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CHANGES IN THE NEPHRON AND NEUROENDOCRINE APPARATUS OF THE KIDNEYS AFTER INJECTION OF SALMONELLA ENDOTOXIN

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One of the most important factors in the pathogenesis of alimentary toxic infections is endotoxemia. Many aspects of the action of salmonella endotoxin that lead to disturbance of homeostatis have already been established. For instance, endotoxin is known to damage the endothelium, which leads to the release of tissue thromboplastin [6], activated prothrombin, and causes intravascular blood clotting and also stimulates the production of prostaglandin E [5]. Besides the hemodynamic disturbances connected with intravascular coagulation, it also leads to the development of trophic changes, edema, and cellular infiltration of organs [1, 3, 6]. The most marked changes arise in the kidneys. Previous investigations have not been aimed at the discovery of the intimate mechanisms of action of salmonella endotoxin and have been mainly descriptive in character. In particular, no morphological evidence has been obtained in support of the fact that endotoxin stimulates the synthesis of prostaglandin E, which is known to be produced by the interstitial cells (IC) of the renal medulla.

The object of this investigation was an ultrastructural analysis of the effect of salmonella endotoxin on different parts of the nephron and on the neuroendocrine apparatus of the kidneys.

EXPERIMENTAL METHOD

Experiments were carried out on 12 rabbits weighing 2.5-3 kg, into which the endotoxin of *Salmonella typhimurium*, purified by Boivin's method, was injected intravenously in a dose of 2 mg/kg. The animals were killed (six at a time) 3 and 24 h later by injection of air into the auricular vein. The body weight of the rabbits 24 h later had fallen on average by 500 g because of diarrhea. The control group consisted of six rabbits. Pieces of renal cortex and medulla were fixed in 1% OsO_4 solution, dehydrated in alcohols of increasing strength, and embedded in Araldite. Light-optical investigation of the material and counting of the lipid granules in IC (50 cells from each animal) were carried out on semithin Araldite sections stained with methylene blue—azure II—fuchsin. To characterize activity of the juxtaglomerular apparatus (JGA) a quantitative method of assessment of granule formation on electron micrographs was used; our previous investigations [2] showed that this method objectively reflects the degree of activity of the JGA. Guided by results obtained by other workers [7], granules of three types and also intermediate forms, depending on the density of the sub-

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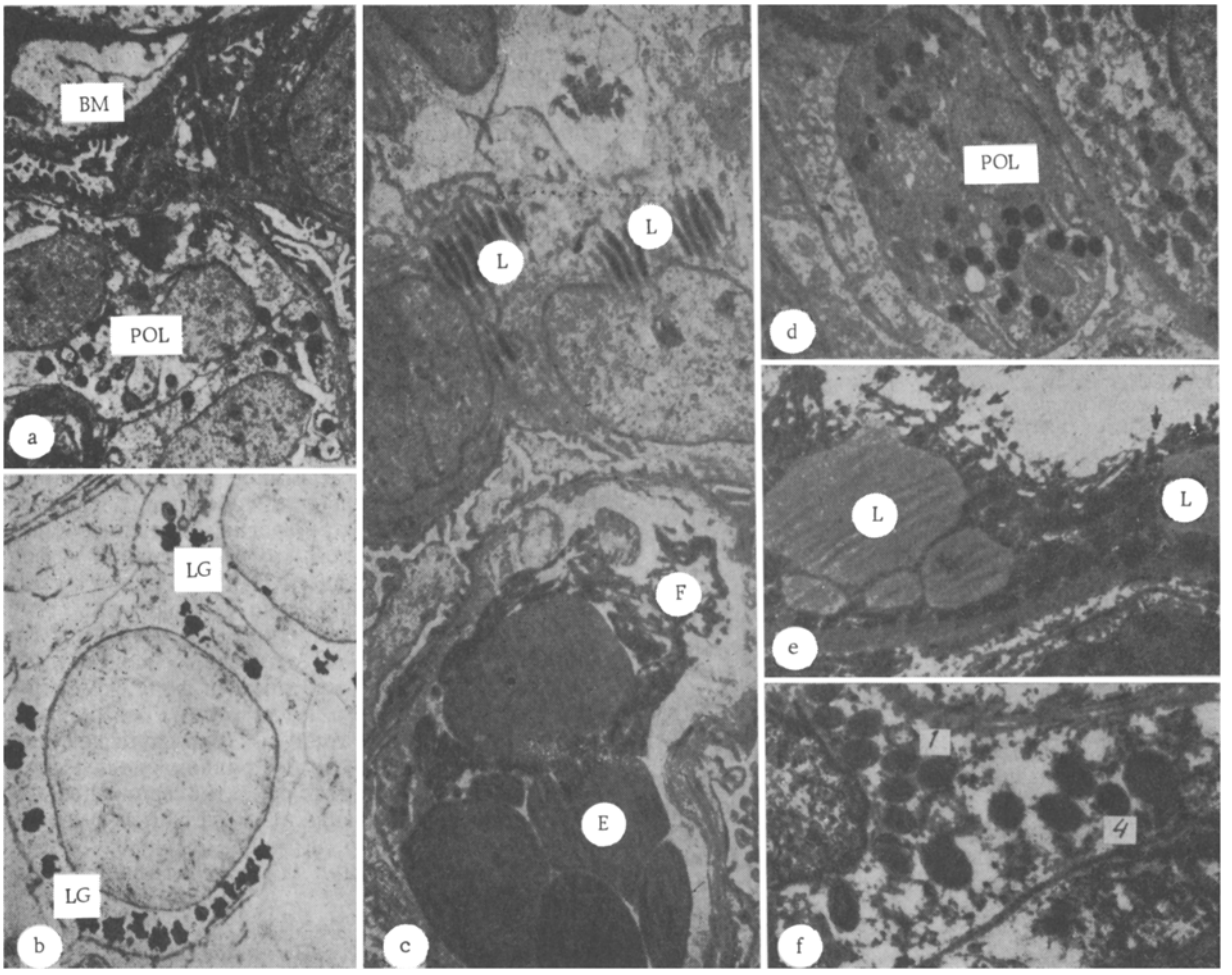

