

# FUNCTIONAL AND MORPHOLOGICAL CHANGES PRODUCED IN THE KIDNEYS OF RABBITS BY AN EXPERIMENTAL IMMUNOPATHOLOGICAL PROCESS

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The mechanism of osmotic concentration of the urine and the role of antidiuretic hormone (ADH) in this process has been intensively studied during recent years. A. G. Ginetsinskii considers that the hyaluronidase-hyaluronic acid system is an important link in the mechanism of the ADH effect [2].

In the present investigation a model of a pathological process in which kidney function was disturbed by the action of immunological factors uncomplicated by infective lesion is described. In these conditions, the glomerular and tubular function was studied in the animals and particular attention was paid to assessment of the concentrating mechanism.

## EXPERIMENTAL METHOD

Experiments were carried out on noninbred rabbits weighing 2-3 kg. Twelve animals received an intravenous injection of a  $\gamma$ -globulin preparation prepared from the serum of rats immunized with an extract of rabbit kidney homogenate. Two other rabbits received an injection of a cytotoxic rat serum against rabbit kidney instead of  $\gamma$ -globulin. The animals were divided into the following groups.

Group 1 consisted of 8 rabbits receiving a single injection of 0.5-2.0 ml of cytotoxic globulin. Four of these animals, which received 2 ml of globulin each, were investigated from the 11th to the 22nd day after the injections; the other four rabbits, each of which received 0.05 ml of immune globulin, were investigated on the 95th day. Group 2 consisted of 6 rabbits receiving injections of 5-7 ml of  $\gamma$ -globulin or cytotoxic serum in the course of one week (three injections); these animals were investigated from the 12th until the 24th day after the last injection. At different times after the last injection of cytotoxic globulin or serum, the titers of antibodies against antigens in the rabbit kidney extract and against rat serum protein in the blood of these animals were determined by the complement fixation reaction.

To assess the kidney function of the experimental and the 13 control (intact) rabbits with a chronic fistula of the urinary bladder, the diuresis (in ml/min/kg body weight) was determined, the glomerular filtration was estimated by the inulin method [8], and the concentration of osmotically active substances in the urine and blood plasma was determined by cryoscopy using a semiconductor thermistor [1]. The sodium concentration was measured by a Zeiss-III flame photometer, and the hyaluronidase activity by a viscosimetric method [5]. These parameters were determined against the background of a water load and after injection of pituitrin P (intravenously in a dose of 0.5 i.u./kg body weight). At the end of the physiological observations, the kidneys were investigated histologically and histochemically (for the content acid mucopolysaccharides by the Ritter-Oleson method) [9].

## EXPERIMENTAL RESULTS

The level of glomerular filtration found in the experimental animals ( $2.5 \pm 0.24$  ml/min/kg) showed no significant variation from the normal level ( $2.47 \pm 0.5$  ml/min/kg). However, the animals investigated 12-25 days after injection of the immune preparations had a diuresis of not more than 0.25 ml/min/kg, while the osmotic concentrations of the urine did not exceed 600 ml/liter even with this low level of diuresis

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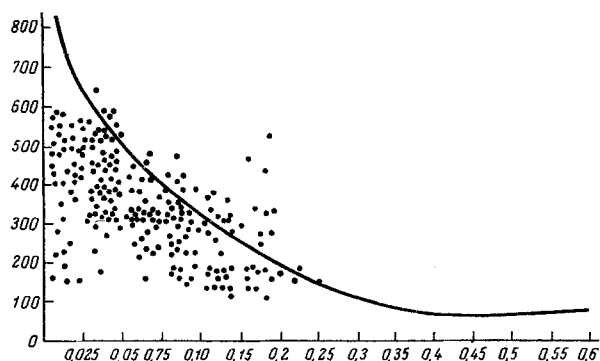


Fig. 1. Relationship between osmolar concentration of the urine level of diuresis in experimental (dots) and control (continuous line) rabbits. Abscissa: diuresis (in ml/min/kg); ordinate: osmolar concentration of urine (in ml/liter).

(Fig. 1). The curve showing the relationship between the concentration of osmotically active substances in the urine and the volume of the diuresis in normal conditions is shown in Fig. 1 for comparison. It may also be seen that the osmotic concentration of the urine varied within wide limits, but was independent of the diuresis. No abnormality was found in the rabbits investigated three months later.

The disturbance of the osmoregulatory function after injection of the cytotoxic antibodies also took the form of a sharp depression of the reaction to ADH. Intravenous injection of the hormone did not inhibit diuresis in the animals except in the rabbits examined 95 days later (Fig. 2). After injection of the hormone the inulin concentration index of the animals remained almost unchanged ( $81 \pm 16.7$  before injection and  $105 \pm 7.3$  after injection), whereas in normal rabbits in similar conditions it was increased twice or three times [3]. The ratio between the inulin concentration in the urine and its concentration in the blood characterizes the degree of reabsorption of water in the nephron. To assess the process taking place in the distal tubules, the ratio between the concentrations of osmotically active substances in the urine and blood was investigated. In these experiments this ratio likewise was unchanged after injection of ADH: before injection it was  $1.3 \pm 0.13$ , and after the injection  $1.4 \pm 0.1$  (in contrast to normal conditions when the concentration index of osmotically active substances was increased three times after injection of ADH) [3]. Consequently, the hyperosmotic reabsorption of water was disturbed. The absence of a sodium diuretic effect can also be taken as evidence of a lesion of the distal portion of the nephron. The sodium excretion was  $2.6 \pm 0.68$  meq/min before injection of pituitrin and  $2.4 \pm 0.70$  meq/min after injection.

