

CHANGES IN THE HYALURONIDASE-HYALURONIC ACID
SYSTEM IN THE KIDNEYS OF RABBITS WITH
EXPERIMENTAL INTERSTITIAL NEPHRITIS

Corresponding Member AMN SSSR the late A. G. Ginetsinskii,
M. G. Zaks, V. I. Ioffe, T. V. Krestinskaya, M. M. Sokolova,
and L. M. Khai

Institute of Evolutional Physiology (Director — Corresponding Member AN SSSR
G. M. Kreps) of the AN SSSR, and Institute of Experimental Medicine
(Director — Active Member AMN SSSR Professor D. A. Biryukov) of the
AMN SSSR, Leningrad

Translated from *Byulleten' Éksperimental'noi Biologii i Meditsiny*, Vol. 57, No. 3,
pp. 30-34, March, 1964

Original article submitted March 20, 1963

Previous investigations [2, 3, 4] have shown that the action of antidiuretic hormone (ADH) takes place as follows. The hormone stimulates the secretion of hyaluronidase in the renal tubules; the enzyme depolymerizes hyaluronic acid in the intercellular junctions of the epithelium of the collecting tubules and of the interstitial tissues; as a result the walls of the tubules become permeable to water, and water is reabsorbed along the osmotic gradient. Evidence was obtained suggesting that in diseases of the kidney this mechanism was disturbed [1]. The object of the present investigation was to analyze the changes in kidney function taking place in certain experimentally induced pathological states of the kidneys.

EXPERIMENTAL METHODS

Experiments were carried out on 36 rabbits weighing 2.0-2.5 kg. The animals were inoculated with an attenuated culture of *Streptococcus haemolyticus* (group A, type 12), by intravenous injection of 1-2.5 billion bacterial cells. During the next 3-5 days the microorganisms disappeared from the blood and spleen, but seedings from the kidneys and urine continued to give a rich growth for 10 days. After this period positive results became less common, and after 3-4 weeks seedings from the kidneys and urine yielded no growth. Hence, this particular culture in fact exhibited selective nephrotropic properties, and this was confirmed by the results of serological analysis. During the first days after inoculation a streptococcal antigen was found in the blood, and its concentration, initially very small, soon increased to reach its maximum on the 6th day. Thereafter it gradually fell as the antibodies began to appear, and the latter persisted for up to 2 months. An antigenic curve of this character indicated the presence of an established infection. In some animals investigated 1 month or later after inoculation, the sera gave a positive complement fixation reaction also with an extract of rabbit kidney, which could be attributed to the appearance "autoantibodies" as a result of injury to the kidney tissue. Since the physiological and morphological evaluation of the kidneys began 40-50 days after inoculation, it may be postulated on the basis of the microbiological and immunological data that at this moment the rabbits were practically free from microorganisms causing kidney damage, but the processes of kidney destruction for which they were responsible had not finally subsided and continued to maintain an immunopathological reaction.

The following indices were used to assess the state of the kidneys: 1) the relationship between the osmotic concentration of the urine (U_{osm}) and the magnitude of the diuresis; U_{osm} was determined cryoscopically [6]; the diuresis was varied by injecting water (5% of the body weight) into the small intestine; urine was collected through a permanent vesical fistula; 2) sensitivity to pituitrin P (ADH); the hormone was injected intravenously in a dose of 0.5 milliunit/kg body weight of the rabbit at the height of water diuresis; 3) the concentration of hyaluronidase in the urine (viscosimetric method [6]); 4) the glomerular filtration (from the inulin clearance) and secretory function (from the excretion of phenol red); 5) the structure of the kidneys — histologically (staining with hematoxylin-eosin) and histochemically — staining for the concentration of acid mucopolysaccharides (with treatment by dialyzed iron in combination with Schiff's reagent by the method of Ritter and Oleson [7]).

EXPERIMENTAL RESULTS

The relationship between U_{osm} and diuresis is shown in Fig. 1. The ability to excrete osmotically concentrated urine was much less in the experimental rabbits than the controls, although they could not develop a high diuresis. In other words, they showed some features of an isosthenuric syndrome.

