PROTEINURIA RELATED TO HYPERPROTEINEMIA IN DOGS FOLLOWING PLASMA GIVEN PARENTERALLY

A Renal Threshold for Plasma Proteins

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The evidence given below is adequate to establish a *renal threshold for plasma proteins.* Whatever the mechanism may be, there is a lag between the start of the plasma injections and the appearance of the proteinuria—a range of 4 to 26 days. We have not yet established how early the proteinuria may appear when very large doses of parenteral plasma are used nor have we made an effort to drive the hyperproteinemia above levels of 11.5 gm. per cent. The level of plasma protein concentration is probably most important in establishing a proteinuria—in normal dogs the values are 9.6 to 10.4 gm. per cent and probably individual variations are greater. Repeat experiments on the same dogs give practically identical values—see Summary Table—dogs 43-346 and 43-141. Prompt disappearance of the proteinuria, normal gross and histological findings in the kidney at autopsy follow discontinuance of plasma injections.

The literature is full of interesting papers dealing with the permeability of the glomerular tuft to substances other than electrolytes—proteins in particular. We do not propose a comprehensive review of this valuable material most of which has been recently discussed by Bing (4). The belief that the glomerular tuft in its normal state is permeable to proteins was slow in gaining acceptance. When the claim was made that the glomerular tuft was permeable to certain proteins, it was often argued that the glomeruli were abnormal or that there must be crevices or stomata between the various cells lining the capillaries and the epithelium covering the tuft.

The preceding paper dealing with the dynamic equilibrium of proteins—an ebb and flow between the plasma proteins and cell proteins—gives at least a suggestion relating to the proteinuria observed. When albumins or globulins are formed rapidly within a *liver cell* because of body demand, the proteins presumably accumulate within the cell until there is an outflow—due to an increased density of intracellular protein or other factors. Conversely in protein fasting when parenteral plasma is given, these liver cells (and others) permit a protein inflow—perhaps due in part to a decreased density of intracellular protein.

We know of no evidence that there are any stomata involved in these ex-

changes by the liver cell or muscle cell so why argue that there must be stomata or some permanent structural change to account for a passage of protein through normal cells just because they happen to be in the glomerular tuft? With increased concentration of proteins in the plasma these proteins in large amounts do pass the glomerular tuft—not only albumin but several varieties of the globulins. Furthermore this passage involves capillaries as well as the epithelium covering the glomerular tuft. When plasma is given intraperitoneally, it appears promptly in the circulation (passage of two cell barriers); and when it supplies the protein needs of the body, it must pass two more barriers (capillaries and cell membrane—muscle or liver cell for example). It seems obvious that plasma proteins within the body must pass cell barriers with ease. It is accepted that body cell membranes or barriers contain proteins and without doubt some enzymes (proteins) are included in the barrier. It has been suggested (19) that these enzymes may participate in the easy passage of albumins and globulins through cell membranes.

Well known publications support the hypothesis that protein molecules can pass the normal glomerular tuft (1, 8–10, 14–18). The problem of orthostatic albuminuria (20) belongs in this discussion. Hyperglobulinemia is discussed by Bing (5) in observed human cases with particular reference to multiple myeloma. Our dogs show a preponderance of globulins at times, especially when large amounts of albumin appear in the urine—increased sedimentation rate was obvious in these dogs and was recorded by Bing in human beings. Some workers explain the passage of proteins through the glomerular filter as purely a physical phenomenon (2). Bell (3) claims that the capillaries of the glomerulus are unlike those elsewhere in the body and the endothelial lining is not continuous. Monke and Yuile (13) discuss the size of the pores in the glomerular tuft as related to hemoglobinuria. Blackman *et al.* (6) discuss the pathogenesis of Bright's disease and emphasize that high urinary globulin concentration gives a bad prognosis in these patients.

EXPERIMENTAL OBSERVATIONS

The experiments in this paper are similar to those in the preceding paper and are to be examined as a single group; the methods are identical. Reference to the Summary Table below will give total figures for all experiments in these two papers.

