

**THE RENAL CLEARANCE OF HEMOGLOBIN IN THE DOG**

by

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## INTRODUCTION

In recent years, the use of quantitative methods (24, 22) in the study of renal physiology has greatly enlarged our understanding of the mechanism by which molecules smaller than inulin (apparent molecular weight, 15,000(3)) are eliminated from the kidney. These methods have not as yet been fully applied (7) to a study of the renal excretion of a molecule as large as that of hemoglobin (molecular weight, 68,800). The need for such a quantitative study is indicated by the discrepancy which exists between the modern concepts of renal function, which maintain as a major premise that the glomerular membrane is impermeable to colloidal molecules, and the fact, well known clinically and experimentally, that hemoglobinuria occurs when free hemoglobin is present in the blood stream.

The data to be presented are concerned with a study of the renal elimination of hemoglobin in the dog, and were obtained by comparing the excretion of hemoglobin with the simultaneous excretion of creatinine. Since in the dog the rate of excretion of creatinine is known to be a measure of the rate of glomerular filtration (23), a determination of its excretion rate may be used as a basis of comparison in obtaining information concerning the excretion pattern of a second substance.

It is generally conceded in the literature (12, 13, 26) that hemoglobin escapes into the urine via the glomerulus, although the manner in which it does so is by no means clear. Some authors accept the idea of a transient injury to the glomerular membrane

(4, 1) but many physiological and histological studies give no evidence of such injury (5). Whipple and his coworkers (14) have implied that a dynamic glomerular mechanism is responsible for the renal threshold of hemoglobin noted by them and others whose work they quote. The possibility of a different explanation for the phenomenon may be drawn from the work of Richards and Walker (20) who postulated the existence of occasional irregular defects in the glomerular membrane large enough to allow the passage of whole plasma.

A controversy has existed regarding the rôle of the tubules in the excretion of hemoglobin. To many observers the presence of hemoglobin within the cells of the convoluted tubules has indicated a phenomenon of tubular excretion. However, the alternative, that the phenomenon is entirely one of tubular recovery, is substantiated by the recent observations of Lison (15) who describes the impermeability of the basal membrane of the tubular cells to any large negatively charged colloids. The actual mechanism of recovery is said by Gerard (10) to be one of athrocytosis in which the apical membranes of the tubular cells pick up colloidal particles from the tubular lumina and store them in much the same manner as the reticuloendothelial cells phagocytose and store particulate matter elsewhere in the body.

#### METHODS

Female mongrel dogs, trained to lie quietly on a board while being catheterized, were used in each experiment.

Approximately 10 per cent solutions of hemoglobin were prepared from the blood of colony dogs to which was added 1 cc. of saturated sodium citrate per 100 cc. The red cells were separated from the plasma

by centrifugation and washed twice with isotonic saline solution. The packed red cells obtained after the final washing were suspended in half their volume of distilled water and then laked by the addition of one-third their volume of anesthesia ether. After shaking the mixture vigorously several times during a period of 15 minutes, the suspension was centrifuged. The clear bright red supernatant solution so obtained was filtered through Whatman No. 50 filter paper and stored overnight at 2° C. Just before injection, the solution was filtered again, and its hemoglobin concentration determined. The amount of solution injected depended upon the initial plasma concentration desired. Injections were made into the jugular vein at a rate not exceeding 20 cc. per minute.

An adequate diuresis was obtained by giving the animals 300 to 500 cc. of tap water through a stomach tube on the evening of the day prior to the experiment, and at intervals of 90 to 120 minutes before and during the experiment. In order to maintain an alkaline urine and thus to avoid the formation of hemoglobin casts which lead to renal obstruction in acid urine, 2 to 6 gm. of sodium bicarbonate were added to each dose of water.

A 10 per cent aqueous solution of creatinine was injected, in part subcutaneously and in part intraperitoneally, in amounts sufficient to produce an initial plasma concentration of 10 to 15 mg. per 100 cc. of plasma.

One hour after the injection of creatinine, the animal's bladder was washed out with warm isotonic saline solution. At the end of each 20-minute period thereafter, urine samples were collected through a curved metal catheter into a 20 cc. syringe. At each collection, the

volume of urine was noted and the bladder flushed with three 14 cc. portions of warm isotonic saline. The urine and saline were collected in a volumetric flask and made up to a definite volume with water. 15 cc. aliquots of the diluted urine were centrifuged in stoppered tubes to free the samples of any red cells or mucus.

The hemoglobin solution was injected at the end of the second or third period. The data obtained during the first periods were useful in determining whether the injection of hemoglobin had produced any noticeable change in the normal excretion of creatinine. The first urine period containing hemoglobin was begun 10 minutes after the end of the injection. If the animal urinated spontaneously at any time during the experiment, the bladder was immediately flushed with saline, and a new period was begun. The experiments were continued until gross hemoglobinuria could no longer be detected.

8 cc. samples of blood were taken before the first period, at the midpoint of alternate periods, and after the last period. One sample was taken just prior to the injection of hemoglobin and another 5 minutes after the injection. The samples were added to 0.1 cc. of saturated sodium citrate and centrifuged. After noting the hematocrit, the plasma was immediately transferred to another vessel. Protein-free filtrates for the determination of total chromogenic material in the analysis of creatinine were prepared by adding 1 cc. of plasma to 12 cc. of Hala's reagent (11). 2 cc. of plasma were added to 12 cc. of Hala's reagent for the determination of total non-creatinine chromogenic material. The protein precipitates were separated by centrifugation and aliquot portions of the supernatant fluid were used for analysis. Plasma and urine creatinine determinations were carried

out according to the method of Shannon, Jollife, and Smith (23).

The hemoglobin concentrations in both plasma and urine were determined colorimetrically after converting the hemoglobin to cyanmethemoglobin as directed by Evelyn and Salter (8).