In some experimental rabbits this was still more clearly apparent. A typical example was rabbit No. 7. In this case, with a considerable increase in diuresis, the osmotic concentration of the urine showed no consistent change, but fluctuated around a constant level.

Diuresis in ml/min/kg	0.05	0.08	0.15	0.21	0.23	0.38	0.43	0.46	0.60	0.63
U_{osm} /liter	413	237	483	569	575	424	392	387	333	337

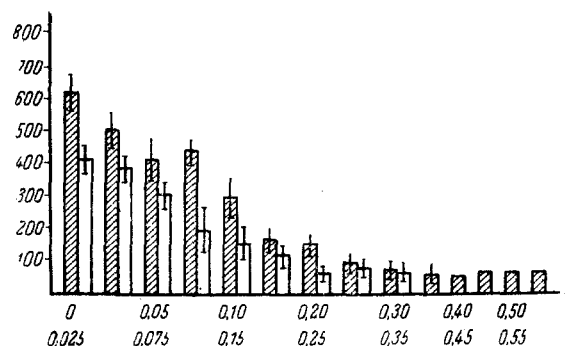


Fig. 1. Relationship between osmotic concentration of urine and level of diuresis in experimental (unshaded columns) and control (shaded columns) rabbits. Along the axis of abscissas — diuresis (in ml/kg/h); along the axis of ordinates — osmotic concentration of urine (in mM/liter). The four columns on the right are results of individual experiments.

Meanwhile, in the experimental rabbits, no significant disturbances in the filtration capacity of the kidneys determined by their inulin clearance and in their secretory activity, determined by the excretion of phenol red, were found. In the control rabbits the average filtration was 2.47 ml/min ($m = \pm 0.05$) and the phenol red excretion 0.1 mg/min ($m = \pm 0.015$); in the experimental animals these values were 2.76 ml/min ($m = \pm 0.31$) and 0.12 mg/min ($m = \pm 0.01$) respectively. The differences between the experimental and control animals were not statistically significant.

All the experimental rabbits showed a more or less marked depression of the reaction to ADH (Fig. 2). Most experimental rabbits did not react to injection of pituitrin or responded with an increase of diuresis. Admittedly, in some repeat experiments on the same rabbit a reaction to ADH sometimes developed and sometimes did not. The decreased diuresis in these cases was probably not tubular, but glomerular in nature, and associated with spasm of the vessels. In any case the control rabbits gave a typical antidiuretic reaction to the hormone in all experiments without exception.

The experimental rabbits showed marked disturbances of the normal relationship between the hyaluronidase concentration in the urine and the level of the diuresis. In the control rabbits (36 determinations) when diuresis was low — from 0.01 to 0.05 ml/kg/min — hyaluronidase was found in 70% of determinations, and when the level of diuresis was from 0.04 to 0.3 ml/kg/min in no case was the enzyme detected in the urine. This means that the relationship between diuresis and hyaluronidase activity of the urine in normal rabbits obeys the law discovered in our laboratory for other mammals. In rabbits with experimental kidney disease (50 determinations) this relationship was upset. When diuresis was low, the enzyme was found in the urine in only 32% of tests, compared with 36% of cases when diuresis was high. Hence, in contrast to normal conditions, no differences were observed between the hyaluronidase activity associated with low and high values of diuresis. A similar phenomenon has been reported earlier in human subjects with various renal lesions [1].

Histological examination of the kidneys of the experimental rabbits showed in all cases the characteristic changes of focal interstitial nephritis. In the cortex, atrophy of the individual glomeruli and tubules was observed, with their replacement by hyperplastic connective tissue, dilatation of some of the remaining tubules, sometimes forming cysts with flattened epithelium, and intercellular infiltration of the stroma, mainly around the vessels and tubules. The epithelium of some of the convoluted tubules, and especially of the ascending limb of the loops of Henle, showed degenerative changes: pycnosis of the nuclei, and lysis and desquamation of cells into the lumen of the tubules. Proliferation and condensation of connective tissue were observed in the papillae, in some places with hyalinosis and collagenization of the ground substance. Hyaline and granular masses (casts) could be seen in the

