DOG PLASMA PROTEIN GIVEN BY VEIN UTILIZED IN BODY METABOLISM OF DOG

HORSE PLASMA AND DOG HEMOGLOBIN NOT SIMILARLY UTILIZED

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Experiments given in this paper support the thesis (4) that dog plasma proteins when given intravenously to dogs are readily utilized in the body with the result that a positive nitrogen balance can be maintained during fasting periods. We knew that the anemic dog (9) promptly utilizes goose or sheep hemoglobin given intravenously to build abundant new hemoglobin and red cells. We anticipated therefore that the fasting dog would utilize *foreign plasma* much as it does dog plasma. This the body does not choose to do. All of the horse plasma protein given intravenously to the fasting dog is probably broken down and thrown away in the urine.

Hemoglobin is constantly being broken down in the circulation and in the presence of anemia the dog utilizes the globin fraction to make new hemoglobin (5). We suspected that when anemia was not present and when the body needed protein (fasting) there might be a demonstrable utilization of the globin fraction to spare or replace body protein. In this we were disappointed and we observe below in the fasting dogs that the bulk of the introduced hemoglobin is broken down and thrown away as extra nitrogen in the urine. There is evidence that some of the introduced hemoglobin is retained to form new hemoglobin for red cells.

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Methods

Method and procedures have been described in an earlier paper (3). Dogs were normal healthy animals unless otherwise noted. All food was withheld for 3 days to allow nitrogen elimination to reach a fasting level. The dog was then catheterized and placed in a clean metabolism cage. Water was available in the cages at all times. When each experiment was terminated with catheterization the dogs were placed on the kennel diet and were not used again until they had regained their former weight, usually periods of 6–8 weeks intervening.

During the periods of dog plasma injection large healthy donors were bled 500 cc. into a flask using as an anticoagulant 22 mg. of *heparin* per 100 cc. of blood, dissolved in 5 cc. of Locke's solution. The blood was centrifugalized for 35 minutes at 3000 R.P.M. in 100 cc. centrifuge tubes, the plasma drawn off with suction, measured, warmed in a warm water bath to slightly above body temperature, and injected into the jugular vein or peritoneal cavity as indicated in each experiment. The total daily dosage was given in two injections 6–8 hours apart. Approximately 15 minutes were required for the plasma to run into the vein from a gravity bottle. A small sample was saved for analysis of total protein, albumin, and globulin. The metabolism cage was kept in the laboratory under close observation throughout the day and urine voided was put aside at once in a separate bottle containing sulfuric acid to prevent loss of nitrogen as ammonia. The urine output per period was diluted to a known volume (usually 500 cc.) and analyzed for total nitrogen and protein. A modification of the gravimetric method of Folin and Denis was used to determine urinary protein excretion.

To furnish horse plasma for the stated injections a normal horse was bled 500 cc. from the jugular vein into a flask, using as an anticoagulant 22 mg. of heparin per 100 cc. of blood. The plasma was analyzed for total protein, albumin, and globulin.

The dog hemoglobin for injection was prepared as described for other experiments (7). Healthy donors were bled 150 cc. into a flask containing 1.5 cc. of saturated sodium citrate. The cells were separated by centrifugalization and washed twice with normal saline. They were then laked with approximately twice their volume of distilled water by moderately severe shaking for at least 15 minutes. Following this the sediment was thrown down by high speed centrifugalization for at least 30 minutes and the clear supernatant fluid was filtered through several layers of cotton gauze. The filtrate was then ready for injection into the vein or peritoneal cavity. Hemoglobin was always used within 8 hours after preparation. All hemoglobin solutions were standardized by the acid hematin method (8). The urine obtained after hemoglobin injection was examined spectroscopically for traces of hemoglobin as described elsewhere (6). Clean but not sterile technique was used for the preparation of the fresh serum and hemoglobin.

Feces were collected promptly after defecation, preserved in sulfuric acid, dried over steam, pulverized, and aliquot portions analyzed for N. Usually the

total feces was handled as a unit and the daily average for the total period indicated in the tables.