All colorimetric measurements were made on the Evelyn photoelectric colorimeter. Care was taken to insure that appropriate blanks were used in each colorimetric determination.

Creatinine and hemoglobin photocalorimetric calibration curves relating the concentration of these substances to galvanometer deflection were constructed with the use of carefully prepared standard solutions. The creatinine standard was prepared by the creatinine zinc chloride method (9), and the hemoglobin standard by the spectrophotometric method of Drabkin and Austin (6).

Values for the plasma concentrations of both creatinine and hemoglobin were plotted against the time of withdrawing the respective blood samples from the animal. By interpolation from this graph, a value was obtained for the plasma concentration of each substance at the midpoint of each period, which was taken as the average concentration of the substance for that particular period. The urinary excretion of both hemoglobin and creatinine in each urine sample was expressed as mg. excreted per minute and graphs were made relating the rate of excretion of each substance to the plasma concentration during the same period. The volumes of plasma cleared of creatinine and hemoglobin per minute during each period were calculated by dividing the mg. of each substance excreted per minute by the corresponding plasma concentration in terms of mg. per 100 cc., and multiplying by 100. Finally, the absolute clearance of hemoglobin and the hemoglobin/

creatinine clearance ratio were each plotted against the hemoglobin plasma concentration.

#### EXPERIMENTAL OBSERVATIONS

A total of seventeen individual experiments were carried out on five animals. Initial plasma concentrations of hemoglobin, following its injection, ranged from 200 to 1300 mg. per 100 cc. of plasma.

The condition of the animals during the course of each experiment remained normal except in experiments 13 and 17, in both of which the animals became rather listless following the injection of hemoglobin, and for several hours thereafter showed an elevation of temperature to about 42° C. It is possible that the hemoglobin solution injected in these instances contained some red cell stroma.

In view of complete reports on the histological findings in dogs which had received multiple injections of hemoglobin (5, 17), it was not deemed necessary to autopsy all of the animals studied in these experiments. However, dog 38-284, which had received a total of 13.67 gm. of hemoglobin in three separate injections, was killed 15 days after the last injection and autopsied immediately. No gross abnormalities were found in any organ. Moderate deposits of iron-containing pigment were observed histologically in the liver and spleen. Hematoxylin and eosin stains of the kidney showed no pathological changes, and special staining of the basement membranes of the glomerular capillaries revealed no abnormalities of these structures. No iron-containing pigment was found in the epithelium of any of the tubules. The absence of such pigment may be related to a moderate degree of anemia produced in the animal by the frequent withdrawal of blood samples.

In Tables I, II, and III, and in Fig. 1, are recorded the detailed data obtained in three characteristic experiments in which the initial plasma concentration of hemoglobin was relatively low (190 mg. per cent), medium (450 mg. per cent), and high (1320 mg. per cent).

The data on the excretion of creatinine in these experiments are analyzed in columns 3 and 4 of the tables and in the graphs of series B in Fig. 1. In the graphs it will be noted that the relationship between the plasma concentration of creatinine and the rate of its urinary excretion per minute is a linear one. The origin of this graph is at zero, indicating that the excretion of creatinine is related only to the rate of glomerular filtration and is not modified by any tubular activity. The volume of plasma, containing the number of mg. of creatinine excreted per minute, designates the volume of plasma water filtered through the glomerulus during each minute. Such clearance values are listed for each period in column 5 of the tables. Variations in the rate of creatinine clearance observed from period to period are attributed to changes in glomerular filtration pressure and are believed to be systemic in origin.

Similar treatment of the data on the plasma concentration and the rate of excretion of hemoglobin is shown in columns 6 and 7 of the first three tables and in the graphs of series A in Fig. 1. Reference to the graphs will show that in each experiment there was a definite plasma concentration of hemoglobin below which none escaped from the kidney. This critical value of plasma concentration is designated as the renal threshold of hemoglobin and above this level the rate of hemoglobin excretion was proportional to the plasma concentration. Since the establishment of these facts is important, all of

TABLE I

Summary of Experiment 8. Dog 38-229. Weight: 12.0 Kilos

Period	Length of period	Creatinine			Hemoglobin			Hb./Cr. clearance ratio	Urine flow
		Plasma	Urine	Clearance	Plasma	Urine	Clearance		
1	2	3	4	5	6	7	8	9	10
	min.	mg./100	mg./min.	cc./min.	mg./100	mg./min.	cc./min		cc./min.
1	15.75	8.80	7.61	86.4	--	--	--	--	0.76
1.66 gm. of hemoglobin dissolved in 45 cc. water injected intravenously (138 mg./kg.)									
2	26.25	7.40	5.60	75.5	--	--	--	--	0.46
3	23.25	7.18	4.83	67.3	148	2.15	1.45	0.0216	0.95
4	16.25	6.40	3.87	60.5	112	1.01	0.90	0.0149	1.26
5	26.25	5.00	3.78	75.5	92	0.56	0.61	0.0080	2.88
6	23.75	3.68	2.99	81.3	81	0.40	0.50	0.0061	2.91
7	22.25	3.20	3.14	98.0	78	0.06	0.08	0.0008	2.94