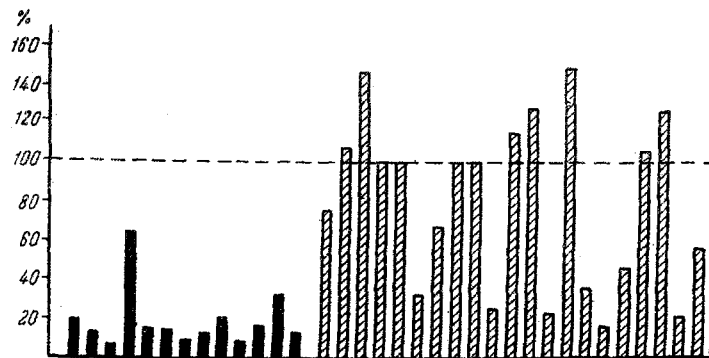


Fig. 2. Reaction of experimental (unshaded columns) and control (shaded columns) rabbits to injection of pituitrin against a background of water diuresis. Along the vertical axis - changes in diuresis (in % of initial value). The horizontal broken line corresponds to the level of the initial diuresis, taken as 100%.

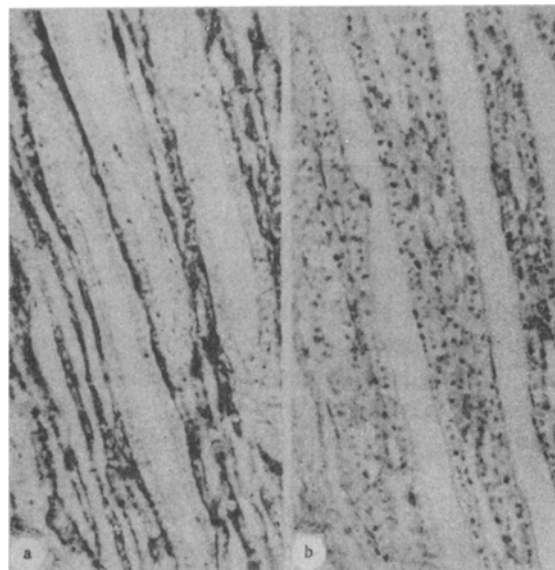


Fig. 3. Identical areas of the kidneys of control (a) and experimental (b) rabbits. Acid mucopolysaccharides in the interstices of the medullary substance. Photomicrograph. Stained by Hale's method and counterstained with hematoxylin.

lumen of the collecting tubules. The changes listed above were focal in character, and the degree of their severity varied from one animal to another.

Histochemical treatment of sections showed that acid mucopolysaccharides, the substrate on which hyaluronidase secreted under the influence of ADH acts, were considerably modified. A clear correlation was observed between the decrease in sensitivity to ADH observed in the animal during life and the changes in the mucopolysaccharide content in the papilla.

In all cases in which the reactions to the hormone either was absent or was considerably depressed, either a marked decrease in the amount of acid mucopolysaccharides detectable by this staining method or their complete absence was observed (Fig. 3). In some cases this absence of the reaction for mucopolysaccharides was focal in

character. Side by side with zones in which mucopolysaccharides were completely absent could be seen others, admittedly small, the structures of which presented a more normal appearance. The residual mucopolysaccharides showed their usual sensitivity to exogenous (testicular) hyaluronidase, and after preliminary treatment of the sections with the enzyme, the reaction with dialyzed iron was negative.

The pathological model which we studied reproduced a number of the features of chronic interstitial nephritis. We were dealing with the changes remaining after recovery of the animals from a bacterial lesion of the kidneys. These changes, on account of their focal distribution, evidently did not give rise to any significant disturbances of the filtrational and secretory functions of the kidneys. It is possible that the residual nephrons were capable of performing these functions on a level indistinguishable from normal. At the same time, the ability of the kidneys to excrete an osmotically concentrated urine was profoundly impaired and their reactivity to ADH — the principal regulator of the facultative tubular reabsorption of water, was sharply depressed. There was undoubtedly a connection between this last phenomenon and the changes in the substrate on which the hormone acts indirectly (through hyaluronidase). The disturbance of the normal production of the enzyme and the relationships between its connection in the urine and the level of the diuresis were equally clearly defined.