EXPERIMENTAL OBSERVATIONS

To obviate some of the slight systemic reactions observed in the earlier experiments (4) which were attributed to sodium citrate we used *heparin* to prevent coagulation in collection of dog and horse plasma. This heparinized plasma can be given either intravenously or intraperitoneally with practically no reaction. In some animals as noted in their clinical histories there was vomiting. This vomitus contained little but mucus and only traces of nitrogen so that it was discarded—rarely did it contaminate the urine and even then it introduced no significant error.

Table 21 shows three separate experiments upon the same dog. This Dog 32-131 had been used in other metabolism experiments and in a previously published paper (4) is recorded a control experiment giving the dog sugar alone. This experiment serves as a base line control for those given in Table 21.

The first experiment (Table 21) is almost perfect. The heparinized plasma caused no clinical disturbance and the dog was normal in all respects. There was no contamination of the urine. In all these experiments the injected plasma was analyzed for protein content so that the figures given for plasma protein injection are accurate and not based on a 6 per cent estimated value as in the earlier report (4). These actual values were determined as 6.75 per cent for total protein. The dog received 160 gm. plasma protein by vein and there was no escape by way of the urine. At the end of the last control period the circulating plasma protein amounted to 55 gm. which is probably 30 gm. above a control on sugar alone. This accounts for about 20 per cent of the injected protein but leaves us 130 gm. retained in or used within the body. The urinary nitrogen is not increased by the plasma injections and the output of urinary N and intake of plasma N practically balance. The evidence seems conclusive that 130 gm. of plasma protein are utilized in the body economy. If this material was stored passively and then released we should expect a subsequent rise in the urinary N in the sugar after period.

During this same experiment there is no change in the A/G ratio although the plasma proteins rise to very high levels (9 per cent). In spite of this increase in plasma proteins there is but little change in

blood plasma volume and no spill over of protein in the urine. The red cell hematocrit falls steadily in proportion to the amount of bleeding which was considerable due to blood volume and other analyses and is reflected in the column "Protein removed from blood as N," Table 21.

TABLE 21

Plasma Protein and Hemoglobin Contrasted—by Vein and Intraperitoneally Dog 32-131.

Experimental periods	Days	Injected N, daily average	Urinary N, daily average	Negative N balance, daily average	Protein removed from blood as N, daily average	Fecal N, daily average	Circulating plasma protein period end	Weight at period end
First	Dog plasma by vein—sugar by stomach tube							
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*kg.*
Fore period—sugar.....	5	0	2.07	2.35	0.182	0.1	39.48	15.3
Plasma protein 80.5 gm.....	7	1.84	1.83	0.32	0.230	0.1	48.5	14.3
Plasma protein 79.4 gm.....	7	1.814	1.99	0.61	0.337	0.1	60.79	13.8
After period—sugar.....	5	0	1.294	1.64	0.242	0.1	55.1	13.0
Second	Dog plasma intraperitoneally—sugar and vegetable oil by stomach tube							
Fore period—sugar.....	5	0	2.50	3.36	0.083	0.78	50.45	15.8
Plasma protein 180.2 gm.....	8	3.603	2.32	+0.34	0.172	0.78	82.72	15.4
After period—sugar.....	5	0	3.21	4.13	0.141	0.78	52.00	14.4
Third	Dog hemoglobin by vein—sugar by stomach tube							
Fore period—sugar.....	5	0	1.744	2.21	0.054	0.41	41.82	14.2
Hemoglobin 30.2 gm.....	7	0.690	2.181	1.96	0.060	0.41	35.48	14.0
Hemoglobin 30.8 gm.....	7	0.70	2.146	1.92	0.068	0.41	38.15	13.2
After period—sugar.....	5	0	1.154	1.67	0.108	0.41	34.84	12.6

Intraperitoneal injections of dog plasma (Dog 32-131, second period) give results in general in accord with the intravenous experiment on the same dog. Larger amounts of plasma can be given (180 gm. in 8 days) and there is no escape by way of the urine. The injected N (3.6 gm. daily) much exceeds the urinary N (2.3 gm. daily) so that there is a positive N balance.

Vomiting developed during the last part of the plasma injection period and continued during the after period of sugar feeding. This may have been due in part to the stomach tube feeding of oil with the sugar causing nausea but probably in part to slight peritoneal irritation due to repeated plasma injections. As a result of the loss of

TABLE 21-*a*

Plasma Protein, Urinary Protein, Plasma and Red Cell Volume

Dog 32-131.

