EXCRETION OF TYPHOID FEVER ANTIGEN BY THE KIDNEYS OF DOGS IN THE COURSE OF CHANGE IN IMMUNOLOGIC REACTIVITY

M. Khakberdyev

Department of Pathologic Physiology (Scientific Director – Corresponding Member Acad. Sci. USSR A.D. Ado), II State Medical Institute (Director – Prof. O.V. Kerbikov), Moscow

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It has long been thought that in healthy kidneys no proteins of molecular weight higher than that of hemoglobin passed through the glomerular membranes [11]. It has now been shown, however, that some substances of molecular weight exceeding that of hemoglobin are excreted by intact kidneys [12, 13, 14].

As far back as 1929 Andrews, Thomas and Welker [10] postulated the hypothesis that in nephritis the kidneys played a detoxicating role by excreting toxic proteins in the urine.

Excretion of bacterial antigens and viruses in the urine has been established in many diseases [3, 5, 8].

L.A. Zil'ber and colleagues succeeded in showing that the viruses excreted by animals are excreted by intact kidneys [6, 7].

N.B. Iafarova-Tumasheva, working in A.D. Ado's laboratory, has shown that dysentery antigens are excreted by intact kidneys in rabbits [9].

Excretion of typhoid antigens by the kidneys has been established in patients by S.O. Blinkinym and T.F. Fesenko [3]. There are no references in the literature to experimental work demonstrating excretion of typhoid antigens.

The present work is concerned with the study of the renal excretion of typhoid antigens in dogs in the process of repeated administration of the antigen, i.e., immunization.

EXPERIMENTAL METHOD

Experiments were performed on female dogs weighing from 13 to 20 kg. Experiments were carried out in the mornings, in the fasting state. One hour prior to administration of antigen, water "loading" was ensured by giving 40-50 ml per 1 kg body weight of water. Specimens of blood and urine were taken before administration of antigen. The antigen was weighed out and diluted with sterile physiologic solution before the experiment. Complete typhoid antigen (series 293, 1955), obtained from the I.I. Mechnikov Institute of Vaccines and Sera, was given on the basis of 0.08-0.4 mg per 1 kg body weight. Sterile 1% solution of inulin or sodium thiosulfate was used as nonthreshold substance. 300-400 ml of mixed solution was prepared from the antigen and inulin or sodium thiosulfate. One of the dog's hind limbs was fixed to the stand by binding. The needle, connected by a rubber tube with the bottle containing the solution, was introduced into the v. metatarsea dorsalis lateralis and fastened with two strips of adhesive tape to the clipped surface of the paw. At first, the clamp on the rubber tube was fully released in order to attain a certain concentration of the test substances in the blood. Subsequently, the clamp controlled the amount of solution which was administered continuously in the form of drops throughout the experiment, at the rate of 3-5 ml per minute. At the end of 6-10 minutes after the beginning of the experiment the bladder was emptied once again and this marked the beginning of the period of clearance. The ex-

periment consisted of two or three periods of clearance each of 20-30 minutes' duration. During the whole clearance period the urine was collected and in the middle of this period a specimen of blood was taken from the other paw. Thus, in order to evaluate the clearance of the substance under investigation the principle of making simultaneous determinations of another, well-known nonthreshold substance (inulin or sodium thiosulfate) was used, as well as continuous drip infusion of the substances concerned which gives a more constant blood level as compared with a single injection [4, 15]. Each investigation consisted of the following stages:

1) continuous intravenous drip of complete typhoid antigen and inulin or sodium thiosulfate;

2) detection of antigen in the blood and in urine by complement-fixation reaction in the cold and determination of the clearance index for the antigen;

3) colorimetric determination of inulin in the blood and in urine or iodometric determination of thiosulfate in the blood and in urine, and determination of the clearance index for these substances;

4) clinical examination of the urine for the presence of blood, albumin, etc.