Tables 4 and 5 should be examined together since the observations were made on the same dog, a rest period of 12 months on a liberal mixed diet intervening. Table 4 is the first experiment of the whole series and was pushed along week by week with the hope of producing "intoxication" with or without loss of nitrogen. Proteinuria was observed in period 6, Table 4, and increased steadily while the plasma injections continued, but vanished within 2 days after plasma injections ceased. When casein by mouth in relatively equivalent amounts replaces the

plasma by vein we note a frugal use of this protein, a continued positive nitrogen balance, and slight weight gain.

Table 5 is a more complete experiment on the same dog (Table 4), after a rest interval of 12 months. The dog in Table 5 weighs 1.7 kg. more and requires a longer period of fasting to settle down to a base line of urinary nitrogen output. About 2.5 times the amount of plasma protein was given at the start as compared with Table 4, and the amount of nitrogen *conservation* is spectacular in periods 5 and 6, Table 5—a total of 25 gm. nitrogen—presumably a repletion of protein reserves. With these larger injections of plasma the proteinuria appears earlier and on the 8th day the urine "boiled solid," the blood plasma protein levels standing at 9.7 gm. per cent. Because of the large amounts of protein in the urine the urea and ammonia fraction is a better index of nitrogen metabolism and shows insignificant changes. A positive nitrogen balance continues. Intraperitoneal injections are then used. Weight loss is very slow and the dog is in excellent condition. Proteinuria cleared 4 days after cessation of plasma injections.

Evidently larger injections of plasma cause an earlier appearance of proteinuria and a higher output of protein in the urine, representing in this dog 24 per cent of the protein injected (Table 5). The levels of blood plasma protein concentration are not strikingly different in Tables 4 and 5. The highest output of protein in the urine is noted in periods 15 to 17 (Table 5) with plasma protein concentration of 10.4 to 11.3 gm. per cent. When the plasma injections cease there is no notable escape of stored nitrogen (periods 18 and 19, Table 5).

Electrophoretic Studies (Dr. Eric Alling)

In general the *urinary protein* concentration ranges as high as 10 to 15 per cent of the plasma protein concentration but the albumin fraction makes up 60 to 75 per cent of the total urinary protein and fibrinogen does not appear in recognizable form in the urine. The other globulins, however, are well represented in the urine and in general follow the percentage representation in the plasma.

Electrophoretic Pattern—Period 7, Table 4

	Plasma	Urine
	gm. per cent	*gm. per cent*
Albumin	4.08	0.6
Globulins—alpha 1	*	0.06
Globulins—alpha 2	*	0.05
Globulins—beta and gamma	3.2	0.13
Fibrinogen		0
Total		0.84
Kjeldahl—total	9.95	0.64

* Obscured by opacities of unknown origin.

TABLE 4

Plasma Intravenously; Sugar by Mouth

Dog 43-141, adult mongrel hound.

Period No. 4 days	Diet Total N	Plasma injected Total N	Urinary nitrogen					Blood plasma concentration	Weight	Urinary N balance
			Total	Urea plus ammonia		Undetermined	Urinary protein			
	gm.	*gm.*	*gm.*	*gm.*	*per cent*	*gm.*	*gm.*	*gm. per cent*	*kg.*	*gm.*
1			6.11	4.67	76.3	1.44			11.3	−6.11
2			3.12	2.28	73.0	1.12		6.20		−3.12
3		7.08	5.10	3.79	74.3	1.31			10.0	1.98
4		7.63	4.37	3.15	72.1	1.22		9.44	9.8	3.26
5		9.58	5.83	4.57	78.3	1.29			9.8	3.75
6		10.00	7.60	5.38	70.8	1.25	0.97		9.7	2.40
7		9.84	8.31	5.65	68.0	0.83	1.83	9.95		1.53
8		9.83	7.99	5.23	65.5	1.03	1.73		9.4	1.84
9		10.91	10.19	6.56	64.4	1.46	2.17	11.50		0.72
			Sugar stopped. Non-protein diet started							
10	0.16	10.30	9.80	5.72	58.6		3.75	9.77	9.4	0.66
			Plasma injections stopped. Casein added to diet							
11	8.00		7.36	4.68	63.7	1.73	1.15	7.64	9.6	1.14
12	8.00		6.85	5.40	78.8	1.45	0	7.97	9.8	1.65
13	8.00		6.35	4.93	77.7	1.42	0			2.15
14	8.00		5.56	4.08	73.4	1.58	0	8.05		2.94
15	8.00		5.27	4.08	77.5	1.19	0	7.08	10.1	3.23

In period 9, Table 4 (Tiselius), plasma proteins—albumin—4.26, alpha 1—0.32, alpha 2, 3, and 4—2.39, beta, gamma, and fibrinogen—4.13. Total—11.1 gm. per cent, and Kjeldhal analysis, total—11.5 gm. per cent.