Experimental periods	Days	Blood plasma Average concentration: Total protein	Albumin	Globulin	A/G ratio	Urinary N, as protein N, daily average	N.P.N. plasma	Plasma volume period end	R.B.C. hematocrit period end
First	Dog plasma by vein—sugar by stomach tube								
		per cent	*per cent*	*per cent*		*gm.*	*mg. per cent*	*cc.*	*per cent*
Fore period—sugar.....	5	5.64	3.02	2.62	1.15	0	13	696	46.0
Plasma—dog.....	7	8.11	4.24	3.87	1.10	0		714	31.6
Plasma—dog.....	7	8.19	4.26	3.93	1.08	0	20	781	26.5
After period—sugar.....	5	7.59	4.16	3.35	1.21	0		652	27.8
Second	Dog plasma intraperitoneally—sugar and vegetable oil by stomach tube								
Fore period—sugar.....	5	6.37	3.21	3.16	1.02	0	20	792	46.0
Plasma—dog.....	8	9.60	5.54	4.06	1.36	0		1043	34.1
After period—sugar.....	5	8.15	4.38	3.77	1.16	0	20	638	42.1
Third	Dog hemoglobin by vein—sugar by stomach tube								
Fore period—sugar.....	5	6.56	3.61	2.95	1.22	0		648	46.0
Hemoglobin.....	7	5.40	2.65	2.75	0.96	0.011		666	49.4
Hemoglobin.....	7	6.28	3.02	3.26	0.93	0.009	19	549	51.6
After period—sugar.....	5	6.25	3.63	2.62	1.39	0.004	17	566	45.5

sugar there is a rise in the urinary N but we must admit that peritoneal irritation might be responsible for a little of this urinary N excess in the after period.

The plasma protein retention following intraperitoneal introduction seems to be the same as with intravenous injection. The rise in circulating plasma proteins is extreme but the rise in plasma volume is

not in proportion although present (Table 21-*a*, second period). The A/G ratio changes but little.

Hemoglobin given by vein (Table 21, third period) in the same dog (32-131) seems to escape in the form of urinary N except for some which may be utilized to form new hemoglobin for new red cells. In giving hemoglobin by vein in these experiments it is necessary to keep below the renal threshold (5) for hemoglobin in the dog. In fact on only a few occasions were traces of hemoglobin noted in the urine and the protein N escape in the urine is trivial. Only 30 gm. of hemoglobin could be given intravenously each week and still keep below the renal threshold and this amounts to 0.7 gm. N per day. As a result of this injection the urinary N rises 0.5–0.6 gm. per day above the expected levels—indicating that there is practically no N retention—and the usual negative urinary N balance continued. Meanwhile the red cell volume and hematocrit do not fall in contrast to an experiment on the same dog with plasma injection (first period) where the red cell hematocrit fell from 46–28 per cent. We may argue therefore that some of the injected hemoglobin is used to make new hemoglobin for red cells and the rest is discarded to account for the excess urinary N. Certainly the reaction to *hemoglobin* is very different from that to *plasma* when either is given intravenously to a protein fasting dog.

Clinical History, Dog 32-131.—A young mongrel hound. See Tables 21 and 21-*a*. First experiment, Sept. 29. Weight 16.7 kg. Plasma protein 5.58 per cent; albumin 2.73 per cent; globulin 2.58 per cent; plasma volume 724 cc.; blood volume 1350 cc.; red cell hematocrit 46.0 per cent. Sept. 29–Oct. 1, all food withheld, weight fell to 15.9 kg. Oct. 2, 50 gm. glucose and 300 cc. water daily by stomach tube. 10 gm. kaolin added on alternate days. Catheterization to start metabolism experiment. Oct. 7, dog plasma injection begun, intravenously. Catheterization. Oct. 9, more or less generalized urticaria ½ hour after injection. 1 cc. of 1:1000 adrenalin was given subcutaneously and by the next day the reaction had cleared and the rest of the experiment was without event. Oct. 14, catheterization. Oct. 17, no reaction using plasma from same donor as on Oct. 9. Oct. 20, last plasma injection. Oct. 21, catheterization. Oct. 26, final catheterization. No fecal contamination of urine during entire period. Dog put on kennel diet.

