STRUCTURAL CHANGES IN THE KIDNEYS IN EXPERIMENTAL *Yersinia pseudotuberculosis* INFECTION

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In pseudotuberculosis the urinary system is only slightly affected, and only if marked toxemia and fever are present is kidney function disturbed; this disturbance is regarded as the result of the toxicoinfectious action of *Yersinia pseudotuberculosis* and its metabolic products [2]. The causative agent of pseudotuberculosis in such patients is excreted not only with the feces, but also with blood and urine [2, 5]. Examination of biopsy specimens from the kidneys of patients infected with *Y. enterocolitica*, with a clinical picture of nephritis, has revealed proliferative glomerulonephritis [4, 6-9].

The aim of this investigation was an experimental study of structural changes in the kidneys of rabbits infected perorally with *Y. pseudotuberculosis*.

EXPERIMENTAL METHOD

Experiments were carried out on 26 rabbits weighing 2-2.5 kg receiving *Y. pseudotuberculosis* strain 1179 serovar 1 per os in a dose of 10^8 bacterial cells. The animals were autopsied starting from 30 min and until 21 days after infection. Material was fixed in 10% neutral formalin and embedded in paraffin wax; sections were stained with hematoxylin and eosin. Kidney fragments measuring 1 × 1 mm were fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer, postfixed in 1% OsO_4 solution, dehydrated in alcohols, and embedded in Araldite. Semithin sections 1 μ thick were cut on the LKB Ultrotome and stained with methylene blue. The sections were examined in the Orthoplan light microscope under a magnification of 1600.

EXPERIMENTAL RESULTS

At autopsy on rabbits from 3 to 14 days after infection with *Y. pseudotuberculosis*, besides the characteristic inflammatory and necrotic changes in the intestine, mesenteric lymph nodes, liver, spleen, and lungs, involvement of the urinary organs also was found. The bladder was distended with cloudy urine. On the 5th-7th days moderate hematuria was observed and the urine was thick with an abundant deposit. The mucous membrane of the bladder was hyperemic and the kidney substance congested. Histologic investigation of kidney sections stained with hematoxylin and eosin revealed vasodilatation in the cortex and medulla, diapedetic hemorrhages, and dystrophic and necrotic changes in the epithelium of the convoluted tubules, which were invaded by yersinias, particularly abundantly on the 3rd-10th days. On the 14th-21st day after infection the interstitial tissue of the renal cortex and medulla of some animals was infiltrated with lymphocytes and histiocytes.

Examination of semithin sections revealed finer changes in the kidneys, which were detectable at the earliest times of infection with pseudotuberculosis, involving not only the tubules, but also the vascular loops of the glomeruli. During the first hours of infection, a few rod-shaped yersinias were found in the lumen of the interlobular vessels and capillary loops of the glomeruli, and some of the bacilli were adherent to the surface of the endotheliocytes. A few bacilli were seen in the lumen of the proximal convoluted tubules. From 12 to 18 h after infection dystrophic changes were found in the epithelium of the convoluted tubules