Further studies of the plasma and urinary proteins by the method of Tiselius were carried out in Table 5, period 14.

	Plasma	Urine
	gm. per cent	*gm. per cent*
Albumin	3.58	1.02
Globulins—alpha 1	1.82	0.10
Globulins—alpha 2	1.65	0.07
Globulins—beta and gamma	2.75	0.24
Fibrinogen	1.31	0
Total	11.11	1.43
Kjeldahl—total	10.79	1.43

TABLE 5

Plasma Parenterally; Sugar and Vitamins by Mouth

Dog 43-141, Experiment 2.

Period No. 4 days	Diet Total N	Plasma injected Total N	Urinary nitrogen					Blood plasma concentration	Weight	Urinary N balance
			Total	Urea plus ammonia		Undetermined	Urinary protein			
	gm.	gm.	gm.	gm.	per cent	gm.	gm.	gm. per cent	kg.	gm.
1	0.3		8.27	6.81	82.4	1.46		5.94	13.0	−7.97
2	0.3		7.18	5.97	83.1	1.21		5.77	12.6	−6.88
3	0.3		5.96	4.70	79.0	1.26		5.56	12.2	−5.66
4	0.3		5.16	3.98	77.2	1.18		5.29	12.0	−4.86
5	0.3	18.42	4.49	3.19	71.0	1.10		8.05	12.2	14.23
6	0.3	19.97	9.26	5.74	62.0	1.24	2.18	9.70	12.3	11.01
7	0.3	16.46	15.15	8.25	54.5	1.52	5.38	9.93	12.2	1.61
8	0.3	12.89	12.53	7.10	56.6	1.29	4.14	9.79	11.9	0.66
9	0.3	13.16	12.41	7.65	61.6	1.42	3.34	9.83	11.7	1.05
10	0.3	12.49	11.30	7.09	62.8	1.28	2.93	9.71	11.5	1.49
11	0.3	12.43	10.10	5.90	58.4	1.48	2.12	9.33	11.5	2.63
Intraperitoneal injections only										
12	0.3	11.82	8.98	5.96	66.4	0.87	2.15	9.81	11.4	3.14
13	0.3	12.99	9.67	6.82	70.6	1.21	2.64	9.55	11.4	3.62
14	0.3	13.77	11.02	6.16	55.9	1.62	3.24	10.42	11.4	3.05
15	0.3	14.35	13.32	7.11	53.4	1.51	4.70	11.13	11.4	1.33
Intravenous injections only										
16	0.3	15.61	16.03	8.76	54.6	1.54	5.73	11.32	10.9	−0.12
17	0.3	15.98	14.65	7.63	52.1	1.71	5.51	10.67	10.7	1.63
Plasma stopped. Sugar and vitamins by mouth continued										
18	0.3		8.09	5.64	69.8	1.39	1.06	8.04	10.4	−7.79
19	0.3		6.31	5.30	84.0	1.01	0	7.32	10.0	−6.01

Again in period 17, Table 5—

	Plasma	Urine
	gm. per cent	gm. per cent
Albumin	2.90	1.03
Globulins—alpha 1	2.45	0.12
Globulins—alpha 2	1.65	0.09
Globulins—beta and gamma	2.50	0.33
Fibrinogen	1.30	0
Total	10.80	1.57
Kjeldahl—total	10.70	1.67

Again in period 18, Table 5, about 102 hours after the last injection of plasma the electrophoretic pattern of the plasma read: Albumin—2.11 gm. per cent, alpha 1 and 2—1.74 gm. per cent, alpha 3—1.86 gm. per cent, beta—0.92 gm. per cent, gamma—0.94 gm. per cent, fibrinogen—0.75 gm. per cent, with a total of 8.32 gm. per cent.

