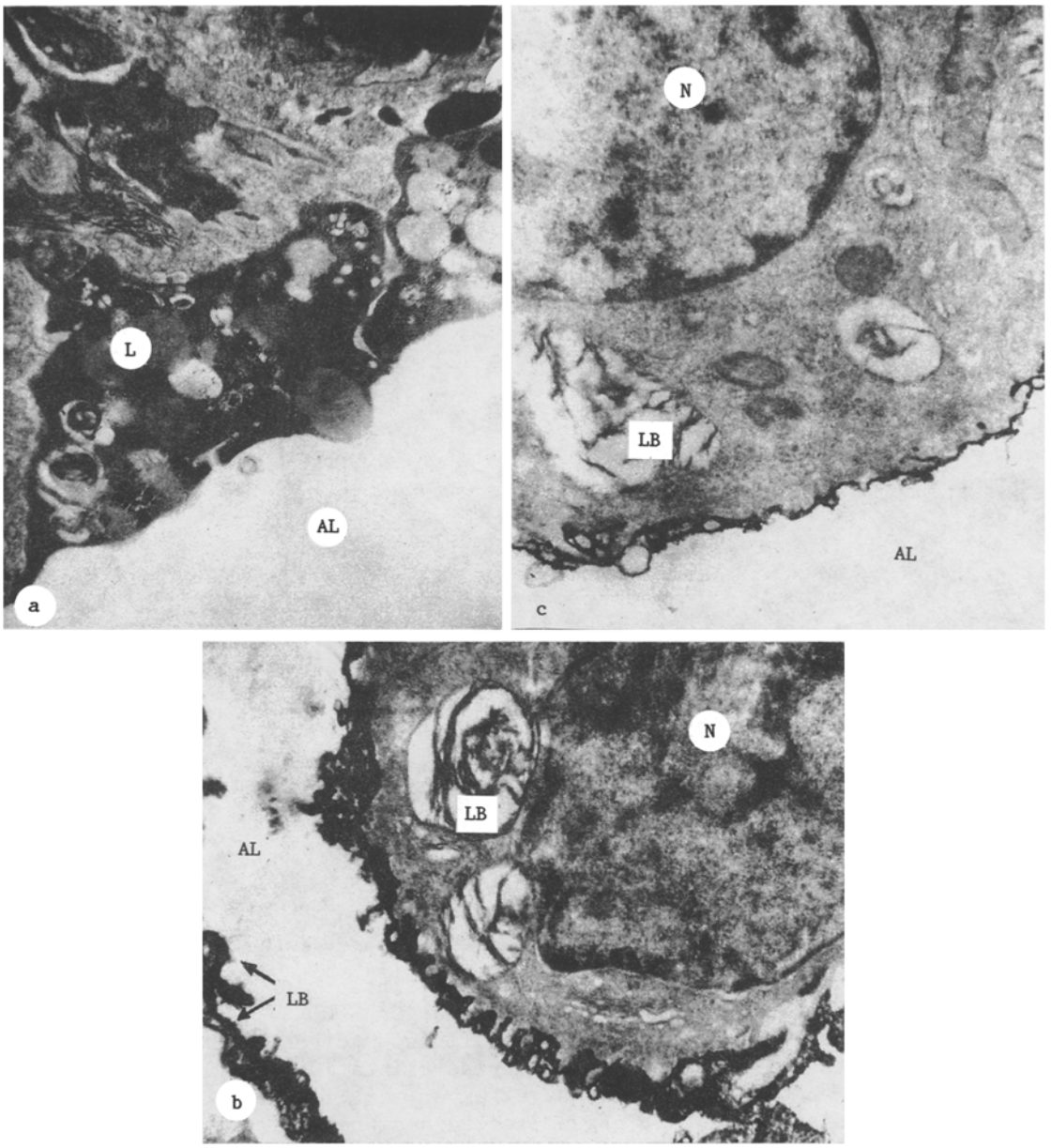

Fig. 2. Changes in lungs of diabetic rats. a) 30th day of experiment: cytoplasm of polymorph contains large lipid drops (L), leukocyte in close contact with type I pneumocyte (PI). 6000 ×. b) Intact lung; product of reaction with ruthenium red can be seen as a dense layer on microvilli of a type II pneumocyte. 14,000 ×. c) 30th day of experiment: thinning of glycocalyx on smoothed surface of type II pneumocyte. 14,000 ×.

basement membrane, and intestitial tissues gave no reaction, due to nonpermeability of the intact alveolar surface layer for ruthenium red. This was proved by experiments in which, after injection of the incubation solution inside the vessels, the glycocalyx of the endothelium could be seen, but the pneumocytes did not react [2]. Investigation of the glycocalyx of the pneumocytes in rats with diabetes revealed a reduction in its thickness, and some loosening of the structure or (occasionally) its complete absence in some parts of the plasmalemma. Similar changes, but starting on the 4th day, were found only in type II pneumocytes.

On the 7th day of the experiment the level of glycosuria did not always reflect the severity of the animals' clincial state (n = 4). Some swelling of the type II pneumocytes was observed microscopically, due to marked dilatation of tubules of the RER (Fig. 1c). The reaction of the glycocalyx with ruthenium red was weaker on the microvilli of the type II pneumocytes, the electron-dense layer was reduced in thickness and looser in texture

in some places, as was clearly visible on comparison with the reaction of the glycocalyx and the type I pneumocytes. Sludging of the erythrocytes and accumulation of platelets, in close contact with the endothelium, were seen in the capillaries.