TABLE II

Summary of Experiment 9. Dog 38-229. Weight: 11.3 Kilos

Period	Length of period	Creatinine			Hemoglobin			Hb./Cr. clearance ratio	Urine flow
		Plasma	Urine	Clearance	Plasma	Urine	Clearance		
1	2	3	4	5	6	7	8	9	10
	min.	mg./100	mg./min.	cc./min.	mg./100	mg./min.	cc./min.		cc./min.
1	22.25	10.87	7.64	70.3	--	--	--	--	1.68
2	24.00	9.15	6.63	77.6	--	--	--	--	2.29
3	15.25	8.47	5.24	61.8	--	--	--	--	2.75
2.59 gm. of hemoglobin dissolved in 32 cc. water injected intravenously (229 mg./kg.)									
4	12.00	8.75	6.75	77.1	434	9.67	2.23	0.0289	3.75
5	21.75	8.34	2.88	34.6	390	3.90	1.00	0.0289	2.12
6	21.00	6.60	3.90	59.1	325	4.88	1.50	0.0254	3.19
7	19.50	5.66	3.76	66.4	282	4.38	1.55	0.0234	4.60
8	17.00	5.18	3.26	63.0	257	3.63	1.41	0.0224	4.56
9	22.75	4.80	3.04	63.4	236	3.34	1.42	0.0224	4.70
10	22.25	3.75	2.14	57.1	166	1.07	0.65	0.0113	0.90
11	21.00	3.30	1.91	57.8	148	0.71	0.48	0.0082	0.33
12	24.00	2.88	1.97	68.4	130	0.47	0.36	0.0053	0.42
13	27.75	2.60	1.74	67.0	115	0.30	0.26	0.0038	4.05
14	22.50	2.30	1.53	66.5	100	0.20	0.20	0.0030	5.60

TABLE III

Summary of Experiment 10. Dog 38-229. Weight: 11.2 Kilos

Period	Length of period	Creatinine			Hemoglobin			Hb./Cr. clearance ratio	Urine flow
		Plasma	Urine	Clearance	Plasma	Urine	Clearance		
1	2	3	4	5	6	7	8	9	10
	min.	mg./100	mg./min.	cc./min.	mg./100	mg./min.	cc./min.		cc./min.
1	18.50	15.55	9.05	58.2	--	--	--	--	1.35
2	26.75	13.05	6.17	47.3	--	--	--	--	1.12
10.00 gm. of hemoglobin dissolved in 70 cc. water injected intravenously (890 mg./kg.)									
3	15.00	11.80	6.13	52.0	--	--	--	--	0.80
4	17.75	10.10	6.56	65.0	1200	12.70	1.06	0.0195	1.18
5	18.00	8.70	3.03	34.9	1088	5.50	0.50	0.0144	3.28
6	19.75	8.02	5.26	65.6	1000	10.70	1.07	0.0158	3.75
7	25.50	7.00	4.38	62.6	933	10.65	1.14	0.0182	2.78
8	27.75	6.26	3.51	56.0	854	9.90	1.16	0.0207	2.24
9	23.25	5.60	2.51	44.8	636	8.16	1.28	0.0286	1.25
10	27.75	5.20	2.21	42.5	569	6.44	1.13	0.0266	1.91
11	22.00	4.55	2.50	55.0	520	6.52	1.25	0.0228	2.90
12	26.25	3.94	2.04	51.8	484	4.85	1.00	0.0193	5.15
13	21.00	3.14	1.82	58.0	437	4.76	1.09	0.0188	2.86
14	25.50	2.86	1.68	58.7	422	3.88	0.92	0.0157	3.45
15	24.50	2.60	1.48	57.0	410	3.78	0.92	0.0162	1.92
16	23.75	2.38	1.16	48.7	404	3.64	0.90	0.0185	1.81