According to A. G. Ginetsinskii's hypothesis, ADH acts on the kidney by stimulating excretion of the enzyme hyaluronidase. A reactivity to ADH in the present experimental conditions was accompanied by absence of hyaluronidase in the urine at all levels of diuresis. In normal conditions it was found when the diuresis was below 0.05 ml/min/kg. This demonstrated injury to the mechanism responsible for the appearance of hyaluronidase in the urine.

The serological investigations in all the rabbits from the 4th to the 17th day after injection of the cytotoxic antibodies revealed high concentrations of antibodies against rat protein. The sera reacted at these same times with extract from normal rabbit kidney. This must be attributed to the injected cytotoxic antibodies circulating in the blood stream and not to newly formed antibodies (of the autoantibody type). The heterologous character of the antibodies discovered is shown by the fact that the titer of anti-tissue antibodies did not rise but, on the contrary, fell until the 10th-14th day by comparison with its level on the 4th-6th day after injection of the cytotoxic antibodies. Only in the rabbits investigated later (until the 24th day) was it discovered that after the titer of anti-tissue antibodies had fallen, it rose again in the subsequent investigation (the possibility cannot be ruled out here that a proportion of the cytotoxic antibodies, which previously were fixed, have become detached). Hence, at the times when the kidney function was investigated, only the initial stages of the immunopathological process were present, resulting from the direct action of the cytotoxic antibodies and also from the action of immune complexes (between rat protein and antibodies against it). No serological changes were found in the rabbits investigated after 95 days.

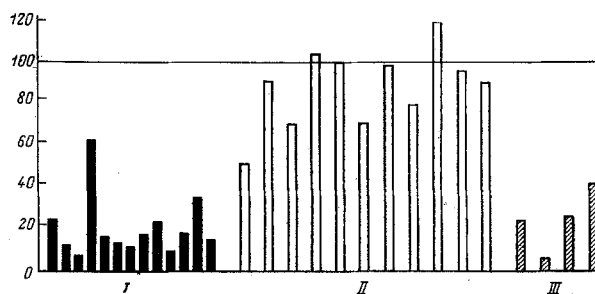


Fig. 2. Reaction of rabbits to injection of pituitrin against the background of water diuresis. I) Control; II) experimental rabbits 10-22 days after end of immunization. Vertical axis) changes in diuresis (in % of initial level). The horizontal line corresponds to the level of the initial diuresis, taken as 100.

The results of the morphological investigation of rabbits at necropsy 10-22 days after the injection of cytotoxic serum deserve great attention. No significant changes were found in the glomeruli, whereas in the proximal convoluted and straight tubules widespread vacuolar and granular degeneration of the epithelium was observed, accompanied in some places by marked dilatation of the lumen of the tubules, forming structures resembling cysts. Similar degenerative changes were found in the cells of the ascending limbs of the loops of Henle; these changes were less marked in the cells of the distal convoluted tubules and the collecting tubules.

It has been shown [7, 11] that in the days following injection of cytotoxic anti-kidney antibodies the kidney function is disturbed, as judged by the presence of proteinuria. When the kidneys of the experimental animals were investigated 50 days or more after the end of the injection [10], gross changes were found in the kidneys, with shrinking of the glomeruli in some cases. These changes are associated with the action of the antibody-antigen complex on the basement membranes of the glomerular capillaries at a time of high antibody production against the foreign protein of the injected cytotoxic sera [11]. The period of the process at the 2nd-3rd week after the last injection of the cytotoxic serum has so far been studied least. According to the results of the present investigation, this period is characterized by a morphological and functional lesion of the tubular part of the nephron. The functional changes are associated, in particular, with disturbance of the ability of the kidney to react to ADH. This could be dependent either on disturbance of the secretion of hyaluronidase or on a change in the acid mucopolysaccharides in the structures of the distal portion. In contrast to the results obtained in experiments on rabbits with chronic experimental nephritis [3], in the conditions of the "pure" immunopathological model of the kidney lesion the acid mucopolysaccharides in the medulla were relatively intact, and they completely retained their ability to be depolymerized by exogenous (testicular) hyaluronidase.

It may therefore be concluded that one of the causes of the reactivity of the kidney to ADH in the conditions of an immunopathological process is disturbance of the mucolytic system in the distal tubules and collecting tubules responsible for osmotic concentration of the urine. These changes are evidently associated with inability of the kidneys to secrete hyaluronidase.

#### LITERATURE CITED

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