The relatively low levels of albumin in the circulating plasma are presumably related in part at least to the more rapid escape of the smaller albumin molecule through the kidney.

TABLE 5-*a*

Period 10 in Table 5

Protein Excreted at Relatively Constant Rate

Time	Urine volume	Volume* plasma injected	Total nitrogen injected	Urinary protein nitrogen		Blood plasma concentration
				Expected	Observed	
	cc. per 12 hrs.	*cc. per 24 hrs.*	*gm. per 24 hrs.*	*gm. per 12 hrs.*	*gm.*	*gm. per cent*
9 a.m.–9 p.m.	550	310	3.17	0.392	0.334	
9 p.m.–9 a.m.	450			0.321	0.362	9.37
9 a.m.–9 p.m.	800	272	2.75	0.570	0.486	10.00
9 p.m.–9 a.m.	275			0.198	0.299	9.86
Total 48 hrs.....	2075	582	5.92	1.481	1.481	
9 a.m.–9 p.m.	690	355	3.56	0.495	0.493	9.59
9 p.m.–9 a.m.	315			0.226	0.294	9.71
9 a.m.–9 p.m.	700	302	3.01	0.502	0.416	9.70
9 p.m.–9 a.m.	300			0.217	0.295	9.77
Total 48 hrs.....	2005	657	6.57	1.44	1.44	

* Plasma injected at 11 a.m.

Table 5-*a* shows a breakdown of period 10 in Table 5. The rate of urine excretion is not constant, and more urine is excreted during the day, but there is a continuous escape of protein in the urine both night and day. Evidently there is not an immediate outpouring of protein through the kidney, following the injection of whole plasma during the forenoon. The evidence favors a relatively constant excretion of proteins by the kidney—see Experimental history, Table 5-*a*.

Tables 6 and 7 should be considered together as the experiments were similar and both animals were *bile fistula* dogs. Duration of both experiments was short—15 and 16 days but the daily injection of plasma was large. Note that the periods are 48 hours in both experiments as compared with 4 day periods in Tables 4 and 5. Bile fistula dogs do not tolerate fasting and these periods were only 2 and 3 days.

Protein reserves are low in these dogs, but the fasting levels were probably not reached before the plasma injections were begun. Proteinuria in traces appeared in 48 hours (Table 6) and on the 4th day (Table 7). Both dogs show a positive nitrogen balance and body weight was maintained. The dog of Table 6 shows definite proteinuria at a plasma protein level of 8.7 gm. per cent but the other bile fistula dog (Table 7) shows proteinuria at a plasma protein level of 10.4 gm. per cent and this level is continuously high while the proteinuria continues (Table 7, periods 6 to 9). Evidently individual variations and not the bile fistula were responsible. Both dogs were autopsied subsequently and showed no significant renal abnormalities.

Table 8 gives an experiment which was started without knowledge that the apparently normal dog was suffering from a mild cystitis and pyelonephritis which became quite active during the later periods 12 to 15. This inflammation explains the strong negative nitrogen balance in periods 13 to 15. Autopsy some 3 months later revealed the extent of this inflammation. A long fasting period (30 days) precedes the plasma injections in period 8, Table 8, to determine whether the onset of the proteinuria would be earlier or more severe in this depleted state. There were traces of proteinuria (the significance not appreciated) before the plasma injections were started, but definite proteinuria began on the 8th day. The degree of proteinuria is not beyond the expected range except in periods 13 to 15 when the infection of the renal pelvis and bladder was causing obvious intoxication. The cause of this inflammation was a series of experiments on the animal some months before that of Table 8, in which daily catheterization was employed to terminate urinary collections. One notes considerable protein utilization and nitrogen retention in periods 8 to 11. The experiment indicates that a long fast does not of itself modify the reaction to plasma injections—also the fact that an infection may disturb the usual picture observed in the normal dogs (compare Tables 1 to 5 in Summary Table).

Experimental History—Table 4.