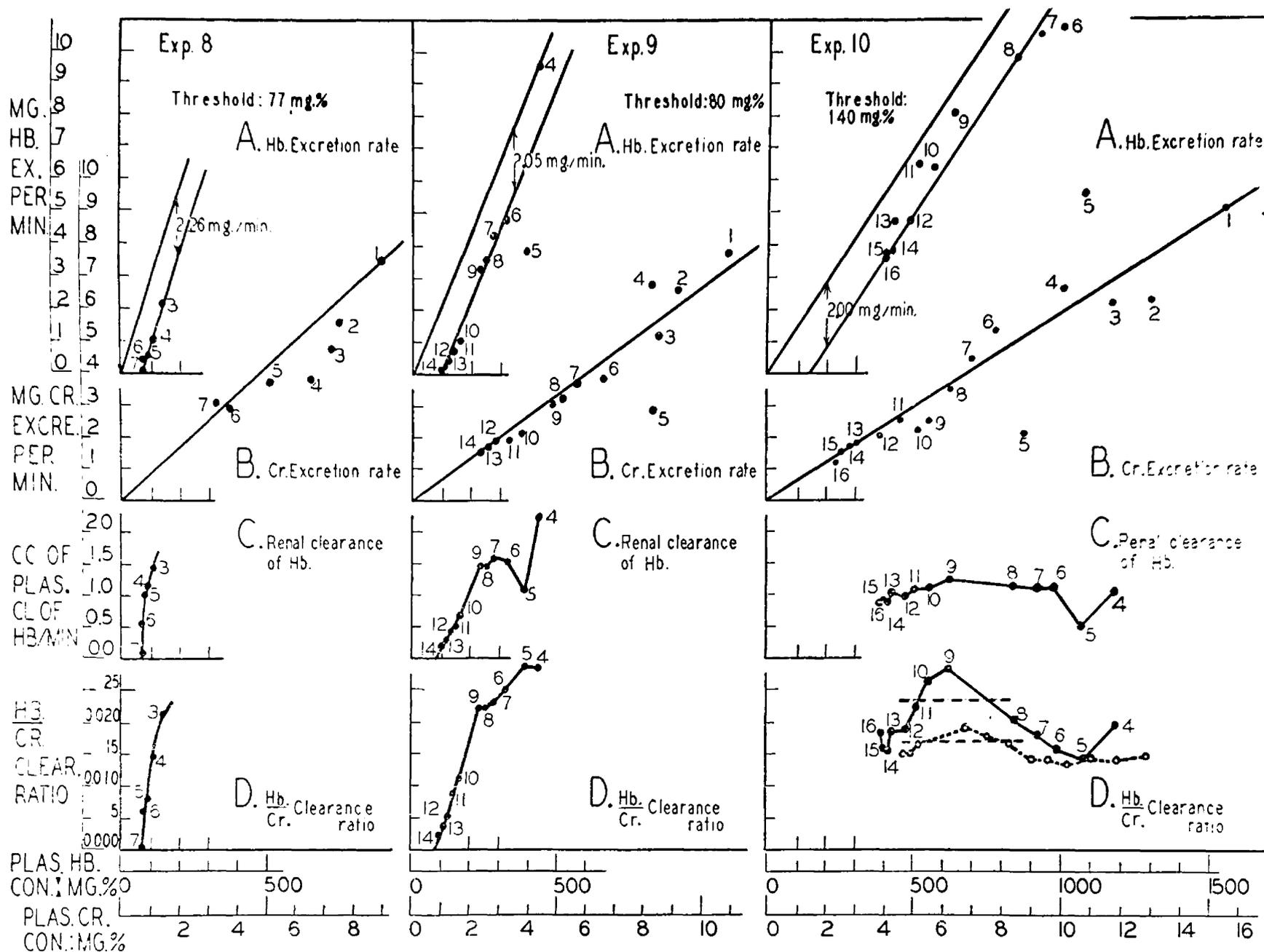


FIG. 1. Dog 38-229. Graphs of the data obtained in experiments 8, 9, and 10. Graphs in series A show the relationship between the rate of excretion of hemoglobin and its plasma concentration. Graphs in series B, similarly, show these relationships for creatinine. The graphs in series C and D relate respectively the absolute renal clearance of hemoglobin and the hemoglobin/creatinine clearance ratio to the plasma hemoglobin concentration.

the values so obtained relating plasma concentration to excretion rate were compiled graphically in Fig. 2. From the distribution of the points on this graph, it is quite clear that the average threshold value is about 100 mg. of hemoglobin per 100 cc. of plasma, and that the direct relationship of the excretion rate of hemoglobin above this level to the plasma concentration holds true for plasma concentrations up to 13 times the threshold value (line A). It is considered that within this range, all of the important aspects of the renal mechanism for the elimination of hemoglobin should have become manifest.

The clearance rate of hemoglobin, expressed as the volume of plasma cleared per minute, is very small when compared to the clearance rate of creatinine (column 8 vs. column 5, Tables I, II, III). In the graphs of series C in Fig. 1, the clearance rate of hemoglobin is related graphically to the plasma concentration. Below the threshold a plasma clearance of hemoglobin is of course not apparent. Increasing concentrations of plasma hemoglobin above the threshold are accompanied by increasing clearance rates. However, the rate of increase diminishes as the plasma concentration rises, and the clearance reaches a maximum value at plasma concentrations of about 250 mg. per 100 cc. Further increases in plasma concentration have no significant effect upon the excretion rate.

The hemoglobin/clearance ratio and its relation to the plasma concentration of hemoglobin in each period of these sample experiments is shown in column 9 of the tables and in the graphs of series D in Fig. 1. The relationships of this ratio to the plasma concentration are similar in nature to those of the hemoglobin clearance curve discussed above. The average value of the hemoglobin/creatinine clearance

