II. RENAL THRESHOLD FOR HEMOGLOBIN IN DOGS UNINFLUENCED BY MERCURY POISONING

By WILLIAM H. HAVILL, JOHN A. LICHTY, JR., GORDON B. TAYLOR, M.D., AND GEORGE H. WHIPPLE, M.D.

(From the Department of Pathology, The University of Rochester School of Medicine and Dentistry, Rochester, N. Y.)

(Received for publication, January 4, 1932)

The first paper submits evidence to show that the initial renal threshold for hemoglobin is depressed by frequently repeated injections of hemoglobin. This depression or minimal threshold is reasonably constant if the hemoglobin injections are continued daily but the renal threshold rises toward the initial threshold level during rest periods with no hemoglobin injections.

The *minimal or depression threshold* for dog hemoglobin is not changed by moderate doses of mercuric chloride given intravenously. Under such conditions there is ample evidence of injury of the renal tubular epithelium but no evidence of glomerular injury. The experiments tabulated below are difficult to explain if we postulate secretory activity on the part of the tubular epithelium related to the escape of hemoglobin from the blood plasma into the urine. The evidence fits much better with the assumption that the hemoglobin passes the glomerular filter and may be absorbed by the tubular epithelium unless that epithelium is injured by mercury or stuffed with previously absorbed hemoglobin coming from many preceding hemoglobin injections. This argument is given further support by experimental data tabulated in Papers IV and V below.

Experimental hemoglobinuria has not been neglected by investigators during the past 30 years and one can assemble evidence to the effect that the hemoglobin passes only through the glomeruli (Ribbert (10), Baker and Dodds (1)), that it is secreted only by the tubules (Miller (9), Lehnert (5)) or that it is filtered through the glomeruli with additions made by the tubular epithelium (Fukuda and Oliver (3)). There have been so many excellent reviews of this whole ques-

tion during recent years that it seems unnecessary to cover this subject again.

The question of the toxicity of mercuric chloride for dogs and the character of the renal lesions have been reviewed by Sansum (11), Haskell, Hamilton and Henderson (4), and MacNider (6). The evidence is overwhelming that the injury of tubular epithelium is the conspicuous feature.

EXPERIMENTAL OBSERVATIONS

The methods were identical with those described in the preceding paper, in fact some of the same dogs were used in both types of experiment. The mercuric chloride was given intravenously dissolved in 150 to 200 cc. of a 10 per cent glucose solution, the solution flowing in slowly by gravity. Giving the mercuric chloride intravenously has many advantages over administration by mouth which may be complicated with vomiting and gastro-enteritis. Occasionally one will produce death within a few hours by this intravenous method and the general picture is that of shock due to colloid injection. Fresh urine samples were examined for albumen and formed elements the day before injection and each day thereafter for 5 days or longer. As the albumen seems to be the most constant index of this type of acute renal injury we record in the tables only data relating to the albuminuria.

TABLE 21

Renal Threshold for Hemoglobin Uninfluenced by Mercury Poisoning

26 hemoglobin injections given before this experiment

Lowest normal or depression renal threshold for hemoglobin = 80 mg. per kilo

Dog 30-32, male, weight 18 kilos

Experiment, *days*.....	1	2	3	4	5	7	8	12	13	14	15	17	18	19	20	21	28
Urine albumen.....	0	Tr.	Tr.	2+	Tr.	Tr.	0	3+	2+	2+	+	Tr.	0	0	0	0	0
$HgCl_2$, *mg. per kg*...	1	0	0	0	0	0	0	1.5	0	0	0	0	0	0	0	0	3*
Hb. intraven., *mg. per kg*.....	0	70	70	70	70	70	80	0	70	130	70	80	90	80	80	80	0
Hb. in urine.....	0	0	0	0	0	0	+	0	0	+	0	0	+	+	+	+	0

* Death 30 minutes after mercury injection and autopsy at once.

Table 21[1] gives satisfactory data to show that the minimum or depression renal threshold for dog hemoglobin is not changed by

[1] The number borne by this table and the later ones is indicative of the paper from which it comes as well as its order in the paper. Thus for example Table 21 is the first table of Paper II, Table 34 the fourth table of Paper III.

moderate injury of the kidney produced by intravenous injection of mercuric chloride in moderate doses.

The clinical condition of the dog was normal up to the day of lethal reaction to the last injection of mercury. He was active and ate food as usual. The urine however gave clear evidence of injury following both the 1 mg. and 1.5 mg. per kilo doses of mercuric chloride. With the presence of albumen we always record the finding of epithelium and granular casts. No red blood cells were found. From the control animals and others killed soon after a sublethal dose we may be sure that there was injury of the tubular epithelium which however was promptly repaired. It is obvious that the lowest or depression threshold of 80 mg. per kilo established in the weeks preceding this experiment (Chart A, Paper I) is not modified by the injury of tubular epithelium. The threshold is very constant and obviously is very close to 80 mg. per kilo as in one instance this dosage gives a negative urine. That the threshold is not lowered even slightly is shown by the frequent injections of 70 mg. per kilo which are invariably negative.