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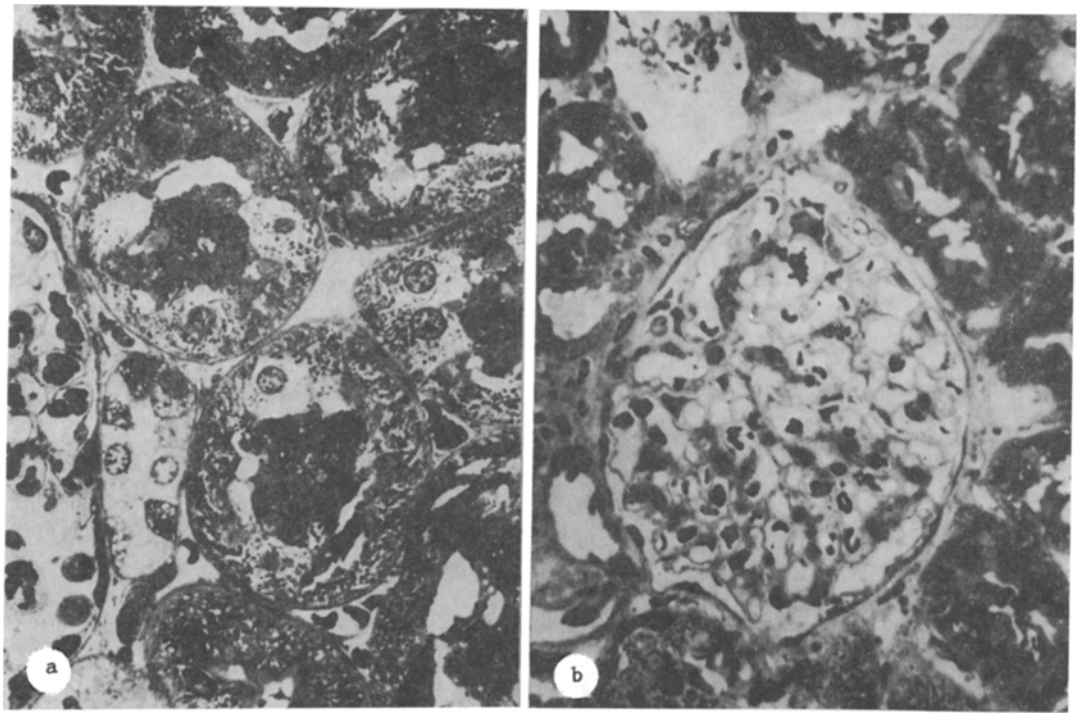

Fig. 1. Histopathologic picture of kidney lesions caused by *Yersinia pseudotuberculosis*: a) Yersinias in epitheliocytes of convoluted tubules with destruction (1000 ×); b) concentration of yersinias in lumen of venules in renal cortex (640 ×). Here and in Fig. 2: semithin sections, methylene blue.