These results do not provide a full explanation of the extent to which the disturbance of sensitivity to ADH is dependent on the changes in the substrate of the final link in its chain of action, and the extent to which the reaction to the hormone is modified on account of the disturbance of hyaluronidase secretion in the tubules under its influence. Both factors possibly play a role in the lowering of sensitivity to ADH. So far as the impaired ability of the kidneys to excrete an osmotically concentrated urine is concerned, besides the undoubted importance of the changes in the sensitivity to ADH, in this case the possibility of a disturbance of the function of the reflex system [8]. In particular, a lesion of the ascending limb of the loop of Henle may be suggested. The cells of the latter create the osmotic gradient responsible for the facultative reabsorption of water in the collecting tubules after their walls have been rendered permeable through the action of ADH. In any case the histological changes in the epithelium of the ascending limb of the loop demonstrate the possibility of injury to this part of the concentrating mechanism of the kidney also. This problem requires further analysis.

The model used in the present investigation is, however, only an approximation to clinical forms of renal pathology. Nevertheless, even in this approximation, it may shed some light on the nature of some phenomena observed in clinical conditions and, in particular, on the syndrome of isosthenuria. Although in our model (excepting a few cases) this syndrome was not reproduced in its classical form, its important features were undoubtedly present. Hence it may be suggested, at the moment purely as a hypothesis, that a connection exists between the isosthenic syndrome, the mechanism of which is still purely a matter for conjecture, and disturbances of the physiological functions of the distal divisions of the nephron, associated with the facultative reabsorption of water and with the concentrating power of the kidney.

SUMMARY

A study was made of functional and morphological changes occurring in the kidneys of rabbits 40 to 60 days after a single intravenous administration of a hemolytic streptococcus culture. A, type Griffiths 12. The infectious process disappears by the 20th-30th day and is then followed by the appearance of autoantibodies in the serum indicating the presence of an immunopathological reaction. In experimental animals there is a considerable reduction of the capacity for urine concentration and developing high diuresis; the sensitivity to pituitrin P is either absent or reduced; after administration of the latter the inulin concentration index does not rise. At the same time glomerular filtration (by inulin clearance C_{in}) and tubular secretion (by phenyl red excretion) show no statistically significant deviations from the normal. As distinct from the normal, hyaluronidase is present in the urine not only in cases of low, but also of high diuresis. Histochemical examination indicated disappearance or marked reduction of acid mucopolysaccharides in the papilla (Ritter-Olsen's method). According to A. G. Ginetsinskii, these mucopolysaccharides serve as a substrate for the action of hyaluronidase secreted under the effect of pituitrin P.

LITERATURE CITED

1. A. G. Ginetsinskii, A. Ya. Broitman, and L. N. Ivanova, *Byull. éksper. biol.*, **8**, 37 (1954).
2. A. G. Ginetsinskii and L. N. Ivanova, *Doklady Akad. Nauk SSSR*, **119**, 5, 1043 (1958).
3. A. G. Ginetsinskii, M. G. Zaks, and L. K. Titova, *Doklady Akad. Nauk SSSR*, **120**, 1, 216 (1958).
4. A. G. Ginetsinskii, *Zh. obshchei biol.*, **1**, 9 (1961).

5. A. G. Ginetsinskii, V. F. Vasil'eva, M. G. Zaks et al., In book: Textbook of Methods of Physiological Investigation of Fish [in Russian], Moscow (1962), p. 204.
6. Yu. V. Natchin, Byull. éksper. biol., 8, 118 (1959).
7. A. G. E. Pearse. Histochemistry, Theoretical and Applied [Russian translation], Moscow (1962).
8. H. Wirz, B. Hargitay, and W. Kuhn, Helv. physiol. pharmacol. Acta, Vol. 9 (1951), p. 196.

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