The autopsy on this dog (Table 21) was done at once after death from intravenous injection. It is obvious that this reaction is not related to the kidneys but to the body as a whole and comprehends whatever the reader carries in his mind as related to peptone shock. The autopsy findings were of no interest to this experiment except the kidneys which in gross were practically normal except for congestion.

Histological sections (Dog 30-32, Table 21) show a normal picture for glomeruli and tubules in almost all fields. The glomeruli show no evidences of injury. A few tubules show occasional desquamated epithelial cells and cell casts which obviously relate to the mercuric chloride injection of 1.5 mg. per kilo given 16 days before death. The repair of the mercury injury however is practically complete and perfect. The pigment in the tubular epithelium is not abundant in this case and this is probably explained by the relatively small number of superthreshold hemoglobin injections which preceded the autopsy. These kidneys show occasional clusters of mononuclear cells and small scars in various parts of the cortex which may include an occasional abnormal glomerulus or tubule. This may indicate some focus of injury or infection but we find these areas in practically all stock dogs of middle age and there is a tendency toward increase with age.

Table 22 shows results identical with Table 21 but this dog tolerated very large doses of mercuric chloride.

The minimal or depression renal threshold was well established at 70 mg. hemoglobin per kilo by a long series of injections preceding these experiments. Attention was directed particularly toward the *lower threshold levels* so that repeated injections of 53 mg. hemoglobin per kilo were done over and over again always with a negative urine in spite of large doses of mercuric chloride. In our experience

the control dog usually dies after a 2 mg. mercuric chloride per kilo dose (Table 33 below) but this dog tolerated 4 mg. mercuric chloride per kilo with but moderate injury of kidney epithelium. This point is taken up again in Paper III. The dose of 66 mg. hemoglobin per kilo is positive and practically coincides with the depression threshold of 70 mg. per kilo. We do not believe it is possible to establish renal threshold closer than 5 mg. hemoglobin per kilo and often the individual will show a fluctuation in this threshold of 10 mg. hemoglobin per kilo.

This dog was clinically normal throughout the experiment up to the time of the injection which caused death within an hour with symptoms of shock. It is obvious that this last large dose of 8 mg. mercuric chloride per kilo had no time to cause histological changes in the kidney so that this organ will give a satisfactory picture of the injury done by the preceding dose of 4 mg. mercuric chloride per kilo given 5 days before death. The autopsy findings are of no interest for this experiment except for the kidneys which in gross were practically normal except for moderate pigmentation.

TABLE 22

Renal Threshold for Hemoglobin Uninfluenced by Mercury Poisoning

85 hemoglobin injections given before this experiment

Lowest normal or depression renal threshold for hemoglobin = 70 mg. per kilo

Dog 29-198, male, weight 22.7 kilos

Experiment, *days*.....	1	2	3	4	5	6	18	19	20	21	25	26	27	28	31
Urine albumen....	0	0	0	—	Tr.	Tr.	0	Tr.	Tr.	+	+	+	2+	Tr.	—
$HgCl_2$, *mg. per kg*..	1.25	0	0	1.4	0	0	2.0	0	0	2.5	0	4.0	0	0	8.0*
Hb. intraven., *mg. per kg*.....	0	0	50	53	0	0	0	66	53	0	53	0	53	0	0
Hb. in urine.....	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0

* Death 1 hour after injection and autopsy at once.

Histological sections (Dog 29-198, Table 22) show some definite evidence of injury and repair but the great bulk of the tubules present a normal appearance. Pigment is abundant in the epithelium of most of the convoluted tubules and represents iron containing residues derived from the injected hemoglobin. This subject is discussed in detail in Paper IV. The glomerular tufts are all normal. A few tubules show evidence of epithelial regeneration and mitotic figures. Occasional cell casts are noted. The mercury obviously caused no injury to glomeruli and only moderate injury to the tubules where some epithelium was destroyed. Other tubular epithelium cells were slightly injured but were repaired during the 5 days which elapsed between the 5th mercury injection and death from a lethal injection.

Table 23 shows an experiment much like the two preceding ones but particular attention was given to threshold values just above the minimal or depression threshold levels.