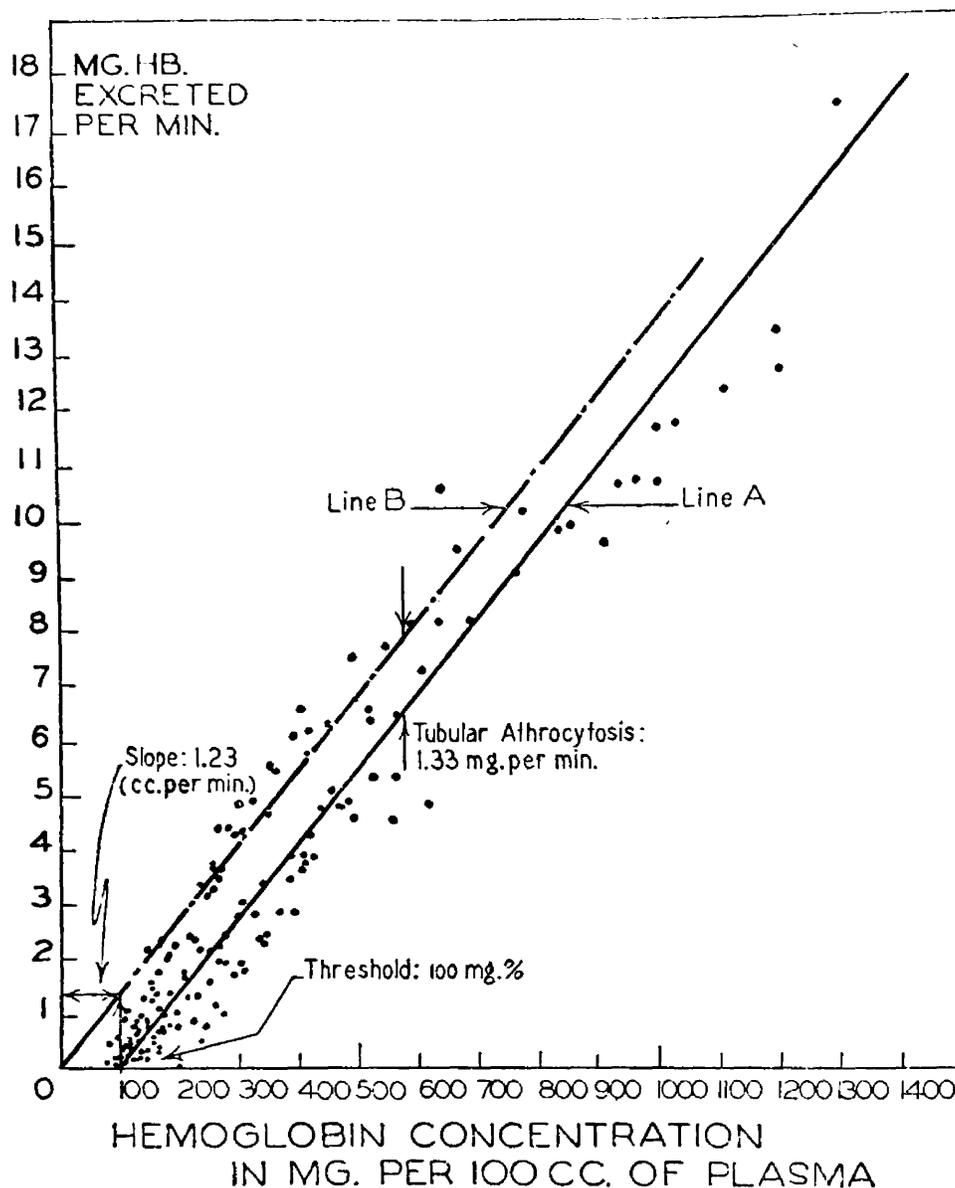


FIG. 2. The points represent a composite of all of the data obtained relating the mg. of hemoglobin excreted per minute to the plasma concentration of hemoglobin. Line A represents the general linear distribution of the points. Line B describes the rate at which plasma is freed of hemoglobin at the glomerulus. Inasmuch as the constants for the rate of glomerular filtration (1.23 cc./min.) and the tubular athrocytosis (1.33 mg./min.) are less than either the mean or the average of the constants obtained from each experiment, they have been dropped in the final discussion in lieu of the average of the values obtained in each experiment as listed in Table V.

ratio obtained from all of the experiments is 0.019 (column 11, Table IV), i.e., the volume of plasma cleared of hemoglobin per minute is only 1.9 per cent of that cleared of creatinine.

A number of the periodic variations found in the graphs of series A and B in Fig. 1, which describe the excretory rates of hemoglobin and creatinine in terms of their respective plasma concentrations, disappear in the hemoglobin/creatinine clearance ratios in the graphs of series D. This indicates that in these instances the same factor was operative in modifying the excretion rate of each substance. The larger variations which occurred during the initial periods of many experiments were frequently associated with a marked but transient oliguria (column 10, Tables I, II, III). These variations are probably related to the transient vasoconstrictor action of hemoglobin on the renal arterial system noted by Mason and Mann (16).

In those experiments in which the initial plasma concentration of hemoglobin was above 1000 mg. per 100 cc., a distinctive type of variation in the hemoglobin/creatinine ratio was noted which must be taken into consideration in further work of this type. It is illustrated by the ratio curve of experiment 10, Fig. 1, graph D. Beginning at period 5, the ratio increases markedly to a maximum in period 9 when the plasma concentration of hemoglobin is 636 mg. per 100 cc. Beyond this point it again falls to a more normal level. The hemoglobin/creatinine ratios of experiment 13, represented by the dotted line in graph D on the same chart, show a similar change in the same range of plasma concentrations. It has been pointed out (19) that during a marked hemoglobinemia, a bilirubinuria may exist, without doubt due to the immediate renal clearance of excess bile pigments formed, from the

TABLE IV

Summary of All Experiments

Dog	Exp. No.	Weight	Hemoglobin	Initial plasma conc. of Hb.	Renal thresh- old	Ave. Cr. clear.	Lowest plasma conc. for max. Hb. clear.	Ave. max. renal Hb. clear.	Ave. max. renal Hb./Cr. ratio	Per cent of injected Hb. in urine	
1	2	3	4 4	5	6	7	8	9	10	11	12
		kg.	gm.	mg./kg.	mg./100	mg./100	cc./min.	mg./100	cc./min.		
38-11	1	12.0	1.31	109	235	87	72	--	--	--	9.8
	2	12.5	1.72	137	235	130	60	--	--	--	--
	3	12.0	1.80	150	275	90*	60	--	--	--	--
	4	14.3	2.15	150	390	125	83	--	--	--	11.7
	5	14.4	7.30	506	630	95	65	300	1.07	0.0192	17.3
	6	12.4	5.76	465	700	140	63	300	0.79	0.0134	12.8
	7	12.4	5.72	461	715	230	61	285	0.69	0.0114	13.5
38-229	8	12.0	1.66	138	194	77*	86	--	--	--	15.4
	9	11.3	2.59	229	450	80	66	230	1.52	0.0252	28.7
	10	11.2	10.00	890	1320	--	60	--	1.03	0.0196	22.0
38-262	11	17.0	2.37	139	330	110*	61	--	--	--	9.4
	12	17.1	4.20	248	680	80	67	180	1.36	0.0233	27.3
	13	17.1	9.76	571	1390	--	70	--	1.14	0.0160	31.0
38-264	14	12.9	3.88	301	480	80*	83	110	1.05	0.0124	18.4
38-284	15	15.7	2.15	137	195	85*	69	175	1.39	0.0210	15.4
	16	16.1	3.52	292	530	85	65	180	1.51	0.0264	21.9
	17	14.6	8.00	549	1160	140	70	250	1.44	0.0209	31.0

\* Threshold value of first experiment in the series.

injected hemoglobin, at a rate above the maximum excretory capacity of the liver. Subsequent to the realization of this possibility, it was found that the addition of a dilute solution of dog bile to the reagents used in the cyanmethemoglobin method for the determination of total hemoglobin caused an appreciable increase in optical density. It may be supposed that between periods 5 and 12 in experiment 10 the excess pigments cleared into the urine were present in concentrations high enough to disturb the hemoglobin determinations. The horizontal lines on this graph represent the ratios of hemoglobin filtration through the glomerulus to the filtration of creatinine, as estimated later in this paper. It is interesting to note for future reference that during periods 9 and 10 in experiment 10 the rate of hemoglobin excretion into the urine exceeded the calculated rate of its filtration through the glomerulus.