Second experiment, Tables 21 and 21-*a*. Apr. 14, weight 17.5 kg. Plasma protein 6.50 per cent; albumin 3.48 per cent; globulin 3.02 per cent; N. P. N. 25 mg.; plasma volume 966 cc.; blood volume 1756 cc.; red cell hematocrit 45 per cent.

Apr. 15–17, all food withheld. Apr. 18, daily 100 gm. glucose plus 100 cc. Wesson oil (vegetable oil) plus 10 gm. kaolin, plus 350 cc. water, by stomach tube. Catheterization to start metabolism experiment. Apr. 23, catheterization. Dog plasma injection intraperitoneally begun. May 1, catheterization. Last plasma injection. Vomiting occurred throughout the period of injection, and at times there was contamination of the urine by vomitus. May 6, catheterization. Dog placed on kennel diet.

Third experiment, Tables 21 and 21-*a*. Nov. 24, weight 16.2 kg. Plasma protein 6.16 per cent; albumin 3.83 per cent; globulin 2.33 per cent; N.P.N. 17 mg.; plasma volume 763 cc.; blood volume 1384 cc.; red cell hematocrit 45.2 per cent. Nov. 24–26, all food withheld. Nov. 27, weight 15.2 kg. Plasma protein 7.04 per cent; plasma volume 627 cc.; blood volume 1160 cc.; red cell hematocrit 46.2 per cent. Daily, 60 gm. dextrose plus 10 gm. kaolin plus 300 cc. water by stomach tube. Catheterization. Dec. 1, catheterization to start metabolism experiment. Hemoglobin injections started, intravenously. Dec. 6, hemoglobin in urine gross +; spectroscopically +. This is the only occasion upon which the dog showed hemoglobinuria either by gross or spectroscopic examination. Dec. 8, catheterization. Dec. 14, last hemoglobin injection. Dec. 15, catheterization. Dec. 19, final catheterization. Dog returned to kennel.

Table 22 gives four experiments on four different dogs. The first experiment (Dog 33-324, Table 22) is much like that described above (Dog 32-131, Table 21). Heparinized dog plasma was given in large amounts and sugar was given in larger doses although to a smaller dog. During the 8 days of plasma injection the dog actually gained 0.2 kg. of body weight and the urinary N is not increased by the plasma injection—there is a small positive balance between the intake N and the urinary N. In the after period we see the urinary N at the level expected from sugar feeding alone and there is no escape of any stored N.

The plasma proteins (Table 22-*a*, Dog 33-324) show conspicuous increases due to the plasma injection but the A/G ratio is unchanged. There is no escape of protein in the urine. The usual fall in the red cell hematocrit is observed due to frequent sampling of blood.

Clinical History, Dog 33-324.—See Tables 22 and 22-*a*, first experiment. May 18, plasma protein 5.56 per cent; albumin 3.48 per cent; globulin 2.08 per cent; N.P.N. 11 mg. May 25–28, all food withheld. May 28, weight 9.7 kg. Daily, 60 gm. glucose, plus 10 gm. kaolin in 300 cc. water, by stomach tube. Catheterization. June 2, dog plasma injection intravenously started. June 4, following the injection the dog developed a marked urticaria and vomited a considerable

portion of its sugar. Vomiting occurred daily thereafter throughout the period of injection. The urine was not contaminated by vomitus however. June 9, last plasma injection. June 10, catheterization. June 15, final catheterization. Dog put on kennel diet.

