MICROBIOLOGY AND IMMUNITY

ON THE PARTICIPATION OF THE RENAL TUBULE IN THE MECHANISM OF EXCRETION OF DYSENTERY ANTIGENS BY THE KIDNEY

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The fact that the kidneys excrete many large molecular substances (serum proteins, dextrins, complete antigens, bacteria of the enteric group and others) can be regarded as established at the present time [3, 13, 16, 18, 19, 20].

Terry, Sandrock, Hye and Whipple [20] and Addis [11] suggest that plasma proteins pass through the glomerular membrane and when the function of the renal tubule is normal are completely reabsorbed in its proximal regions. Under conditions of increased plasma protein concentration (10-11%), a larger amount of protein, having passed the glomerular membrane, is not completely reabsorbed, and a significant portion of it appears in the urine. The amount of protein in the glomerular filtrate of a man is estimated at 50 g per day, and the concentration in the filtrate as 0.03 [3, 18].

There are data on the influence of the immunological state of the body on the excretion of many antigens and viruses [1, 4, 5, 8, 10].

N. B. Yafarova [10] suggested a role of the tubules in the excretion of dysentery antigens by the rabbit, considering that it is secreted by the epithelium of the renal tubules.

In the present study we attacked the following problems: 1) to estimate the excretion of dysentery antigens by the kidneys of the dog, 2) to elucidate the influence of changes in diuresis on the extent of excretion of dysentery antigen, 3) to study the influence of maximum reabsorption of glucose on the excretion of dysentery antigen, 4) to elucidate the influence of dysentery antigen on the reabsorption of glucose, 5) to determine the influence of change in the immunological state of dogs on the processes just mentioned.

EXPERIMENTAL METHODS

Dogs weighing 8 to 18 kg were used as the experimental animals. The investigations were made during a prolonged experiment on 16 dogs. About an hour before the start of administration of the antigen the dogs were given a water load of 40-45 ml per kg body weight. Before administration of the antigen blood and urine samples were taken. We administered the antigen in the amount of 0.1-0.4 mg per kg of body weight in a solution of 500-800 ml sodium thiosulfate or glucose. The substance under investigation was given by intravenous drip during the course of each experiment. The experiment consisted of 5-6 clearance periods of 10-20 minutes each. The bladder was emptied by catheterization or the urine was collected through a Pavlov-Orbeli fistula. In the middle of the clearing period blood was taken.

The concentration of thiosulfate in the bllod and urine was determined iodometrically, and the amount of antigen in the blood and urine was determined by complement fixation in the cold.

The immune serum used in the reactions was a polyvalent precipitating anti-dysentery serum with a titer of 1:800,000, obtained from the Moscow Municipal Bacteriological Institute; it had a complement-fixing titer of 1 : 2,000,000. Using this serum, it would have been possible to detect in 1 ml of the fluid being investigated 0.0000005 g or 0.0005 mg of antigen. The amount of glomerular reabsorption of glucose was calculated from the formula

$$Rg = Pg. Ot - Mg. D \text{ (mg/min.)}$$

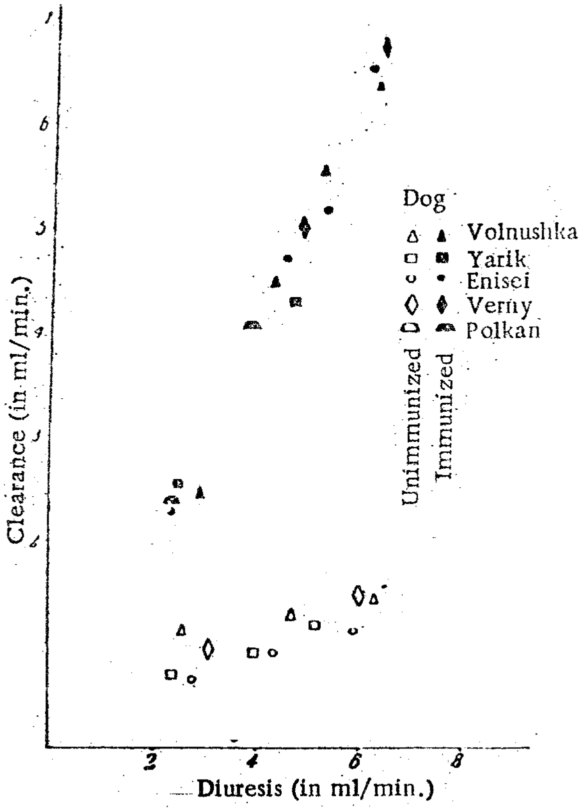
where Rg is the reabsorption of glucose, Pg the plasma glucose in mg%, Ot the thiosulfate clearance, Mg the urine glucose in mg%, and D minutes of diuresis.

