THE EXCRETION OF DYSENTERY ANTIGENS BY THE KIDNEYS IN DOGS WITH UNCHANGED AND CHANGED IMMUNOLOGICAL REACTIVITY

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According to the latest findings, the mammalian kidney is permeable to macromolecular substances such as hemoglobin, serum and foreign proteins and toxins, antigens and the microorganisms of scarlet fever, croupous pneumonia, dysentery and typhoid fever.

Excretion of viruses in viral diseases was proved by L. A. Zil'ber [2], who also raised the question of the importance of this process in immunity.

It was shown by G. Wallenius [12] that the clearance index of the simplest macromolecules of the polysaccharide dextran was inversely proportional to the molecular weight. It was found that large-molecular substances undergo reabsorption in the tubules, as was shown in the case of protein marked with Evans blue and fluorescein [3, 11]. The question of the excretion of microorganisms by the healthy kidney has been examined even in the earliest stages of the development of immunology (V. Vysokovich, I. I. Mechnikov cited by [1, 2, 4], and later by L. Ascher and J. Sokol (cited by [1, 2, 4]). The majority of workers answered this question in the negative.

Excretion by the kidneys of bacterial antigens, toxins and viruses has been proved, and their removal may be regarded as one of the pathological mechanisms of immunity [1].

In the present work the excretion of dysentery antigens from the kidneys of dogs was studied during experimental dysentery poisoning.

EXPERIMENTAL METHODS

The investigation was made in chronic experiments on dogs. A constant infusion by the drip method of a 1% solution of inulin (or a 1% solution of thiosulfate) and simultaneously of Flexner dysentery antigens (prepared at the I. I. Mechnikov Institute, series 360) was set up. The antigens injected amounted to 0.1-0.4 mg per 1 kg body weight for 15-30 days. During this time complete clearance of antigens from the animals took place. After administration of water (40 ml per 1 kg body weight) to the dog, it was placed on a board, and one of its hind paws was fixed to the board with bandages. A needle, joined by a rubber tube to a vessel containing the solutions of substances to be injected, was inserted into the dorsal lateral metatarsal vein and fixed with two strips of adhesive plaster to the shaved or depilated surface of the paw.

In order to produce a definite concentration of the injected substances in the blood, their rate of flow was regulated by compression of the lumen of the tube with a rubber band.

The experiment consisted mainly of 2 clearance periods each of 20 minutes. The urinary bladder was emptied by catheterization or else the urine was collected through a Pavlov-Orbeli fistula. In the middle of the clearance period blood was taken from the dorsal lateral metatarsal vein on the side opposite to that in which the injections were being given.

The concentration of thiosulfate in the blood and urine was determined by an iodometric method, and the inulin colorimetrically by means of a thiourearesorcin reagent. Quantitative determination of dysentery antigens in the blood was carried out by means of the cold complement fixation reaction. The clearance index of the injected substances was then calculated. Further, the antibody titer in the blood was checked by the precipitation reaction and control analyses of the urine were made.

Experimental Results of Administration of Dysentery Antigen to Nonimmunized Dogs

Experiments were carried out on 8 dogs not previously immunized. In view of the fact that antigen was injected repeatedly into many of these animals, experiments in which dysentery antigen was injected for the first time had to be specially selected.

On primary injection, the antigen was excreted in the urine of all 8 dogs, although in two of them it was only found in traces, but in the remaining six in amounts which permitted calculations to be made.

The magnitude of clearance from dysentery antigens varied from 0.08 to 0.7 ml/min, which corresponds to 0.15 to 1.5% of the index of clearance of the simultaneously injected inulin or sodium thiosulfate (Table 1).

Repeated injections of dysentery antigen, which may be regarded as a process of immunization, were accompanied by increase in the indices of clearance of these antigens from the animal. This process is illustrated in Fig. 1, showing the results obtained in the dog Kashtanka.

The antigen clearance indices of the dogs subjected to primary and repeated injections but not immunized with vaccine were between 0.08-1.3 ml/min, which corresponded to 0.09-5.42% of the degree of filtration.