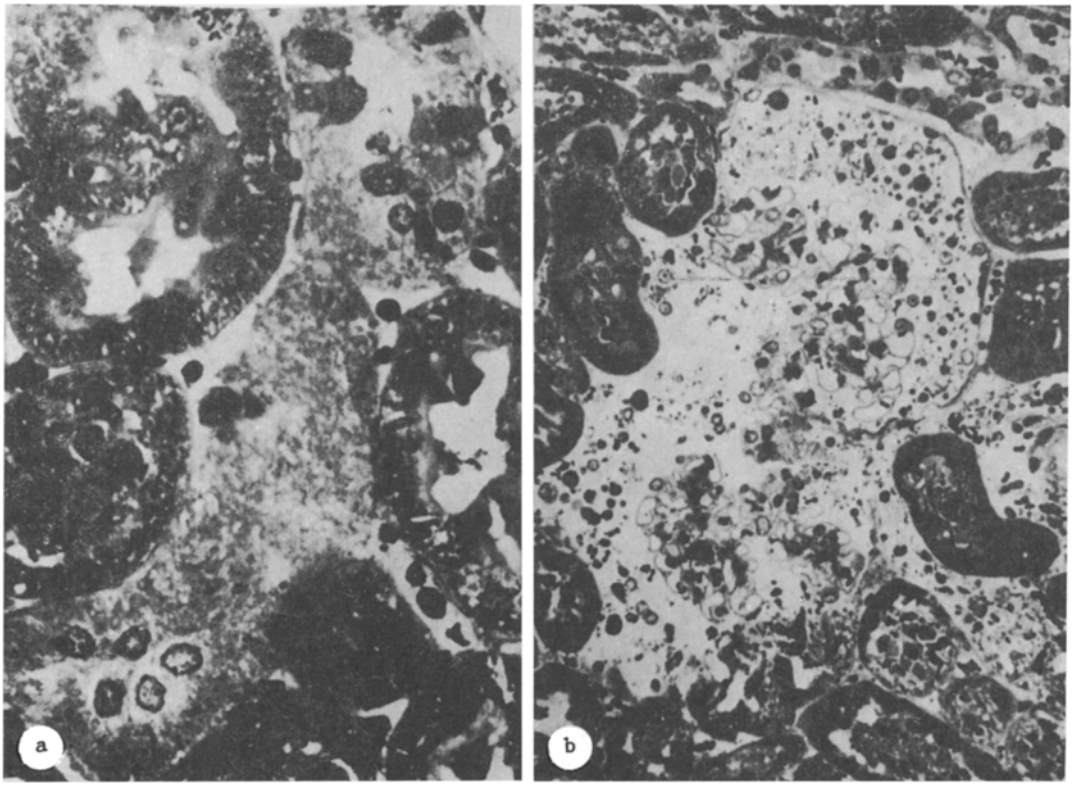

Fig. 2. Histopathologic picture of kidney lesions caused by infection with *Yersinia pseudotuberculosis*: a) Necrotic nephrosis, cytolysis of tubular epithelium (1600 ×); b) "half-moon" in lumen of glomerular capsule, microorganisms and erythrocytes in interstitial tissue (400 ×).

of the kidneys, associated with invasion and intraepithelial multiplication of the yersinias. More marked changes were observed in the proximal convoluted tubules. In one case different stages of involvement of the epithelium of the tubules could be detected (Fig. 1a). In the first stage, in the basal part of the epitheliocytes multiplication of yersinias was observed, with the formation of self-like masses and displacement of the nucleus into the apical part of the cell. The epitheliocytes were not destroyed and nucleoli were present in their nuclei. The intraepithelial yersinias were in the form of coccobacilli. In the second stage destruction of the epitheliocytes took place, with the discharge of numerous coccoid yersinias into the lumen of the tubules; among them could be seen fragments of destroyed epitheliocytes, erythrocytes, and microscopic casts, in the form of homogeneous round formations.

From 1 to 3 days after infection the number of microorganisms in the capillaries of the renal glomeruli and in the intertubular vessels was greater than at the previous time (Fig. 1b). Massive dissemination of yersinias could be seen in the proximal and distal convoluted tubules and the loops of Henle. The cytoplasm of the epitheliocytes was vacuolated and fragmented. No epithelial nuclei could be seen in some tubules. Differences in the staining properties of the epitheliocytes were noted: intense or pale staining of the cells.

On the 4th-5th day serious disturbances of the microcirculation and destructive changes in the epithelium of the convoluted tubules were observed. The intertubular capillaries were dilated, and erythrocytes formed rouleaux (sludge syndrome). Marked edema of the interstitial tissue and diapedesis of erythrocytes were observed, with necrobiotic changes and metachromasia of the walls of the blood vessels, which contained both viable and degenerated yersinias in their lumen. In the interstitial tissue between the glomeruli and convoluted tubules there were many microorganisms, bacilli but mainly coccoid forms. Destruction of the tubular epithelium reached the degree of necrosis (Fig. 2a). In tubules with severe epithelial dystrophy the borders of the cells were invisible and they formed something resembling a syncytium, filled with multiplying bacteria, and the solitary remaining epithelial nuclei were displaced toward the apical part of the cells. Most convoluted tubules and loops of Henle were tightly packed with masses of microorganisms, casts, and desquamated epitheliocytes. Heterogeneity of the epithelium was found in tubules with less marked destruction, very pale cells with large vacuoles containing many microorganisms, and also darker, hyperchromic cells, with signs of clasmatosis, were present.

Besides changes in the tubular epithelium, involvement of the renal glomeruli also was found; their capillaries revealed a sludge syndrome, and a few microorganisms were present in the cytoplasm of the mesangial cells. Severe destruction of capillary loops was found in some glomeruli, with "half-moons" consisting of collections of microorganisms, cell debris, desquamated epithelium, erythrocytes, and single polymorphonuclear leukocytes, present in the lumen of Bowman's capsule (Fig. 2b). The capsular epithelium of the glomerulus was greatly swollen.