TABLE 22

Foreign Plasma Protein and Dog Hemoglobin Not Conserved by the Protein Fasting Dog

Experimental periods	Days	Injected N, daily average	Urinary N, daily average	Negative N balance, daily average	Protein removed from blood as N, daily average	Fecal N, daily average	Circulating plasma protein period end	Weight at period end
Dog 33-324 Dog plasma by vein—sugar by stomach tube								
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*kg.*
Fore period—sugar.....	5	0	1.920	2.07	0.047	0.1	18.04	9.0
Plasma protein 98.4 gm.....	8	1.968	1.920	0.20	0.092	0.16	41.29	9.2
After period—sugar.....	5	0	1.440	1.66	0.113	0.11	27.48	8.3
Dog 33-136 Horse plasma by vein—sugar by stomach tube								
Fore period—sugar.....	5	0	3.388	4.02	0.110	0.52	33.86	14.7
Plasma protein 103.7 gm.....	7	2.371	4.169	2.47	0.150	0.52	44.87	13.6
Plasma protein 40.8 gm.....	3	2.183	7.553	5.91	0.017	0.52	56.92	13.6
After period—sugar.....	4	0	7.748	8.54	0.272	0.52	47.17	13.6
Dog 31-138 Horse plasma intraperitoneally—sugar by stomach tube								
Fore period—sugar.....	5	0	3.126	4.10	0.101	0.87	50.76	22.6
Plasma protein 105.0 gm.....	7	2.401	3.619	2.20	0.112	0.87	54.37	21.7
After period—sugar.....	4	0	3.297	4.31	0.140	0.87	59.59	21.3
After period—sugar.....	4	0	3.900	4.90	0.127	0.87	54.15	20.1
Dog 33-178 Dog hemoglobin intraperitoneally—sugar by stomach tube								
Fore period—sugar.....	5	0	2.094	2.76	0.066	0.60	29.61	15.6
Hemoglobin 80.0 gm.....	7	1.83	3.091	1.92	0.061	0.60	37.66	14.8
Hemoglobin 44.8 gm.....	4	1.79	3.505	2.378	0.063	0.60	43.20	14.3
After period—sugar.....	4	0	2.225	3.01	0.185	0.60	39.59	14.0

The second experiment (Dog 33-136, Table 22) shows the result of giving *horse plasma* intravenously in amounts corresponding to the dog plasma experiments. The injections were continued for 10 days

at which time evidences of "serum hypersensitivity" developed together with albuminuria and the dog was clinically quite sick. The N.P.N. of the blood rose (Table 22-*a*). Several days later the dog was

TABLE 22-*a*

Plasma Protein, Urinary Protein, Plasma and Red Cell Volume

Experimental periods	Days	Blood plasma Average concentration: Total protein	Albumin	Globulin	A/G ratio	Urinary N as protein N, daily average	N.P.N plasma	Plasma volume period end	R.B.C. hematocrit period end
Dog 33-324 Dog plasma by vein—sugar by stomach tube									
		per cent	*per cent*	*per cent*		*gm.*	*mg. per cent*	*cc.*	*per cent*
Fore period—sugar.....	5	4.89	2.97	1.92	1.6	0	18		53.2
Plasma—dog.....	8	7.91	4.75	3.16	1.5	0		522	26.1
After period—sugar.....	5	7.25	4.34	2.91	1.5	0	18	379	36.1
Dog 33-136 Horse plasma by vein—sugar by stomach tube									
Fore period—sugar.....	5	4.87	2.84	2.03	1.40	0.005	16	525	47.3
Plasma—horse.....	7	8.13	3.81	4.32	0.88	0.061	23	904	37.4
Plasma—horse.....	3	6.20	2.72	3.48	1.28	1.660	48	746	33.0
After period—sugar.....	4	5.66	2.43	3.23	1.33	0.927	49	845	25.5
Dog 31-138 Horse plasma intraperitoneally—sugar by stomach tube									
Fore period—sugar.....	5	5.97				0	15	791	53.0
Plasma—horse.....	7	6.83	3.48	3.35	1.04	0.004	21	947	46.0
After period—sugar.....	4	6.11	3.33	2.78	1.19	0.010	23	896	44.0
After period—sugar.....	4	5.95	3.12	2.83	1.10	0.012	23	900	46.0
Dog 33-178 Dog hemoglobin intraperitoneally—sugar by stomach tube									
Fore period—sugar.....	5	5.69	3.23	2.46	1.31	0	17	570	54.0
Hemoglobin.....	7	6.46	2.48	3.98	0.62	0.002	18	671	53.6
Hemoglobin.....	4	6.60	2.34	4.26	0.55	0.003	15	652	50.0
After period—sugar.....	4	5.38	3.20	2.18	1.47	0.002	16	670	51.0

killed with ether (clinical history below). A chronic nephritis found at autopsy explains in part the high urinary escape of protein.