For the previous history and hemoglobin injection consult Table 1 and Chart D, Dog 30-154, Paper I. It gives clear evidence that there is no rise of the threshold values except in the very last observation when an injection of 90 mg. hemoglobin per kilo gives a negative urine. The dog died a few days later as the result of renal injury due to 3 mg. mercuric chloride per kilo and these kidneys showed extreme epithelial necrosis which blocked completely the lumina of many tubules. It would seem that this extreme change would give adequate explanation for this single negative observation with a superthreshold dose. We believe that this is purely a mechanical factor which is present only when extreme tubular injury and obstruction is caused by the mercury. Anuria is the next stage which often supervenes in these lethal cases.

This dog 30-154 (Table 23) tolerated small doses of mercuric chloride very well and showed little evidence of renal injury even to a large dose of 2.5 mg. mercuric chloride per kilo. There was no clinical evidence of renal disturbance up to the last dose of 3 mg. mercuric chloride per kilo when some diarrhoea and vomiting appeared shortly before death. The dog died 8 days following the last large dose of mercury and presented all the evidences of renal insufficiency. Autopsy was done promptly after death. The findings are of no interest to this experiment except for the kidneys which were surprisingly normal in gross. Histological sections show a typical picture of *mercuric chloride nephrosis*. The majority of the tubules show necrotic epithelium which in many causes complete blockage of the tubules. Some of this necrotic material is impregnated with lime salts giving the tissue a deep blue granular appearance. The glomerular tufts are all normal. Some tubules have escaped the necrosis and show the usual yellow granular pigment due to the hemoglobin injections. Casts are everywhere numerous. Some efforts to repair the tubular epithelium are noted with proliferation of new epithelium.

Table 24 is much like the preceding experiments and this dog tolerated large doses of mercury with minimal evidence of renal injury. During this time there was no change in the lowest renal threshold values.

A single dose of 100 mg. hemoglobin per kilo gave a negative urine but all other like doses showed a positive urine. This indicates that the 100 mg. hemoglobin level was very close indeed to the absolute renal threshold. The last dose of 2.5 mg. mercuric chloride per kilo gave practically no evidence of renal injury as there was no albuminuria.

Following this last experiment this dog was not injected with any mercury nor

TABLE 23

Renal Threshold for Hemoglobin Uninfluenced by Mercury Poisoning

17 hemoglobin injections given before this experiment

Lowest normal or depression renal threshold for hemoglobin = 60 mg. per kilo

Dog 30-154, male, weight 23.2 kilos

Experiment, *days*	1	2	3	5	12	15	16	17	18	19	22	23	24	26	37	38	40	43	44	45
Urine albumen	0	0	0	0	0	0	0	0	Tr.	0	0	+	0	0	0	3+	3+	3+	3+	
$HgCl_2$, *mg. per kg*	1.25	0	0	0	0	1.75	0	0	0	0	2.5	0	0	0	3.0	0	0	0	0	
Hb. intraven., *mg. per kg*	0	80	50	100	120	0	120	120	90	90	0	0	90	100	0	0	90	0	0	
Hb. in urine	0	+	0	+	2+	0	2+	+	+	+	0	0	+	+	0	0	0	0	0	
																		D	D	
Clinical condition	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	D	V	V	*

N = normal, D = diarrhoea, V = vomiting.

* Death and autopsy at once.

TABLE 24

Renal Threshold for Hemoglobin Uninfluenced by Mercury Poisoning

83 hemoglobin injections given before this experiment

Lowest normal or depression renal threshold for hemoglobin = 100 mg. per kilo

Dog 29-297, female, weight 10.2 kilos

Experiment, *days*	1	2	3	4	8	10	11	12	13	14	15	17	18	19	20	21	36	37	38	39
Urine albumen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Tr.	0	0	0	0	0
$HgCl_2$, *mg. per kg*	1	0	0	0	0	1.5	0	0	0	0	0	2	0	0	0	0	2.5	0	0	0
Hb. intraven., *mg. per kg*	0	80	80	80	150	0	0	100	80	150	100	0	150	100	0	0	0	80	80	100
Hb. in urine	0	0	0	0	2+	0	0	+	0	2+	+	0	2+	0	0	0	0	0	0	+

given hemoglobin for 3.5 months but was bled about twice a week 100 to 200 cc. whole blood which reduced her red cell hematocrit from the initial level of 42 per cent to a level ranging from 35 to 25 per cent. This moderate grade of anemia was produced to note whether the iron containing pigment was removed from the renal tubular epithelium and whether the kidneys subsequently were less resistant to mercury poisoning. After 3.5 months this dog was given an identical dose of mercuric chloride 2.5 mg. per kilo which previously had caused no disturbance. This second dose caused a decided albuminuria but not a lethal injury. Five days later the dog was in good clinical condition and the urine had returned to normal. She was given gas and killed and the autopsy done at once. The anatomical findings are of no interest except for the kidneys which were practically normal in gross.