The investigations began with the excretion of dysentery antigens by 9 dogs which had not been immunized, and 6 dogs immunized with dysentery vaccine. The results of the experiment are presented in Table 1, and agree with the data from the investigations of N. B. Yafarova, A. A. Polner and I. M. Khakberdyev (1956).

In the following series of experiments, with the aim of elucidating the role of the glomerular capsule and the renal tubule in the process of excretion of dysentery antigen by the kidney, we carried out experiments with different water loads, i.e., the infusion of 300 to 800 ml of sodium thiosulfate solution along with the antigen.

EXPERIMENTAL RESULTS

The results of the experiments are shown in the accompanying figure.



Effect of change in diuresis on antigen clearance.

As is seen from the figure, increased diuresis in the unimmunized dogs produced a negligible increase in excretion of the antigen. When diuresis was 2.3-2.75 ml/min. the excretion of antigen per minute was 0.003-0.005 mg; when diuresis was increased to 5-6 ml/min. the amount of antigen excreted was 0.005-0.006 mg/min. What explains such an insignificant increase of antigen excretion when diuresis is increased 2-3 times? Experiments showed that increase of diuresis in the unimmunized dogs was accompanied by a decrease in the concentration of the indicator substance. With diuresis of 2.3-2.5 ml/min., the concentration of the indicator substance reached 0.5, with diuresis increased to 5-6 ml/min. the concentration of the indicator fell in 2 cases to 0.25. In the immunized dogs increase in diuresis exhibited a positive effect on the elimination of dysentery antigens by the body. This was confirmed by repeated experiments on the same animals (Volnushka, Polkan, Verny, Enisei, Yarik; see Figure).

During relatively low diuresis (2.25-3.5 ml/min.) and with indicator concentrations equal to unity, clearance of antigen varied within range 2.43-4.5 ml/min., and under conditions of increased diuresis (4-6.6 ml/min) amounted to 4-6.6 ml/min., (see Figure). The concentrations of the indicator, in spite of considerable increases in diuresis, did not decrease.

It must be pointed out that raising the water load increase of diuresis are not accompanied by any significant change in the glomerular filtrate. From this it may be conjectured that the increase in excretion of antigen, observed when the water load is raised, is connected first of all with the activity of the tubules. This conjecture is supported by the fact of considerable activitation of antigen excretion by increased diuresis, especially in immunized animals (see Figure).

The influence of maximum secretion of para-aminohippuric acid of maximum reabsorption of glucose on reabsorption of ascorbic acid was demonstrated by the investigations of Selkurt, 1944.

Pitts, 1944, established a blocking of the reabsorption of ceratin by increased reabsorption of glycine.

TABLE 1
Excretion of Antigens by Dog Kidneys

No. p/p	Dog's name	Date of experiment (1955)	Antigen: Concentration in plasma (mg%)	Antigen: Concentration in urine (mg%)	Antigen: Indicator concentration	Antigen: Glomerular filtration of thiosulfate	Antigen: Diuresis per minute (in ml)	Antigen: Antigen clearance (ml/min.)
					Unimmunized			
1	Volushka	5/V	1.4	0.2	0.5	188.2	2.5	1.25
2	Enisei	6/V	0.4	0.1	0.25	209 0	2.75	0.68
3	Yarik	30/V	0.2	0.1	0.5	187.6	2.3	0.75
4	Kudrash	6/V	0.4	—	—	188.3	7.0	—
5	Verny	11/V	0.4	0.1	0.25	218.4	6.0	1.5
6	Saturn	25/V	0.4	0.1	0.25	210.0	5.0	1.25
7	Zhuchka	31/V	0.1	—	—	206.0	2.0	—
8	Jack	30/III	0.1	—	—	206.0	2.8	—
9	Ace	31/V	0.1	—	—	206.0	2.0	—
					Immunized			
1	Kudrash	13/X	0.8	0.8	1	125.2	3.9	3.9
2	Polkan	17/V	0.8	0.8	1	140.0	4.0	4.0
3	Martyshka	28/IX	0.8	0.8	1	116.0	4.75	4.75
4	Volnushka	15/IX	0.4	0.4	1	139.5	4.5	4.5
5	"	27/IX	0.4	0.8	2	136.9	3.4	6.8
6	"	17/X	0.4	0.8	2	115.15	2.45	4.9
7	Verny	5/VII	0.8	0.8	1	164.3	6.6	6.6
8	"	15/VII	0.8	0.8	1	132.4	6.5	6.5
9	Yarik	1/IX	0.4	0.4	1	123.5	2.25	2.25
10	"	14/X	0.4	0.4	1	126.0	2.5	2.5