EXPERIMENTAL RESULTS

Thirty-one experiments were performed in the course of 10 months on five healthy dogs, not previously immunized. The intervals between the experiments were from 9-13 to 20-30 days. The results of the experiments are presented in Table 1. With the exception of one dog (Alma) the typhoid antigen was not excreted in the urine after the first administration. The antigen began to be excreted in the urine beginning with the second experiment in the dogs Palma and Zhuchka, the third experiment in Chernushka and the fifth one in Ryzhulia. The minimal amount of plasma antigen which is detectable by the complement-fixation reaction is 0.1 mg%. At this concentration the antigen is excreted in the urine. According to our data, there is no relation between the antigen clearance and its concentration in the blood. With identical concentration of the antigen in the blood different clearance indices are noted and, conversely, approximately the same clearance is seen with different concentrations of the antigen in the blood. This, in our opinion, indicates a complex mechanism of antigen excretion rather than simple filtration.

The antibody titer of the blood rises on repeated administration of the antigen to the dogs. However, no relation is noted between the renal excretion of the antigen and the blood antibody titer (Table 1). In the case of Ryzhulia, no antigen was excreted in the urine during the first four experiments despite a high blood antibody titer (1:128000) and subsequently different antigen clearance indices (0.14-0.37 ml/min) were found with constant blood antibody titer (1:256000). Quite different antigen clearance values were found in Chernushka with a blood antibody titer of 1:256000. In the remaining three dogs the clearance values were comparable with those in Ryzhulia despite a higher antibody titer in the latter.

The question of the mechanism of antigen excretion is as yet unsolved. As can be seen from Table 1, some relation between antigen clearance and inulin clearance is observed in the case of Ryzhulia and Alma. The antigen clearance is seen to rise with a rise in inulin clearance index and, conversely, in Chernushka and Zhuchka, the beginning of antigen excretion coincided with the highest sodium thiosulfate and inulin clearance. Quite possibly the antigen is excreted as the result of increased permeability of the renal glomeruli. This suggestion is favored by the relation between inulin clearance and antigen clearance as well as the transient slight proteinuria which always accompanies antigenuria.

From the moment of antigen excretion the inulin clearance index (or sodium thiosulfate clearance index) diminished in the case of Palma while in Zhuchka and Chernushka it first rose and then also decreased. This indicates that in these dogs there was toxic action of the antigen which was manifested by lowered renal filtration. Despite this, these dogs continued to excrete the antigen in their urine, and sometimes a rise in antigen clearance was noted. In two dogs – Ryzhulia and Alma – the antigen excretion was not accompanied by lowering of inulin clearance, but, on the contrary, a rise in the latter was observed in subsequent experiments. This indicates that in these dogs the filtration function of the kidneys was not lowered.

It was stated above that antigenuria was always accompanied by slight (up to 0.5%) transient (three to six hours) proteinuria. No blood or renal epithelial cells were present in the urine. In order to discover the nature of the excreted protein, experiments were staged with anaphylactic shock in guinea pigs. These experiments show that serum proteins are excreted in the urine (Table 2). There are no indications that the slight transient proteinuria signifies morphological damage to the kidneys.