TABLE 1

Excretion of Dysentery Antigen by the Kidneys after Primary Injection

Name of dog	Clearance index of dysentery antigen (in ml/min)	Relation of degree of clearance of antigen to degree of filtration (in %)	Remarks
KashtanRa	0.7	1.54	Filtration estimated by thiosulfate
Khriza	Traces	—	"
Beliak	0.125	0.21	Filtration estimated by thiosulfate. Dog died after injection.
Laska	0.09	0.15	Filtration estimated by thiosulfate
El'ba	0.5	0.49	Filtration estimated by inulin
Seraia	0.08	0.40	" "
Ovcharka	0.1	0.23	" "
Martyshka	Traces	—	" "

Experimental Results of Administration of Dysentery Antigen to Dogs Previously Immunized with Dysentery Vaccine

Three dogs (Umnik, Laska and Lada) were immunized with alcoholized dysentery vaccine, obtained from the Moscow Institute of Epidemiology, Microbiology and Hygiene, according to the scheme: 0.5-1.0-3.0-5.0-5.0 ml at intervals of 5 days. Antigen was administered repeatedly to these dogs at intervals of 15 to 30 days.

The intensity of its excretion was such that the clearance index was from 0.20 to 2.43 ml/min, and in one case it amounted to 5.1 ml/min. Figure 2 shows the excretion of antigens from a dog after previous immunization with vaccine.

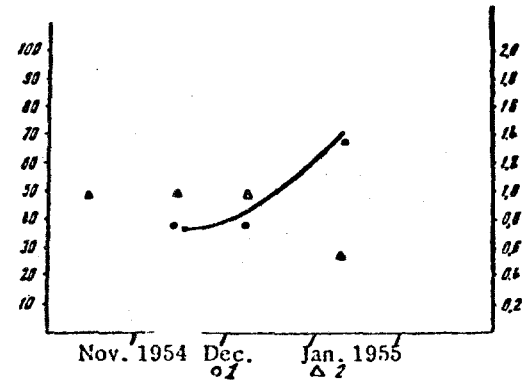

Fig. 1. Excretion of dysentery antigens after repeated injection into the dog Kashtanka.
1) Clearance index of antigen (on the right hand scale); 2) clearance index of thiosulfate (on the left hand scale).

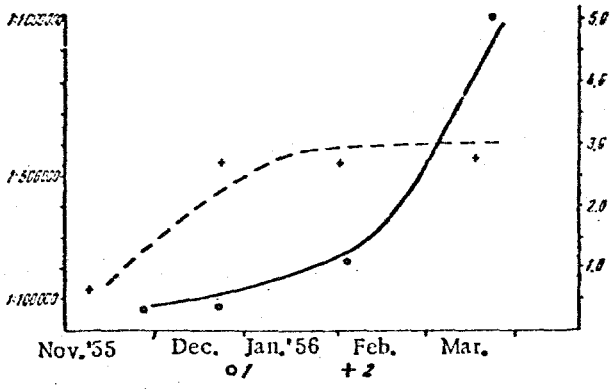

Fig. 2. Excretion of dysentery antigens on repeated injections into the immunized dog Umnik.
1) Antigen clearance index (on the right hand scale); 2) precipitin titer (on the left hand scale).

In order to solve the problem of specificity, we thought it desirable to set up an experiment in which a dog, immunized against dysentery, would be injected with another antigen, namely typhoid fever, in order to compare the degree of clearance of the latter with the degree of clearance of dysentery antigen injected in the same quantity.

The dog Lada, immunized with alcoholized dysentery vaccine, was given 2 injections of dysentery antigen, after which typhoid antigen was injected. Clearance of typhoid antigen was found to be 0.30 ml/min, amounting to 0.34% of the inulin filtration determined at the same time. Clearance of dysentery antigen amounted to 0.39 ml/min (0.53% of the filtration) if injected before the typhoid antigen and 0.85 ml/min (0.98% of the filtration) if injected afterwards.