Histological sections (Dog 29-297, Table 24) show a renal picture which could hardly be differentiated from a normal stock dog. The glomeruli and tubules are normal so that evidently the slight injury had been completely repaired. A very few tubules show in their epithelium a few tiny yellow pigment grains but practically all of the deposited pigment was removed during this rest period combined with a moderate anemia. This point will be referred to again in Papers IV and V.

This dog was somewhat more resistant to mercuric chloride than control dogs even after she had been made anemic to remove almost all the pigment in the tubular epithelium. Whether the preceding series of mercury injections was a factor we cannot say—refer to MacNider's (7) work with uranium and increased tolerance following a previous injury.

DISCUSSION

We do not propose to plunge into the troubled waters of debate relating to the secretory mechanism of the kidney glomeruli and tubules. We refer merely to the admirable papers of MacNider (6), Richards (12), Edwards (2), Marshall (8) and many others. We will attempt to limit our discussion to the behaviour of dog hemoglobin when it is eliminated from the blood plasma into the urine. Possibly some of the confusion in the literature comes from an attempt to correlate findings in cold blooded animals with those in warm blooded animals. It surely is possible that the mechanism of urinary secretion may differ in the frog as compared with the dog, just as it must be different in some of the primitive fishes which possess no glomeruli within their kidneys.

The experimental evidence tabulated above is strongly supported by Tables 31, 32 and 36 in Paper III. The minimal or *depression*

renal threshold is evidently not modified by moderate doses of mercuric chloride and consequent injury done the renal tubules. The same is true even for somewhat severe renal tubular injury, short of actual obstruction to the flow through the tubules. There is no evidence for glomerular injury. We believe this means that from the blood plasma the injected hemoglobin passes out through the glomerular tuft into the convoluted tubules. It passes through these tubules unchanged if there have been frequent preceding hemoglobin injections even if the tubular epithelium has been injured by mercury injections. The normal kidney before it has been subjected to frequent hemoglobin injections will take up much hemoglobin from the tubule into the tubular epithelium and this explains the high initial renal threshold for hemoglobin and its subsequent depression to a relatively fixed or minimal threshold. It is not surprising that this tubular epithelium reaches a repletion stage beyond which it can take in no more pigment material until it has disposed of this pigment and other related substances according to its individual method.

We are unable to explain our findings if the renal tubular epithelium in the dog participates actively in the passage of dog hemoglobin out of the plasma into the tubular lumen.

We believe that this minimum or depression renal threshold comes quite close to the absolute base line or glomerular threshold. The picking up of hemoglobin from the tubular lumen is an important factor in conservation of hemoglobin and hemoglobin "building stones."

SUMMARY

The minimal or depression renal threshold for dog hemoglobin is not modified by moderate doses of mercuric chloride.

This type of renal injury involves the epithelium of the convoluted tubules but the glomeruli escape.

We are unable to explain our findings if we assume that the tubular epithelium takes an active part in the passage of dog hemoglobin from the blood into the urine.

The evidence points toward the glomerular tuft as responsible for the passage of the hemoglobin from the blood plasma into the tubules. The glomerular tuft establishes the true hemoglobin threshold under these conditions.

If the convoluted tubules are normal, we note that hemoglobin is taken into the epithelium and this explains the high initial renal threshold.

With repeated hemoglobin injections this tubular epithelium becomes stuffed with hemoglobin pigment fractions and can absorb no more, which explains the minimal or depression threshold. Further injury of this tubular epithelium with mercury causes no change in this minimal renal threshold, unless we produce actual tubular obstruction.

BIBLIOGRAPHY

1. Baker, S. L., and Dodds, E. C., *Brit. J. Exp. Path.*, 1925, **6**, 247.
2. Edwards, J. G., *Am. J. Physiol.*, 1930, **95**, 493.
3. Fukuda, Y., and Oliver, J., *J. Exp. Med.*, 1923, **37**, 83.
4. Haskell, C. C., Hamilton, J. R., and Henderson, W. C., *J. Lab. and Clin. Med.*, 1926, **11**, 707.
5. Lehnert, F., *Beitr. path. Anat. u. allg. Path.*, 1912, **54**, 443.
6. MacNider, W. deB., *Physiol. Rev.*, 1924, **4**, 595.
7. MacNider, W. deB., *Science*, 1931, **73**, 103.
8. Marshall, E. K., *Physiol. Rev.*, 1926, **6**, 440.
9. Miller, J. W., *Frankf. Z. Path.*, 1912, **11**, 403.
10. Ribbert, H., *Centr. allg. Path. u. path. Anat.*, 1913, **24**, 241.
11. Sansum, W. D., *J. Am. Med. Assn.*, 1918, **70**, 824.
12. Wearn, J. T., and Richards, A. N., *Am. J. Physiol.*, 1924, **71**, 209.