TABLE 1

Relation Between Blood Antigen and Inulin Clearance in Dogs

Dog's name	Time of antigen administration	Blood antigen concentration (mg%)	Urine antigen concentration (mg%)	Antigen clearance index (ml/min)	Inulin clearance index (ml/min)	Antigen / inulin %	Blood antibody titer
Alma	21/I 1956	0.8	0 05	0.14	54.11	0.25	
	3/II	0.3	0.15	0.35	86.26	0.4	1 : 16 000
	16/II	0 7	0.4	0.08	68.08	0.11	
	5/VI	0 6	0.25	0.424	86.05	0.49	1 : 64 000
	17/VI	0.6	1.05	0.70	82.64	0.84	
	9/VIII	0.9	1.20	0.95	120.65	0.78	
Palma	23/XI 1955	—	—	—	62.66	—	
	6/XII	0.2	0 3	0.33	12.86	2.57	
	22/XII	0 2	0.35	0.36	16.45	2.18	1 : 64 000
	12/I 1956	0.2	0.35	0.28	17.75	1.58	
	28/I	0.2	0.05	0.8	33.34	2.4	1 : 64 000
	15/II	0.7	0.35	0.3	27.01	1.11	
Zhuchka	15/II 1956 Inulin only				107.37		
	21/III Inulin only				131.36		23/III 1956 1 : 8 000
	11/IV	—	—	—	78.98		
	20/V	0.2	0.05	0.36	147.82	0,24	
	1/VI	1.2	0.9	0.376	79.33	0.46	1 : 32 000
	18/VI	0.6	0.6	0.40	78.67	0,50	1 : 128 000
	6/VIII	0,6	0.45	0 46	77.58	0.51	
Cher-nushka	30/XI 1955	—	—	—	99.52	—	
	13/XII	—	—	—	117.59	—	
	4/I 1956	0.6	0.35	0.83	199.21	0.41	
	14/I	0.1	0.35	3.25	125.00	2.6	
	23/I	0.8	0.75	3.68	92.97	3.96	1 : 25 600
	1/II	0 1	0.35	2.69	83.6	3.21	
Ryzhulia	13/XI 1955	—	—	—	35.35	—	
	9/XII	—	—	—	46.31	—	
	29/XII	—	—	—	41.31	—	
	9/I 1956	—	—	—	68.36	—	
	25/I	0.4	0.15	0,24	96.77	0.27	1 : 128 000
	14/II	0.6	0.05	0,145	67.16	0.215	
	10/IV	0.2	0.2	0,3	64.87	0.46	1 : 256 000
	25/V	0.55	0.3	0,378	117.00	0,31	1 : 256 000

Note: 1. On April 10, 1956 Ryzhulia was subjected to an experiment in which dysentery antigen was administered. 2. In the case of Chernushka clearance was determined with the help of sodium thiosulfate.

At the beginning of the discussion it was noted that in all dogs with the exception of one, there was no excretion of antigen when it was first administered. How could this be explained? It must be mentioned that in those cases in which antigen was absent from the urine it was also absent from the blood. It may be supposed that on initial administration of antigen healthy reticuloendothelial cells quickly capture the administered antigen. The concentration of the antigen in the blood is then too low for excretion to occur. In subsequent exper-

TABLE 2

Results of Experiments with Sensitization of Guinea Pigs by Urine of the Experimental Dogs

Animal No.	Sensitization	Experimental conditions and results (resolving injection)	Degree of shock
1	Twice repeated sensitization (February 10 and 12, 1956) with dog urine taken prior to antigen administration. Urine given subcutaneously (2 ml each time).	April 26, 1956 intravenous injection 1 ml whole dog serum. Animal died after five minutes.	++++
2	Ditto	April 26, 1956 intravenous injection 1 ml whole dog serum. No manifestations of shock.	–
3	Twice repeated sensitization (April 10 and 12, 1956) with dog urine taken after antigen administration. 2 ml urine given subcutaneously. Amount of antigen 1γ in 1 ml urine, amount of protein 0.066‰	April 26, 1956 intravenous injection 1 ml whole dog serum. Animal died after six minutes.	++++
4	Ditto	April 26, 1956 intravenous injection 1 ml whole dog serum. Animal died after four minutes.	++++
5	Twice repeated sensitization (April 10 and 12, 1956) with complete typhoid antigen in 1:1000000 dilution, 2 ml subcutaneously.	April 26, 1956 intravenous injection 1 ml whole dog serum. Piloerection, rapid respiration, restlessness.	+
6	Ditto	Intravenous injection 10 γ antigen.	+
7	Animal not sensitized.	Intravenous injection 1 ml whole dog serum. No manifestation of shock.	–

iments the toxic action of the antigen reduces the functional activity of the reticuloendothelial cells and they do not capture such large amounts of antigen as previously. This results in sufficient concentration of the antigen in the blood to ensure excretion in the urine.

SUMMARY

It was established that during immunization of dogs by the antigen of typhoid fever the latter was excreted by the kidneys.

The index of clearance of the antigen varies from 0.08 to 3.68 ml per minute, which constitutes from 0.11% to 2.57% of inulin clearance and from 0.41% to 3.96% of sodium thiosulfate clearance.

Excretion of typhoid fever antigen is not a process of simple filtration, but a complicated process in which both filtration and possibly reabsorption take part.

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