Somewhat smaller values of clearance of typhoid antigens compared with clearance of dysentery antigens administered previously were obtained also in the dog Umnik which had been immunized to dysentery. Clearance of typhoid antigens in Umnik amounted to 1.62 ml/min (2.43% of the filtration), and of dysentery antigens injected subsequently, preceding the injection of typhoid – 1.8 ml/min (3.01% of the filtration).

Although these experiments do not permit the question of specificity of excretion of antigens from animals immunized to a given antigen to be finally answered, they do nevertheless suggest the existence of some degree of specificity in the excretion of dysentery antigens.

We also studied the problem of damage to the renal parenchyma. Control analyses of the urine before and after injection of dysentery antigen showed the appearance of traces of albumin in the urine after injection of antigen (transient proteinuria). The protein was estimated by the Robers-Stol'nikov method. In order to confirm the excretion of protein and to determine its nature we carried out anaphylaxis experiments with guinea pigs.

The plan and results of these experiments are shown in Table 2.

As seen in Table 2, in animals sensitized to urine taken from the dog after injection of antigen, and containing proteins, a strong anaphylactic shock was observed on injecting as an assaulting dose the serum proteins of a dog (with fatal results in 2 out of the four guinea pigs). In guinea pigs sensitized to urine taken from the dog before the injection of antigen and not containing proteins according to Heller's test, anaphylactic shock was also observed although to a weak degree. This fact suggests the presence even in normal urine of quantities of protein adequate for sensitizing a guinea pig, and in accordance with the findings of Rigas and Heller [10], who demonstrated by the methods of ultrafiltration and electrophoresis the presence of proteins in the healthy human urine.

Anaphylaxis experiments on guinea pigs, confirming the excretion of antigen in the urine, showed that at the same time there occurs excretion of serum protein. Such signs of damage to the kidneys as the presence of erythrocytes, hemoglobin, etc., were not found in our experiments.

TABLE 2

Schedule of Anaphylaxis Experiments on Guinea Pigs

Animal No.	Sensitization		Reaction in response to injection of an assaulting dose on May 17, 1956	Evaluation of severity of shock
	1st injection May 3, 1956	2nd injection May 5th, 1956		
1	2 γ of dysentery antigen	The same as in the 1st injection	Injection of 50 γ of dysentery antigen. Two min. later slight shivering and 8 minutes later, slight convulsions	+
2	1 ml of urine without protein (before injection of antigens)	Ditto	Injection of 1 ml of dog serum. One minute later severe convulsions in the lateral position	++
3	1 ml of urine containing proteins (after injection of antigen).	"	Injection of 1 ml of dog serum. One minute later severe convulsions in the lateral position. After 4 minutes, death.	++++
4	Ditto	"	Injection of 50 γ of dysentery antigen. After 7 minutes, periodic shivering, twitching.	+
5	"	"	Injection of 1 ml of dog serum. After 4 minutes strong convulsions, defecation and urination.	++
6	"	"	Injection of 1 ml of dog serum. One minute later convulsions in the lateral position, defecation and urination. Death after 6 minutes.	++++
7	Animal not sensitized		Injection of 1 ml of dog serum. No shock	−

It may be suggested that activation of the excretory function of the kidneys during excretion of antigens is accompanied by increased permeability of the glomerular membrane. This also shown by the appearance of a slight transient proteinuria. It must be pointed out that the fact of penetration of protein through the glomerular membrane is not regarded as a sign of renal damage and takes place in healthy animals (the so-called benign albuminuria) [5].

Phenomena similar to those which we observed, consisting of transient excretion of homologous serum protein have been noted in response to injection and excretion of hemoglobin and foreign protein ([7] and others).

From an assessment of the magnitude of the clearance indices of dysentery antigens obtained, and comparison with the degree of filtration as determined simultaneously in our experiments, we observe that percentage ratio between the clearance index of dysentery antigens and the total filtration is smaller than that calculated in the same way with dextran fractions with molecular weight of 28,500 (11%; [11]). The clearance indices for dysentery antigens are closer to that of dextran with a molecular weight of 91,700 (1.2% of the total filtration), determined by Giebish et al., [6], and to the clearance of hemoglobin (according to Monke and Yuile) equal to 3% of the total filtration [9].