On the basis of these investigations one may suggest the possibility of a reciprocal effect of antigen and glucose on their excretion by the kidneys. With the object of elucidating this we carried out the experiments of the following series. It is well known that glucose in concentrations below its threshold value is completely reabsorbed and not excreted in the urine. In otder to make possible an estimate of the speed of excretion we maintained concentrations of glucose in the blood of the order of 400-463 mg%.

We carried out the experiments on 6 unimmunized and 6 immunized dogs. The studies carried out to investigate the effect of antigen on the reabsorption of glucose are presented in Table 2. From Table 2 it is evident that in unimmunized dogs the excretion of glucose in the urine amounted to 401.4-406.3 mg/min., while the reabsorption varied from 347.8 to 385.1 mg/min., i.e., somewhat exceeded the maximum reabsorption of glucose established by Shannon, Farber and Troast (1941).

Evidently antigen, when first introduced into the body, stimulates the reabsorbing system of the kidney and exalts the processes of absorption. In immunized dogs, under the influence of dysentery antigens the maximum reabsorption of glucose declined about 30-33% on the basis of the data of Shannon, and amounted to 162.8-247.6 mg/min.

Study of the influence of maximum reabsorption of glucose on the excretion of dysentery antigens by the kidney revealed several pecularities of the process of clearing the body of antigen.

Excretion of dysentery antigens by the kidneys of unimmunized dogs under conditions of maximum reabsorption of glucose was accomplished at a rate 3-4 times that in control experiments and amounted to 0.017-0.018 ml/min. The concentration index of the antigen, in spite of the 2-2½ fold increase in diuresis, did not decrease. Antigen clearance reached 4.3-4.5 ml/min., exceeding that in control experiments 3-4 times (see Table 2). Our experiments showed that maximum reabsorption of glucose increases the excretion of antigen by the kidneys of unimmunized dogs 3-4 times. In dogs immunized with dysentery vaccine the excretion of antigen by the kidneys during maximum reabsorption of glucose reached 0.075-0.096 ml/min. and was increased 2-3 times in comparison with control experiments.

In spite of the elevated diuresis, leading to glycosuria, the concentration index of the dysentery antigen remained equal to unity. Antigen clearance varied from 9.4 to 12.9 ml/min.

On the basis of study of the results of the experiments of the series we suggest that maximum reabsorption of glucose, producing an overload of sugar, promotes increased excretion of antigen by the kidneys of unimmunized and immunized dogs.

Investigation of the variations in filtration by the clearing coefficient of sodium thiosulfate reveals some depression. It might be suggested that complicated phenomena of reabsorption and secretion of antigen take place simultaneously in the glomerular apparatus of the kidneys, similar for example to the excretion of urea. If a determining importance of reabsorption is recognized, then we should admit the possibility of significant filtration of antigen through the capsular membrane.

However, according to the available data of Wallenius (1954) and Giebish, Lauson and Pitts (1954) filtration of the macromolecular fractions is inversely proportional to the molecular weight. The proteins of human blood plasma, having molecular weights from 35,000 to 170,000, appear in the filtrate up to 0.03%. It may be supposed that the dysentery antigen, consisting of various fractions with molecular weights from 10^6 to 10^4 and less, behaves in the blood as a substance consisting of various amounts of fractions with the above mentioned high molecular weights. If we assume that 1/10 of the total antigen present in the blood falls into the fraction having molecular weights of 10^5 and less, then the amount of antigen appearing the glomerular ltrate amounts to about 1/2000 of that found in the plasms (Webster, Sagin, Anderson, Breese, Freeman and Landy, 1953).

On this assumption, as is seen from Table 2, the amount of antigen filtering through in one minute is several times smaller than the amount excreted in the final urine in the same time. Under the stated condition of antigen filtration, in unimmunized dogs the amount of antigen excreted in the final urine is 13.2 times the amount in the filtrate. In immunized dogs the ratio of the excreted and filtered antigen rises to 61.9.