Fig. 1. Changes in kidneys after injection of salmonella endotoxin: a) polymorph (POL) with sequestrated lysosomes in lumen of glomerular capillary, BM) Basement membrane of glomerulus. 3 h after injection of endotoxin, 4000 ×; b) accumulation of lipid granules (LG) in IC 3 h after injection of endotoxin, 4000 ×; c) erythrocytes (E) and fibrin (F) in lumen of glomerular capillaries. Lipid inclusions (L) in cytoplasm of mesangial cells, 24 h after injection of salmonella endotoxin, 6000 ×; d) polymorph (POL) with sequestrated lysosomes in lumen of peritubular capillary, 24 h after injection of endotoxin, 4000 ×; e) destruction of microvilli (arrows, on apical surface of nephrocytes of proximal tubule, lipid inclusions (L) in their cytoplasm, 24 h after injection of endotoxin, 4000 ×; f) granules of first (1) and fourth (4) types in cytoplasm of endothelial cells of JGA 24 h after injection of salmonella endotoxin, 15,000 ×.

stances composing them, were counted in the epithelioid cells of the JGA of the rabbits, and it was considered that these various types and forms reflect successive stages of granule formation. The density of the contents of the granules was analyzed by means of a microdensitometer (Joyce Loebl, Great Britain), with a resolving power of 10 μ. Measurements of optical density of the granules on photographic plates were carried out in two mutually perpendicular directions passing through the center of the granule; the ratio between mean amplitude of the optical density curve of the granules and the background value (density of the photographic plate) were calculated. Statistical analysis revealed four characteristic types of granules ($P < 0.05$). Granules of the first type had relatively translucent contents, with density approximately the same as the background; group 2 consisted of granules with areas of condensation of the contents; group 3 of dense granules surrounded by a narrow pale rim; and group 4 were uniformly electron-dense granules. For objective description a rating factor (K) was attached to each of these types of granules: $K_1 = 0.1$, $K_2 = 0.5$, $K_3 = 0.6$, $K_4 = 1.0$. The coefficient of density of the granules (T) was calculated by the following equation:

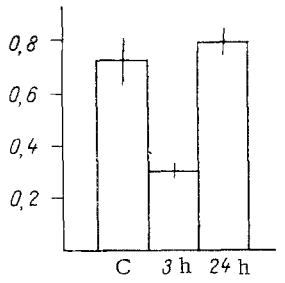

Fig. 2

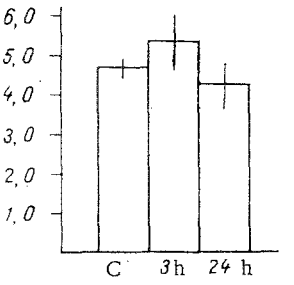

Fig. 3

Fig. 2. Changes in density of granules in epithelioid cells of JGA 3 and 2 h after injection of **salmonella** endotoxin. Here and in Fig. 3: C) control.