Regarding the mechanism of excretion of antigens it can be tentatively concluded that during excretion they are subjected to filtration and to reabsortive processes in the tubules. The difficulty in determining the renal threshold for antigens does not exclude tubular activity and it may depend, as with other proteins, on variations in the size of the molecules of different fractions of antigen.

Excretion of dysentery antigens is not accompanied in all animals by a uniform change in filtration. Increase of filtration, noted in certain animals, shows activation of filtration in the process of antigen excretion. However, such an increase in filtration does not always take place.

It is of interest that during excretion of phage, studied in L. A. Zil'ber's laboratory, uniform changes in filtration were also observed (V. A. Parnes et al., [3]). The absence of any parallel between filtration and antigen clearance shows that some process other than filtration is also concerned in the excretion of the antigen molecules. This process is the activity of the renal tubules. In this connection, dysentery antigens evidently are not distinguished from other macromolecular compounds undergoing filtration and reabsorption.

No strict parallel between antigen clearance and antibody titer in the blood could be observed in our experiments. The role of excretion of antigens by the kidneys was more pronounced in immunized animals, but no close relationship between the variations in antibody titer and antigen clearance index could be observed.

The increase in antigen clearance arising during immunization may depend on a lowering of the reabsorptive activity of the tubules and to an increase of filtration, brought about by immunization. This increase in the excretory function of the kidneys in respect to bacterial antigens arising during immunization may be regarded as a peculiar manifestation of the protective mechanisms, the pathophysiological mechanisms of immunity, not directly connected with antibodies or phagocytosis.

SUMMARY

In dogs dysentery antigens are excreted by the kidneys following their intravenous introduction. The indicator of clearance determined for dysentery antigens increases with their repeated introduction or after preliminary immunization by dysentery vaccine. Excretion of antigens is connected with a transitional proteinurea. However there are no signs of disturbed function of the kidneys. As to the mechanism of excretion of these macromolecules – one may assume that they are excreted by filtration in glomeruli with the following partial reabsorption in the tubules. It is probable that increased filtration of the antigens and decrease of their reabsorption takes place in immunization.

LITERATURE CITED

[1] A. D. Ado, Antigens as Extraordinary Stimulants of the Nervous System,* Moscow, 1952.

[2] L. A. Zil'ber, Voprosy virusologii, No. 1, 49-54 (1956).

[3] V. A. Parnes, N. D. Petrova, E. V. Volina and Z. A. Avenirova, Zhur. Mikrobiol., Epidemiol. i Immunobiol., No. 5, 40-45 (1950).

[4] N. B. Iafarova, Excretion of Dysentery Antigens by the Kidney of the Rabbit, Dissertation,* Kazan, 1954.

[5] F. S. Fowweather, Brit. Med. J., 1955, pp. 1419-1423.

[6] G. Giebish, H. D. Lauson and R. F. Pitts, Am. J. Physiol., 1954, v. 178, pp. 168-170.

[7] D. R. Gilligan, M. D. Altschule and E. M. Katersky, J. Clin. Invest., 1941, v. 20, pp. 177-188.

[8] H. Mayersbach and A. G. E. Pearse, Brit. J. Exper. Pathol., 1956, v. 37, pp. 81-89.

[9] I. V. Monkel and C. L. Yuile, J. Exper. Med. 1940, v. 72, pp. 149-165.

[10] D. A. Rigas and C. G. Heller, J. Clin. Invest., 1951, v. 30 N. 8 pp. 853-861.

[11] A. D. Sellers, N. Griggs, I. Marmorston and H. Goodman, J. Exper. Med., 1954, v. 100, pp. 1-9.

[12] G. Wallenius, Acta Soc. med. Upsal. Supp., 1954, v. 59, pp. 1-91.

* In Russian.