From this there arises the necessity of admitting the existence of a second mechanism ofsecretion of antigen, i.e., its secretion by the epithelium of the renal tubules, similar to the excretion by the kidney of colloidal dyes and molecularly dispersed substances (phenol red, uric acid, phosphate, penicillin, para-aminohippuric acid, and others).

TABLE 2

Influence of Maximum Reabsorption of Glucose on Excretion of Dysentery Antigens by Kidneys of the Dog

No. p/p	Name of dog	One minute's urine in ml	Thiosulfate clearance (in ml/min.)	Antigen: concentration in plasma (in mg %)	Antigen: concentration in urine (in mg %)	Antigen: concentration index	Antigen: filtration (in mg/min.)	Antigen: excretion of antigen in urine (in mg (in mg/min.)	Antigen: relation of excreted antigen to that filtered off	Antigen: antigen clearance (in ml/min.)	Glucose: concentration (in mg % in plasma)	Glucose: concentration in urine (in mg %)	Glucose: filtration (in mg/min.)	Glucose: excretion in the urine (in mg/min.)	Glucose: reabsorption (in mg/min.)
1	Volushka	2.5	188.2	0.4	0.2	0.5	0.0003764	0.005	13.2	1.25	—	—	—	—	—
2	Enisei	2.25	209.0	0.4	0.1	0.25	0.000418	0.00225	6.5	0.68	—	—	—	—	—
3	Yarik	2.3	187.6	0.2	0.1	0.5	0.0001876	0.0023	12.2	0.75	—	—	—	—	—
4	Kaiya	8.9	192.5	0.4	0.2	0.5	0.000385	0.0178	46.2	4.45	404	4 565	777.7	406.3	371.4
5	Lada	9.0	185.5	0.4	0.2	0.5	0.000371	0.018	48.5	4.5	424	4 460	786.5	401.4	385.1
6	Spark	8.6	164.0	0.4	0.2	0.5	0.000328	0.0172	52.4	4.3	463	4 690	251.1	403.3	347.8
7	Kydryash	3.9	125.2	0.8	0.8	1	0.0005008	0.031	61.9	3.9	—	—	—	—	—
8	Polkan	4.0	140.0	0.8	0.8	1	0.00051	0.032	62.7	4.0	—	—	—	—	—
9	Martyshka	4.75	116.0	0.8	0.8	—	0.000464	0.038	81.8	4.75	—	—	—	—	—
10	Kudryash	9.4	120.0	0.8	0.8	1	0.00048	0.0752	156.6	9.4	400	2 640	480	258.7	231.3
11	Lisa	12.8	118.0	0.8	0.8	1	0.000472	0.0924	195.7	12.8	405	2 540	477.9	325.1	152.8
12	Renka	12.0	128.0	0.8	0.8	1	0.000512	0.096	187.8	12.0	406	2 200	511.6	264.0	247.6
13	Martyshka	12.9	120.0	0.8	0.8	1	0.00048	0.0932	194.1	12.9	422	2 470	506.4	308.6	197.5

Secretion of antigen by the cells of the renal tubules of unimmunized and immunized animals is significantly different. Before immunization secretion exceeded filtration of antigen 10-13 times, in immunized dogs secretion was 50-60 times filtration.

The phenomenon of depression of maximum reabsorption of glucose under the influence of dysentery antigen, found by us, may be regarded as a partial blocking of tubular reabsorption of glucose, brought about by an increase in secretion of dysentery antigens by the epithelium of the renal tubules, similar to the relations found for example, in studying the influence of secretion of para-aminohippuric acid on the reabsorption of ascorbic acid (Selkurt and others).

SUMMARY

In 56% of cases primary introduction of dysentery antigen to unimmunized dogs was accompanied by slight excretion of antigens. Excretion of antigens by kidneys after immunization was accelerated by 5-6 times. Increased diuresis in immunized dogs increased antigen excretion correspondingly. Prior to immunization, accelerated diuresis decreased the concentration index, the antigen excretion not being pronounced. Glucose loading, instituted to maximal reabsorption, increased dysentery antigen excretion. The dysentery antigen depressed the maximal reabsorption of glucose in immunized dogs. A certain relation in connection with increased dysentery antigen excretion by kidneys in the process of immunization may be attributed to functional changes of cells of the glomerular apparatus.

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