The disappearance rates of hemoglobin from the plasma are graphically shown in Fig. 3, each curve representing an individual experiment. In each instance there is a rapid drop in plasma concentration during the first 60 to 90 minutes, after which the rate of disappearance becomes slower. In view of the fact that renal elimination proceeds at a regular rate proportional to the plasma concentration, it is suggested that the rapid drop is related to the equilibration during this time of phagocytic activity and the diffusion of hemoglobin into extravascular body spaces and fluids. There is no evidence that high initial concentrations of hemoglobin initiate any special mechanism of removal which does not function at lower levels. This observation differs from that of Ottenberg and Fox (18).

A summary of the pertinent data obtained from each experiment is

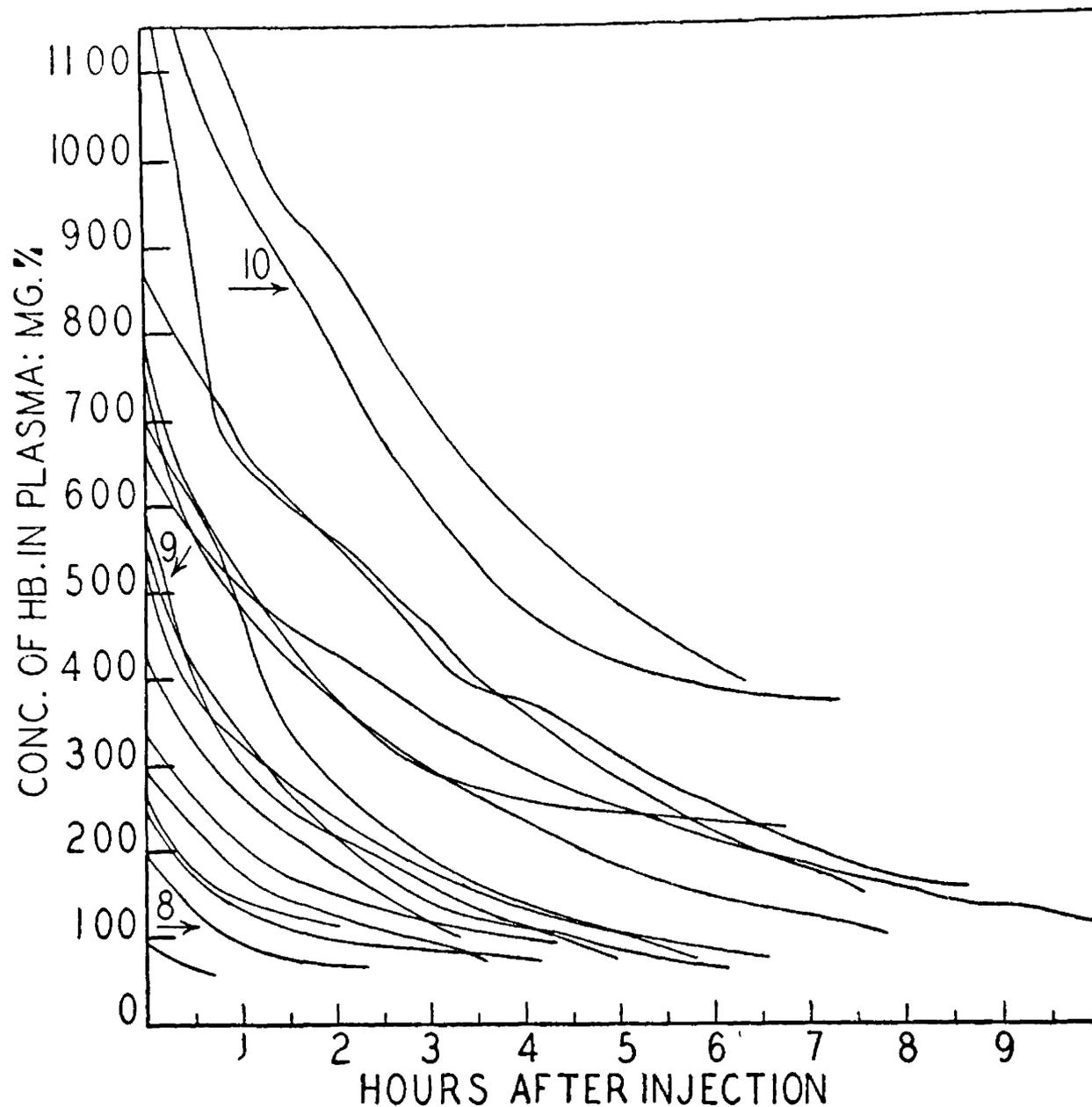


FIG. 3. Each line represents the rate at which hemoglobin disappeared from the blood stream during an individual experiment. Lines 8, 9, and 10 are the disappearance curves of the respective experiments chosen for the protocols recorded in the text.

presented in Table IV. It may be noted that the initial plasma concentration of hemoglobin (column 6) shows no direct relationship to the amount of hemoglobin injected per kilo of body weight (column 5). This would indicate that the plasma volume is not a constant proportion of the body weight, and that, therefore, previous work by other investigators, who based their interpretations solely on the amount of hemoglobin injected per kilogram, can no longer be considered reliable.

The renal threshold values for hemoglobin (column 7, Table IV) were obtained by extrapolating the curves, relating hemoglobin excretion to plasma concentration, to the point of zero excretion. The average level is 100 mg. per 100 cc. of plasma and those marked with an asterisk were obtained at the time of the first hemoglobin injection. The threshold is unrelated to the initial level of hemoglobin concentration in the plasma and no relationship is found between the number of injections previously given nor the time interval separating them. In the present series of experiments, no lowering of the "threshold" (i.e., the minimum amount of injected hemoglobin required to cause hemoglobinuria), as reported by Lichty, Havill, and Whipple (14) following repeated daily injections, was observed. It is felt that their results could be better explained by a slow accumulation of hemoglobin in the plasma after each injection caused by a decreasing phagocytic capacity of the reticulo-endothelial system.