Dog 43-141, adult female mongrel hound; was given regular kennel ration for several months prior to this experiment. Two days of fasting were followed by 8 days of feeding 50 gm. glucose by stomach tube daily. Each period was terminated by catheterization. Plasma protein determinations were done on blood samples drawn immediately prior to the time of plasma injection—about 11 a.m. Plasma administered intraperitoneally in periods 3 and 4 and intravenously thereafter. During periods 9 and 10 there was some vomiting and slight diarrhea. This ceased as soon as the plasma injections were discontinued, and no significant intoxication was noted at any time. 150 gm. of low protein diet plus 200 mg. choline and 25 mg. nicotinic acid was given daily during periods 10 to 15. Casein (15 gm. daily) was supplied during the after period so that the total N in diet was 2.0 gm. per day.

Experimental History—Table 5.

Dog 43-141 (Table 4), after a rest period of almost a year on a diet of kitchen scraps, was fasted for 4 days and then given 50 gm. of glucose and 10 cc. of the special vitamin mixture by stomach tube daily for 16 days. The dog lost almost 2 kg. during these 3 weeks but seemed otherwise active and healthy. Large volumes of homologous plasma (357 to 548 cc.) were

given in single doses half intravenously and half intraperitoneally for the first 10 days of plasma administration. During periods 8 and 9 and 12 to 15 the plasma was given intraperitoneally only. At all other times the plasma was injected intravenously. Diarrhea was noted only twice (periods 14 and 19). The dog remained in excellent general condition throughout the experiment except for the expected loss of weight. Plasma was the sole source of nitrogen throughout the experiment except for the 0.074 gm. N present in the daily dose of vitamins.

Experimental History—Table 5-*a*.

Dog 43-141. Period 10, Table 5, was divided into 12 hour intervals by catheterizing the dog and washing out the bladder with normal saline. No difficulty was encountered. An at-

TABLE 6

Plasma Intravenously; Low Protein Diet and Vitamins by Mouth

Dog 46-79, bile fistula.

Period No. 2 days	Diet Total N	Plasma injected Total N	Urinary nitrogen					Blood plasma concentration	Weight	Urinary N balance
			Total	Urea plus ammonia		Undetermined	Urinary protein			
	gm.	*gm.*	*gm.*	*gm.*	*per cent*	*gm.*	*gm.*	*gm. per cent*	*kg.*	*gm.*
1	0.24		4.60	3.37	73.2	1.23		5.83	11.0	−4.36
2*	0.12		2.96	2.22	75.0	0.74		6.65	10.7	−2.84
3	0.24	6.95	3.15	2.45	77.8	0.70	Trace	7.84	10.8	4.04
4	0.24	7.34	3.78	2.36	62.4	0.86	0.56	8.72	10.9	3.80
5	0.24	7.29	4.39	2.31	52.6	0.87	1.21	9.45	10.9	3.14
6	0.24	7.85	5.72	2.28	39.9	1.18	2.26	10.25	10.9	2.37
7	0.24	7.42	6.48	3.12	48.2	1.12	2.24	9.14	10.7	1.18
8	0.24	7.36	6.35	2.68	42.2	1.20	2.47	9.45	10.9	1.25
9	0.24	7.54	5.46	2.56	45.3	1.00	2.08	9.95	10.7	2.32
10*	0.12	3.85	2.95	1.23	41.7	0.69	1.03	9.80	10.7	1.02
11	0.15		3.79	1.90	50.0	1.06	0.83	9.26	10.1	−3.64
12	0.15						0.06	8.44	10.2	
13	0.15		4.62	3.29	71.1	1.33	Trace	8.47	9.9	−4.47
14	0.15		4.45	3.45	77.5	1.00		8.72	9.3	−4.30
15	0.15		4.32	3.37	78.0	0.95		8.34	9.1	−4.17
16	0.15		2.79	2.00	71.8	0.79		8.03	9.2	−2.54

* 1 day period.

tempt was made to produce a constant urine volume without success. The night output was always less than the daytime volumes. One column of the table gives the values of urinary protein nitrogen calculated as follows:

$$\text{Expected urinary nitrogen in 12 hour period} = \frac{\text{Urine volume in 12 hour period}}{\text{Total urine volume in 48 hours}} \times \text{Total urinary nitrogen in 48 hours}$$

Experimental History—Table 6.