On the 15th day intensification of the structural changes in the lungs was observed. Marked dilatation of tubules of the RER, with migration of organelles and nuclei toward the periphery, was observed in many type II pneumocytes, the number of lamellar corpuscles was frequently increased, and they merged with one another to form curiously shaped lamellae, and the microvilli in these cells had almost completely disappeared (Fig. 1d, e). The reaction with ruthenium red was often considerably reduced on type II pneumocytes. The alveolar macrophages and polymorphonuclear leukocytes (polymorphs) showed marked changes, with a sharp increase in their phagocytic activity but, probably, a decrease in their digestive power. Many phagocytosed lamellar bodies and large lipid drops appeared in their cytoplasm (Fig. 2a).

On the 30th day the general state of the animals (n = 2) was of average severity, but on the 60th day, one animal was in a serious state. Microscopically, the number of areas in which the alveolar walls were almost completely lined by type II pneumocytes remained increased. On electron microscopy the same changes were found in the type II pneumocytes as at the previous time of the experiment. The state of the glycocalyx of the type II pneumocytes, with their smooth surface, shortened microvilli, and dilated tubules of RER, was characterized by a weaker reaction with ruthenium red and by a decrease in its thickness (Fig. 2c). In the late stages of the experiment, it became very difficult to differentiate the altered type II pneumocytes from alveolar macrophages and polymorphs, whose cytoplasm constantly contained an increased number of phagocytosed lamellar bodies and collections of lipid drops (Fig. 2a).

In experimental diabetes mellitus considerable changes arise in the structure, primarily of the type II pneumocytes. The most characteristic and constant feature of the changes in these cells was marked dilatation of the tubules of RER. Plopper and Morishige [13] counted the number of type II pneumocytes with dilated tubules of RER on the 14th day in diabetic rats: There were 67 such cells in a total of 81. We found dilatation of tubules of the RER as early as on the 4th day of the experiment; later, until the end of 2 months of observation, these changes not only were still present, but were increased in severity. Evaluation of the cause of the abrupt dilatation of the tubules of RER in the literature is unequivocal. It is associated with inhibition of secretion and synthesis in type II pneumocytes. For instance, after administration of puromycin, an inhibitor of protein synthesis [4], to animals marked dilatation of the tubules of RER was observed in the type II pneumocytes. Meanwhile, the morphology of the lamellar bodies in most cases was the same as that in intact rats. The presence of unchanged lamellar bodies and of marked dilatation of RER can probably be regarded as evidence, not of blocking of biosynthesis in RER, but only of a decrease in the activity of certain enzymes of the network. In particular, a decrease in acetyl-CoA-carboxylase and fatty acid synthetase [5], enzymes located in RER, has been established. One result of this is undoubtedly a decrease in the oxidation of glucose [10] and in the rate of its incorporation into neutral lipids and phospholipids [11] in the lungs of diabetic rats; inhibition of leucine utilization also has been observed [10]. The view can be accepted that insulin plays a key role in the normal function of RER of type II pneumocytes [6, 13].

The glycocalyx is supposed to play an active role in cell permeability, to maintain the linkage between cells, to stabilize the cell membrane, and to control intercellular interactions. The glycocalyx contains in its composition specific receptors which determine the antigenic and immunogenic properties of the cells. It has been suggested that polysaccharides of pneumocytes, present in the composition of surfactant, perform the stabilizing role of this compound [8], bind large amounts of water, and form the aqueous phase of the surfactant complex. Changes which we observed in the type II pneumocytes of diabetic rats are evidence of hypoproduction of the supramembranous substance, which is manifested by thinning of the glycocalyx. Experiments [15] have shown reduction of secretion and diminished incorporation of phosphatidylcholine by 36% and of phosphatidylglycerol by 66% into the surfactant of diabetic rats. A decrease in the surfactant content lowers the surface tension of the alveoli and may contribute to the development of various lung injuries [1].

The pathological process in the lungs of diabetic rats also is aggravated by a deficiency in the phagocyte system – polymorphs and alveolar macrophages. Diabetic patients are well known to be prone to serious infectious diseases. Investigations on diabetics have demonstrated the functional insufficiency of the polymorphs and, in particular, a decrease in chemotaxis [9] and phagocytic activity [12, 14].

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ULTRASTRUCTURE OF THE DUODENAL EPITHELIAL CELLS IN THE EARLY STAGES OF EXPERIMENTAL COLIBACILLOSIS

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UDC 616.98:579.842.11]-092.9-07: 616.342-018.7-076.4

KEY WORDS: colibacillosis; duodenum; brush-border epitheliocytes; endocrine cells; adenylate cyclase

The leading role in the diarrheal syndrome caused by enterotoxigenic strains of Escherichia coli is ascribed to the thermolabile enterotoxin of these bacteria. The enterotoxin, binding firmly with the specific receptor of the apical plasmalemma of the epithelial cell, activates adenylate cyclase [7-9], as a result of which the cAMP concentration is increased and secretion of water and electrolytes stimulated. Activation of the adenylate cyclase of the epithelial cells also takes place as a result of the action of certain gastrointestinal hormones of the endocrine cells of the gastrointestinal tract [9]. The endocrine cells are known to secrete their contents either into the vascular system or into the interstitital space, through which they exert their action on neighboring target cells [6]. Endocrine cells of the APUD system of the gastrointenstinal tract have been shown to be most numerous in the proximal part of the small intestine [5], which is the most sensitive part to the action of the enterotoxins of E. coli [10]. Although the ultrastructure of the epithelial cells of the small intestine has been studied in some intestinal infections, problems concerned with changes in the duodenum in colibacillosis, especially in the earliest stages of the disease, remain virtually unstudied.

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(Presented by Academician of the Academy of Medical Sciences of the USSR N. K. Permyakov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 109, No. 1, pp. 78-82, January, 1990. Original article submitted June 12, 1989.

0007-4888/90/0001-0102$12.50