The average creatinine clearance figures (column 8, Table IV), indicating the approximate level of glomerular filtration for each experiment, are comparable to those reported by other investigators (23).

In column 9, Table IV, are listed the plasma levels above which the clearance rate of hemoglobin was found to be at a maximum. In

column 10 the average maximum hemoglobin clearance rates are given in terms of the cc. of plasma cleared per minute. The maximum hemoglobin/creatinine clearance ratios are listed in column 11.

The approximate percentages of injected hemoglobin recovered in the urine during the course of each experiment are listed in column 12 in Table IV. The recovery was roughly proportional to the amount injected.

### DISCUSSION

The most important facts disclosed by these experimental data are that the excretion of hemoglobin has a true renal threshold, and that above this threshold hemoglobin is eliminated by the kidney at a rate which is directly proportional to its concentration in the plasma. Significant, also, is the finding that the renal clearance rate of hemoglobin is of low magnitude when compared with the total glomerular filtration rate. On the basis of these facts and on the related evidence found in the literature, it is possible to arrive at an explanation of the phenomenon of hemoglobinuria which is in agreement with the modern concept of renal function.

With the establishment of these relationships, tubular excretion of hemoglobin must be excluded as a possible mechanism on several grounds. Tubular thresholds are not known to exist and the basal membrane of the tubular cell is said to be impermeable to large electronegative colloidal molecules (15). All previously studied tubular phenomena have been shown to have a maximal limit beyond which their functional capacity does not increase no matter how great the plasma concentration becomes (22).

From the experimental data it can be assumed with reasonable assurance that hemoglobin filters through the glomerulus at a constant rate much lower than the filtration rate of creatinine. In view of the mass of evidence which points to the purely mechanical nature of the glomerular membrane, it is very unlikely that in the case of hemoglobin it should possess a dynamic secretory activity and thus be responsible for the threshold phenomenon. If there can be no glomerular threshold, then the absence of hemoglobinuria below a certain plasma level implies that the tubules have the capacity to reabsorb all of the hemoglobin which escapes through the glomerulus at subthreshold plasma concentrations. Since the capacity reaches its maximum at the threshold, any increase in plasma concentration above this level will cause the hemoglobin, filtered in excess of that reabsorbed, to appear in the urine. As the plasma concentration is increased to higher and higher levels, the amount of hemoglobin recovered by the tubules becomes a smaller and smaller percentage of that which escapes through the glomerulus. At these higher plasma levels, therefore, the clearance of hemoglobin and the hemoglobin/creatinine clearance ratio tend to become constant in value.

The actual magnitude of the glomerular and tubular factors in this phenomenon may be ascertained by a further analysis of Fig. 2. Line A in this figure represents an average of all the experimentally determined points relating the mg. of hemoglobin excreted per minute to the plasma concentration. Since the tubular reabsorption rate remains constant above the threshold, the filtration rate of hemoglobin at the glomerulus, in relation to all the points on line A, may be represented graphically by a line B which is parallel to line A and

which passes through the origin of the coordinate system. The slope of this line (0.0123) indicates that the average volume of plasma cleared of hemoglobin each minute is 1.23 cc. It is constant both above and below the threshold. (See legend of Fig. 2.)

The average rate of hemoglobin filtration per minute at the glomerulus for each experiment is recorded in column 7 of Table V. In column 8 the average creatinine clearance, i.e., the total filtration, is listed for each experiment. These two values are compared in column 9, in which are recorded the glomerular hemoglobin/creatinine filtration ratios. The range of these values is 0.016 to 0.048 and the average is 0.029, i.e., the filtration rate of hemoglobin is 2.9 per cent of the filtration rate of creatinine. This average rate of glomerular filtration of hemoglobin during each experiment (column 7) appears to be greater at low initial plasma concentrations than at higher initial levels. The factor effecting this inverse relationship is obscure, but it operates uniformly throughout an entire experiment.

The average magnitude of the tubular reabsorption rate can also be determined by an analysis of the type of graph shown in Fig. 2. If a perpendicular is erected at the threshold level on the abscissa and extended to intersect line B, the length of the new line measured on the ordinate scale, will represent the magnitude of maximum tubular recovery in terms of mg. of hemoglobin recovered per minute. Actual values for the maximum rate of tubular recovery were determined by such graphical analysis for each experiment and are recorded in column 10 of Table V. The range of magnitude was found to be from 0.90 to 3.80 mg. per minute and the mean value 2.00 mg. per minute. Except for three instances at low plasma concentrations, and two at higher

TABLE V

Summary of Derived Experimental Constants

Dog	Exp. No.	Initial plasma conc. of Hb.	Total renal elimination			"Estimated" average rate of glomerular filtration			"Est." average tubular recovery of Hb
			Threshold	Average max. clear.	Hb./Cr. clear. ratio	Hb. filtration*	Cr. filtration	Hb./Cr. clear. ratio	
1	2	3	4	5	6	7	8	9	10
		mg./100	mg./100	cc./min.		cc./min.	cc./min.		mg./min.
38-11	1	235	100	--	--	2.14	72	0.0298	1.87
	2	235	130	--	--	2.88	60	0.0480	3.80
	3	275	90	--	--	2.14	60	0.0357	1.95
	4	390	125	--	--	1.74	83	0.0210	2.72
	5	630	95	1.07	0.0192	1.83	65	0.0281	2.60
	6	700	140	0.79	0.0134	1.37	63	0.0217	2.23
	7	715	230	0.69	0.0114	1.06	61	0.0174	1.30
38-229	8	192	77	--	--	3.00	86	0.0349	2.26
	9	450	80	1.52	0.0252	2.30	66	0.0348	2.05
	10	1320	140	1.03	0.0196	1.38	58	0.0230	2.00
38-262	11	330	110	--	--	2.60	61	0.0426	3.50
	12	680	80	1.36	0.0233	1.84	67	0.0275	1.68
	13	1390	75	1.14	0.0160	1.14	70	0.0163	0.90
38-264	14	480	80	1.05	0.0124	1.93	82	0.0235	1.54
38-284	15	194	85	1.39	0.0210	2.38	69	0.0345	2.00
	16	530	85	1.51	0.0264	2.15	65	0.0331	1.95
	17	1160	130	1.44	0.0209	1.40	70	0.0200	1.25