Dog 46-79, adult female mongrel hound with bile fistula, in the course of bile salt metabolism studies to be reported later by Dr. William B. Hawkins *et al.*, was given kennel diet plus 50 to 100 cc. of her own bile by mouth daily for 50 days prior to the start of this ex-

periment. The dog received 200 gm. of low protein diet plus 10 cc. of special vitamin mixture daily for 4 days prior to intravenous administration of plasma, and this diet was continued throughout the period of plasma injections and for 2 days after plasma was stopped. The dog evidenced some allergic reaction on the 1st and 3rd days of plasma administration, in the form of facial edema and transient flushing of skin with itching. This cleared spontaneously. Vomiting occurred following injection during periods 5 and 6, probably owing to large volumes of plasma (374 to 385 cc.) given in a short time (10 to 15 minutes). Slight jaundice appeared during periods 9 and 10 and continued for several days. The dog received only 50 gm. glucose plus 10 cc. of the vitamin mixture by stomach tube daily during periods 12 to 16.

Further experiments not related to this paper were finished and about 6 weeks after period 16 the dog was killed with ether. The kidneys in gross and in histological sections showed no significant abnormalities. The 2 weeks' proteinuria left no mark on these organs.

TABLE 7

Plasma Intravenously; Low Protein Diet and Vitamins by Mouth

Dog 46-9, bile fistula.

Period No. 2 days	Diet Total N	Plasma injected Total N	Urinary nitrogen					Blood plasma concentration	Weight	Urinary N balance
			Total	Urea plus ammonia		Undetermined	Urinary protein			
	gm.	*gm.*	*gm.*	*gm.*	*per cent*	*gm.*	*gm.*	*gm. per cent*	*kg.*	*gm.*
1	0.24		2.62	1.89	72.3	0.73		6.15	8.8	−2.38
2	0.24	7.47	2.59	1.80	69.4	0.79		8.04	9.0	5.12
3	0.24	7.24	3.00	2.05	68.6	0.95		9.34	9.1	4.48
4	0.24	7.33	3.20	2.39	74.6	0.81	Trace	9.90	9.1	4.37
5	0.24	7.56	3.54	2.23	63.0	0.70	0.61	10.37	9.1	4.26
6	0.24	7.30	6.89	3.67	53.3	1.11	2.11	10.34	9.1	0.65
7	0.24	7.23	6.55	3.20	48.9	0.77	2.58	10.47	9.0	0.92
8	0.24	7.46	5.71	2.83	49.3	0.88	2.00	10.48	9.0	1.99
9	0.24	6.85	3.69	1.80	48.9	0.71	1.18	10.44	8.9	3.40
10	0.24		4.58	3.01	65.8	0.80	0.77	9.53	8.9	−4.34
11	0.24		2.73	1.81	66.1	0.92	Trace	8.20	8.6	−2.49
12	0.24		2.30	1.72	80.0	0.58		7.83	8.4	−2.06

Experimental History—Table7.

Dog 46-9, adult female mongrel with bile fistula, in the course of other experiments to be reported later by Dr. W. B. Hawkins *et al.*, was given kennel diet plus 100 cc. of her own bile daily for 25 days prior to the start of this experiment. The dog received 200 gm. of low protein diet plus 10 cc. of vitamin mixture, 200 mg. choline, and 25 mg. nicotinic acid for 7 days prior to intravenous administration of plasma, and this diet was continued throughout the period of plasma injections and for 6 days after the plasma was stopped. Facial edema, flushing and itching of the skin occurred once in period 6 and vomiting was noted once in period 9. Otherwise the dog was in excellent health and lost practically no weight during the 16 days of plasma injection. The proteinuria disappeared completely after 2 days on kennel diet. The dog was used for other experiments and was sacrificed several months later. No gross or histologic abnormalities were noted in the kidneys.

Experimental History—Table 8.

Dog 43-290, adult female mongrel bull, used previously for various experiments. The dog

was given regular kennel ration for several months prior to this experiment and was somewhat overweight. Two days of fasting was followed by 28 days of feeding 50 gm. of glucose by stomach tube daily. Faint traces of protein were detected in the urine during this starvation period. Catheterization was not employed in this experiment but had been used as routine in previous experiments. Heavier traces of protein appeared in the urine during the first period on plasma injections. The dog rapidly became toxic and began vomiting after the second period of plasma injections. Small ulcers appeared on the oral mucous membranes during period 11 (Table 8) and diarrhea became frequent. Vomiting became a conditioned reflex

TABLE 8

Plasma Intravenously; Sugar by Mouth

Dog 43-290, cystitis and pyelonephritis.