Fig. 3. Changes in number of granules in IC of renal medulla 3 and 24 h after injection of salmonella endotoxin.

$$T = \frac{\sum_{i=1} K_i n_i}{\sum_{i=1} n_i},$$

where n is the number of granules assessed. Analysis of the values of the coefficient thus obtained showed that the closer its value to unity, the more mature electron-dense granules (type 4) were present in the JGA.

EXPERIMENTAL RESULTS

Slowing and vacuolation of the cytoplasm of the endothelial cells of the glomerulus, an increase in the number of polymorphs with sequestrated lysosomes in the capillaries of the glomerulus, and the appearance of fibrin (Fig. 1a) were observed 3 h after injection of salmonella endotoxin; sludging of erythrocytes was observed in the peritubular capillaries. Moderate edema of the interstitial tissue of the renal medulla was observed. The nephrocytes were swollen, with electron-transparent cytoplasm, with vacuolated mitochondria and endoplasmic reticulum. Vacuolation of the endoplasmic reticulum, swelling of the mitochondria, and translucency of the cytoplasm also were observed in the epithelioid cells of the JGA; most granules were oval or round in shape, with contents of low electron density. Nerve endings on the epithelioid cells were swollen. The number of granular synaptic vesicles containing adrenergic mediator was reduced.

Mathematical analysis of granule formation in **the JGA revealed** a decrease in the coefficient of density of the granules to 0.44 ± 0.04 compared with the control value of 0.71 ± 0.1 (Fig. 2). Counting the number of granules in IC of the medulla showed that the increase in their number to 5.09 ± 0.7 was not statistically significant compared with the control — 4.91 ± 0.25 (Fig. 3). Lipid granules were seen to accumulate in some IC **(Fig. 1b).**

Congestion of the capillaries of the glomeruli was observed 24 h after injection of salmonella endotoxin, with the accumulation not only of erythrocytes, but also of platelets, polymorphs, and fibrin in the glomeruli. Lipid inclusions were found in the cytoplasm of the mesangial cells and in some of the podocytes. The endothelial cells were swollen and vacuolated (Fig. 1c). Collections of polymorphs with sequestrated mesosomes were seen in the peri**tubular capillaries** (Fig. 1d). Some nephrocytes of **the proximal** tubules were without microvilli, and large lipid drops could be seen in the cytoplasm of the cells (Fig. 1e). Hydropic degeneration was observed in the nephrocytes in other parts of the nephron. Granular or hydropic degeneration also developed in the cells of the macula densa and the epithelioid cells **of the** JGA. The granules consisted almost entirely of type 1 (Fig. 1f). Some IC had a denser cytoplasm than normally, with reduced processes, whereas others appeared swollen and had a vacuolated endoplasmic reticulum, swollen mitochondria, and solitary granules in their cytoplasm. Just as 3 h after injection, nerve endings in JGA were swollen but the number of adrenergic synaptic vesicles was reduced. Estimation of the density of the granules at this stage of the experiment revealed an even greater decrease in the coefficient T — down to 0.29 ± 0.1 (Fig. 2).

This investigation of the kidneys at different times after injection of salmonella endotoxin thus revealed some new facts not described previously [3, 6]. The results of the use of the suggested method for quantitative analysis of the changes in JGA and IC under the influence of the endotoxin demonstrate that these structures are concerned in the hemodynamic disturbances. The increase in activity of the JGA, especially after 24 h, when signs of intravascular coagulation were more marked, could be a sign of a response to the developing hypoxia. The decrease in the number of granules in IC was connected, on the one hand, with activation of the JGA and, on the other hand, possibly with the direct action of the endotoxin which, as we know, stimulates prostaglandin synthesis [4, 5]; the fatty degeneration of the mesangial cells, podocytes, and nephrocytes discovered now for the first time requires special anaysis.

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