Multiplication of microorganisms in the tubular epithelium and destructive changes in the kidneys were visible until the 21st day (time of obervation) after infection, and repair processes were observed after the 14th day.

These investigations revealed for the first time the marked affinity of *Y. pseudotuberculosis* for the epithelium of the renal tubules. In the early times of infection (in the presence of bacteriemia) the microorganisms disseminate into kidney tissue, and in the composition of the glomerular filtrate they enter the lumen of the tubules, where they penetrate into the epithelium, especially of the proximal part of the nephron, where they multiply intensively and cause destruction of the epitheliocytes, leading in some cases to necrotic nephrosis. On destruction of the epitheliocytes the yersinias are disseminated in the lumen of the tubules, which may explain the bacteriuria and pathological changes in the urine. The presence of microorganisms in the kidneys continued for a long time, ending with severe disturbance of the microcirculation, penetration of yersinias into the interstitial tissue, and the probability of their re-entry into the bloodstream, thus maintaining the bacteriemia. Involvement of the glomeruli in the form of extracapillary glomerulonephritis, caused by the cytopathic action of *Y. pseudotuberculosis* and its antigens, was discovered for the first time. Destructive changes in the tubular apparatus of the nephrons may cause a long-term disturbance of kidney function, which is found in some cases of infection in man in the form of hypo-isosthenuria, and sometimes acute renal failure. Disturbance of tubular secretion is confirmed by the results of radioisotope renography [1]. The results are evidence of the epidemiologic hazard from the urine of pseudotuberculosis patients, and this must be taken into consideration in clinical practice.

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TRANSFORMATION AND ULTRASTRUCTURAL CHANGES OF ERYTHROCYTES ON SENSITIZATION TO SERUM PROTEIN

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Investigation of transformation of erythrocytes (loss of their usual discoid shape) has revealed many different varieties of these cells in blood diseases, carcinogenesis, and surgical suppurative infection [2, 3, 5]. However, as yet insufficient attention has been paid to morphological changes in erythrocytes in response to injection of a foreign protein, although the role of these cells in immunologic reactions is extremely important [1, 4].

In the investigation described below the shape and ultrastructure of erythrocytes was studied during sensitization of laboratory animals with normal horse serum (NHS).

EXPERIMENTAL METHOD

Experiments were carried out on 40 male Wistar rats (20 experimental and 20 control) weighing 220 ± 21 g. NHS, diluted 1:2 with 0.15 M sodium chloride solution, was injected subcutaneously into the dorsal region in a volume of 1.8-2.4 ml (dose rate 0.6 mg protein/100 g body weight). Control animals were injected with the same volume of sodium chloride solution. Twice the volume of NHS or physiological saline was injected 7 and 22 days later. The use of these doses of serum as sensitizing and reacting doses was chosen because with them the anaphylactic reaction which developed was mild or moderately severe, anaphylactic shock was not observed, and all the animals survived until the end of the experiment. Blood (1 ml) was taken from the tail vessels of the animals 30 min after injection of the reacting dose of NHS and also during the background investigation. As a control of the degree of sensitization of the animals, the leukocyte count and leukocyte formula were determined, with calculation of the morphologic reactivity index (MRI), whereby the intensity of the hemoimmune response of cellular type (HIRCT) could be estimated, the number of cells producing autoantibodies (hemolysins) was studied [4], and the number of circulating immune complexes (CIC) determined [6].

Erythrocytes were sedimented by centrifugation and washed off three times with cold (to 4°C) 0.15 M phosphate buffer, pH 7.2. Next, 0.25 ml of the cell residue was incubated at 4°C for 3 h in 2.5 ml of 0.25% glutaraldehyde solution in phosphate buffer, postfixed for 1.5 h in 1% OsO_4 solution, dehydrated in alcohols of increasing concentration and acetone, and embedded in Araldite. Ultrathin sections were examined in the JEM-100 CX electron microscope. Prepara-

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