Period No. 4 days	Plasma injected Total N	Urinary nitrogen					Blood plasma concentration	Weight	Urinary N balance
		Total	Urea plus ammonia		Undetermined	Urinary protein			
	gm.	*gm.*	*gm.*	*per cent*	*gm.*	*gm.*	*gm. per cent*	*kg.*	*gm.*
1							7.35	14.8	
2						Trace			
3									
4						Trace		13.2	
5									
6		4.42	3.17	71.8	1.25			12.5	−4.42
7		5.08	4.02	79.2	1.06		6.38	12.1	−5.08
8	8.32	4.44	3.30	74.4	1.14	Trace			3.88
9	8.50	4.38	3.28	74.9	1.10	Trace	9.54	11.8	4.12
10	8.91	6.79	4.98	74.0	0.81	1.00	9.83		2.12
11	8.58	5.27	3.47	65.6	0.65	1.15			3.31
12	9.49	10.00	5.46	55.0	1.95	2.59	9.55	11.6	−0.51
		Sugar discontinued. Dog toxic—vomiting							
13	9.35	16.00	9.57	59.4	1.71	4.72		10.9	−6.65
14	9.48	24.86	16.05	64.6	2.83	5.98	10.04	10.3	−15.38
15*	5.24	11.21	16.97	62.5	1.82	2.42	7.67	10.0	−5.97

* 2 day period.

and occurred whenever one of the observers entered the room. It was not prevented by adequate oral and parenteral doses of atropine. Because of vomiting, sugar was stopped and the dog was given Ringer's solution fortified with potassium chloride and injectable vitamin B complex (solu-B, Upjohn) in an attempt to restore electrolyte balance and correct the obvious vitamin deficiencies. Dehydration became severe and the animal was almost moribund. Non-protein nitrogen on the last day of plasma injection was 63 mg. per cent. Generous amounts of hamburger steak followed by regular kennel ration restored the dog's weight and appearance of well-being, but proteinuria and occasional spontaneous hematuria continued for 3 months. The dog was killed with ether and autopsy revealed acute and chronic cystitis with a moderately severe chronic pyelonephritis. This condition was probably related to repeated catheterization in previous experiments and it helps explain the high figures for urinary nitrogen in periods 12 to 15, Table 8.

The *Summary Table* brings out the important points of the paper. It is to be emphasized that the first 5 tables (1 to 5) giving data procured by the use of three normal dogs, show a reasonably uniform response. The two bile fistula dogs, Tables 6 and 7, show differences but the fistula was probably not wholly responsible. The last experiment, Table 8, deals with an abnormal dog in which the cystitis and pyelonephritis was lighted up by the long period of protein fasting. A large negative nitrogen balance is to be noted.

It is apparent that larger amounts of plasma given parenterally will cause proteinuria to appear earlier and to a greater degree—compare two experiments on the same dog (43-141), in which the nitrogen of the injected plasma is increased by more than 50 per cent, the proteinuria appears on the 8th day (14th day with less plasma), and the percentage of injected plasma protein appearing in the urine measures 24 per cent (as compared with 16 per cent with less plasma).

SUMMARY TABLE

Table No.	Dog No.	Duration of plasma administration	N from plasma total	N from diet total	Urinary nitrogen			Urinary protein N as per cent of protein N injected	Time elapsed before proteinuria	Concentration of protein in plasma		
					Total	Protein	Balance			Proteinuria appeared	Maximal level	Average of all levels
		days	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*per cent*	*days*	*gm. per cent*	*gm. per cent*	*gm. per cent*
1*	43-346	92	204	1.0	184	13.4	21	7	14	10.4	11.5	10.3
2*	43-346	76	178	3.0	154	14.0	27	8	26	10.3	11.5	10.0
3*	44-98	76	180	52.0	168	38.6	64	21	22	9.9	10.7	9.6
4	43-141	32	75	0.2	59	11.6	16	16	14	9.7	11.5	10.1
5	43-141	52	191	3.9	149	45.7	46	24	8	9.7	11.3	9.9
6	46-79	15	56	1.8	38	12.7	20	23	4	8.7	10.2	9.3
7	46-9	16	58	1.9	35	9.2	15	16	6	10.4	10.5	9.6
8	43-290	30	68	0	83	18.0	−15	26	8±	9.5	10.0	9.7

* See tables in preceding paper.