\* Corrected for  $T_m$  (tubular absorption). The values recorded are the slopes of the lines which in the excretion rate:plasma concentration graph of each experiment were obtained in a manner similar to line B in Fig. 2.

concentrations, the values lie within a relatively narrow range.

Some confirmation of this hypothesis is supplied by the experimental data of Bogniard and Whipple (2). These investigators accurately measured the increased iron content of the kidneys of dogs which had received multiple intravenous injections of known amounts of hemoglobin. In one dog, weighing 7.5 kilos, which had received a total of 31 gm. of hemoglobin in seven daily injections, each amounting to 625 mg. per kilo, the actual iron increase above normal was approximately 18 mg. Using the average tubular reabsorption rate obtained experimentally as a basis, the iron content of the hemoglobin, which would have been reabsorbed by the tubules of this animal in the 7-day period, was calculated. If all the iron contained in this hemoglobin had remained in the renal epithelium, the iron content of the kidneys would have increased by somewhat over 23 mg.

On the basis of these deductions, it is possible to state precisely a concept of the mechanism by which hemoglobin undergoes renal excretion. Since the average hemoglobin/creatinine glomerular filtration ratio in the dog is about 0.030 (column 9, Table V), it is suggested that three of each hundred pores in the glomerular membrane are large enough to permit the passage of a hemoglobin molecule. As a result, that fraction of the plasma which loses its hemoglobin by filtration at the glomerulus is 3 per cent of the total amount filtered. The proximal convoluted tubules recover hemoglobin by a process of athrocytosis at a maximal rate, which has a mean value of 2 mg. per minute (column 10, Table V). When more than this amount of hemoglobin passes through the glomerular membrane, hemoglobinuria occurs. The fate of the protein moiety of the recovered hemoglobin following in-

tracellular digestion, is unknown, but the iron-containing pigment is stored for future release into hematopoietic channels.

Although it is impossible to ascertain the true nature of the pores in the glomerular membrane, it is necessary to consider them as graded in size, as are those of an artificial membrane, in order to meet the demands of the experimental data. The pore walls are conceived as carrying an electrostatic charge as a result of which the effective diameter of the pore, with respect to the passage of large charged molecules, may be smaller than its structural diameter. The validity of this interpretation is attested by the fact that the perfusion of a kidney with a solution containing some surface active substance such as saponin renders the glomerular membrane completely permeable to protein molecules as large as hemoglobin (21). This phenomenon, which is common to the glomerular as well as some artificial membranes, is reversible and is not believed to be the result of a change in the actual structural dimensions of the pores. That the charge on large molecules such as hemoglobin is a determining factor in their passage through the glomerular membrane has also been demonstrated by the fact that the pH of a hemoglobin-Ringer's solution perfused through the kidneys of frogs markedly affects the rate at which hemoglobin escapes into the urine (26). It is very possible that the difference in the isoelectric point is one of the deciding factors in the normal impermeability of the glomerular membrane to plasma albumin, and its partial permeability to hemoglobin.

That hemoglobin produces a glomerular injury must be largely ruled out, not only because of the absence of any definite proof of such an action in the literature, but also because the hypothesis is incom-

patible with the constancy of the threshold, the regularity of the hemoglobin clearance curves, and the finding of an inverse ratio between the amount of hemoglobin injected and the clearance rate.

The question has been raised whether the hemoglobin which escapes from the glomerulus in these experiments is not dissociated hemoglobin with a molecular weight smaller than 68,800. Such an assumption is improbable in view of the drastic conditions required for hemoglobin dissociation (25).

Although the glomerular membrane has been shown to behave in many respects as a mechanical filter, it must be borne in mind that it is a living structure and that its ultimate integrity is unconditionally dependent upon the living state. It is not improbable that the application of this experimental technique to the excretion of other large molecules of varying size will more fully reveal a graded glomerular pore size.

#### SUMMARY AND CONCLUSIONS

Simultaneous hemoglobin and creatinine renal clearance studies have been presented which indicate that hemoglobin is eliminated by the kidney at a rate which is 3 per cent of the creatinine clearance, above a plasma hemoglobin concentration of approximately 250 mg. per 100 cc. In dogs whose average glomerular filtration rate is 66 cc. per minute, about 2 cc. of plasma are cleared of hemoglobin per minute.

A definite renal threshold exists for hemoglobin at a plasma concentration of about 100 mg. per 100 cc., below which hemoglobinuria does not occur.

The uniformity of the process indicates that hemoglobinuria is not the result of a transient glomerular injury induced by the hemoglobin.

It is tentatively suggested that the experimental results obtained may be interpreted in terms of the following concept. The glomerulus permits the filtration of hemoglobin in amounts directly dependent upon plasma concentration. However, only 3 per cent of all the pores of the membrane are electrostatically large enough to permit the passage of an undissociated hemoglobin molecule. Of that hemoglobin which passes down the tubule, a relatively constant though small amount is recovered by the tubules by a process not unlike that of phagocytosis found elsewhere in the body. An average value for this "athrocytic" capacity in a medium-sized dog is 2 mg. of hemoglobin per minute. This pattern of renal hemoglobin excretion is in agreement with the principles of the modern theory of kidney function.

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