Tables 6 and 7—bile fistula dogs.

Table 8—cystitis and pyelonephritis.

There seems to be adequate evidence that there is a threshold for plasma proteins. The values for the initial proteinuria in terms of plasma protein concentration run from 9.7 to 10.4 gm. per cent in the normal dogs and the average figures in these same experiments are 9.6 to 10.3 gm. per cent—not a wide spread. The highest levels attained for plasma protein concentration are 11.5 gm. per cent. The overflow of protein is very considerable in some experiments—yet the proteinuria cleared in 1 to 4 days when plasma injections were discontinued, and the kidneys were demonstrably normal subsequently.

DISCUSSION

Our interest in the renal threshold for plasma proteins came about by accident. We attempted to repeat some earlier experiments (7) in which a pecu-

liar intoxication was observed in dogs receiving plasma by vein and sugar by mouth. As the repetitions did not bring on this intoxication, the experiments were lengthened and finally protein was found in the urine. The proteinuria increased as larger amounts of plasma were given parenterally, and when plasma injection was discontinued it promptly cleared. There was no evidence of renal damage following proteinuria at a high level maintained for 5 to 7 weeks. The first experiment is given in Table 4 and for contrast an animal with a diseased kidney (pyelonephritis—Table 8) was included in the series.

Renal thresholds for hemoglobin in the dog have interested workers in this laboratory for years. Thresholds for dog hemoglobin, for dog muscle hemoglobin, for goose and sheep hemoglobin have been established (12). The threshold for muscle hemoglobin was about 5 per cent that of blood hemoglobin. The renal threshold for blood hemoglobin can be lowered by repeated injection of hemoglobin day by day, which results in accumulation of it in the epithelium of the convoluted renal tubules (11). Further study of hemoglobin renal clearance is reported by Monke and Yuile (13, 21, 22).

It is obvious that the small protein molecules pass the renal threshold in greater amounts—albumin 3 or 4 times that of the globulins in these experiments. *Fibrinogen* stands out as distinct from the other globulins by present methods of analysis. Fibrinogen may in fact pass the glomerular tuft, be precipitated as fibrin in the tubules, to be absorbed or perhaps digested by the tubular epithelium (not unlike some hemoglobin in hemoglobinuria). The amounts of fibrinogen concerned would be very small and could readily escape detection.

Some may believe that the renal threshold is wholly dependent upon the level of plasma proteins in the blood and this may be a fact. However, we must consider the possibility of some absorption of proteins by the convuluted tubular epithelium, which might for a time delay the appearance of proteinuria.

SUMMARY

Proteinuria in normal dogs can be produced at will by parenteral injections of dog plasma.

As the plasma injections are continued the plasma protein concentration rises and at some point protein begins to appear in the urine. The level of plasma protein concentration at which proteinuria appears in normal dogs ranges from 9.6 to 10.4 gm. per cent. This may be termed the renal threshold for proteinuria. Repeat experiments in the same dog show threshold levels to be practically identical.

An interval of days (4 to 26 days) has been noted between the start of plasma protein injections and the appearance of the proteinuria. Larger doses of plasma shorten this interval and the critical plasma protein level is attained sooner.

Considerable amounts of protein may appear in the urine—298 gm. protein during a 52 day period in one instance studied—yet the urine clears in 1 to 4 days after cessation of protein injections. Autopsy shows undamaged kidneys.

Maximal levels of plasma protein concentration range from 10.0 to 11.5 gm. per cent. The highest levels are usually associated with maximal output of protein in the urine.

It seems clear that plasma proteins readily pass cell barriers (or membranes) within the body, including the endothelium and epithelium of the renal glomerulus.

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