Horse plasma intravenously (Dog 33-136, Table 22) reacts entirely differently from dog plasma. The urinary N rises to high levels fol-

lowing these injections and in the after period continues at even higher levels. In fact there is an escape of more N in the urine above control levels than was introduced, to be explained no doubt by the body reaction to a foreign protein with the associated clinical symptoms of intoxication. In spite of this loss of urinary N the circulating plasma contains an excess of protein during and after the horse plasma injection periods. The A/G ratio is disturbed in the 1st week (Table 22-*a*) and the N.P.N. rises to 3 times normal. The surplus of circulating protein may be thought to be a mixture of horse and dog plasma protein and this in fact may be the case but during the after period this excess might be due to a contribution from the reserve store which we know can furnish much new plasma protein. The reaction to foreign serum complicates this experiment and makes it unsatisfactory.

The rise in plasma volume (Dog 33-136, Table 22-*a*) is of some interest and may be wholly due to the excess of plasma protein but some may choose to explain this apparent rise in plasma volume as due to increased permeability of the capillaries sufficient to permit escape of the blood volume dye thus giving high readings for blood volume

The red cell hematocrit shows the usual fall due to repeated blood samplings although some of this fall may be explained by the increase in blood volume if this is real and not due to dye escape.

Clinical History, Dog 33-136.—See Tables 22 and 22-*a*, second experiment. Jan. 5, weight 16.6 kg. Plasma protein 5.07 per cent; albumin 3.25 per cent; globulin 1.82 per cent; plasma volume 858 cc.; blood volume 1619 cc.; red cell hematocrit 47 per cent. Jan. 5–7, all food withheld. Jan. 8, plasma protein 5.21 per cent; albumin 3.38 per cent; globulin 1.83 per cent; N.P.N. 22 mg.; plasma volume 775 cc.; blood volume 1490 cc.; red cell hematocrit 48.1 per cent. Daily, 60 gm. glucose plus 300 cc. water, by stomach tube. Catheterization. Jan. 13, catheterization. First injection of horse plasma, intravenously. Jan. 22, last plasma injection. Dog quite lethargic. Icteric. Areas of hyperemia are prominent over the body surface. Jan. 23, catheterization. Dog more active. Jan. 26, catheterization. Dog returned to kennel diet. Feb. 3, plasma protein 3.17 per cent; albumin 1.43 per cent; globulin 1.64 per cent; N.P.N. 56 mg. Dog has become progressively more listless since returned to kennel. Now comatose. Shows marked edema. Dog killed and autopsied. Autopsy findings: Necrotizing bronchopneumonia. Pulmonary edema. Acute splenic tumor. Acute lymphadenitis. Chronic nephritis. Cloudy swelling of kidneys. Ulcerations of skin. Ascites. Marked edema of lower extremities.

Horse plasma given for 7 days *intraperitoneally* is a satisfactory experiment (Dog 31-138, Table 22) and again gives no positive evidence that the body can utilize these foreign proteins as it does dog plasma protein. Horse plasma protein (105 gm.) is equivalent to 16.8 gm. of N and if this dog had been on sugar alone we believe its final after period would have shown a urinary daily output of 2.5 gm. N. If we calculate the excess due to the plasma injections we find 120 gm. N or about 75 per cent of the injected N. This dog on sugar alone would show a shrinkage of circulating proteins probably to 30 gm. or less, a level 25 gm. below the end period in this experiment. This surplus plus the protein escaping in the urine will account for all of the injected horse plasma protein. This assumes that horse plasma protein remains in the circulation. It would be of interest to expand the after period to observe whether for a time an excess of urinary N would be observed while the blood plasma proteins are returning to normal. Certainly it would be a rash claim that the foreign protein is utilized within the body metabolism.

Clinical History, Dog 31-138.—See Tables 22 and 22-*a*, third experiment. Feb. 26, weight 23.8 kg. Plasma protein 6.12 per cent; albumin 3.80 per cent; globulin 2.32 per cent; N.P.N. 20 mg.; plasma volume 913 cc.; blood volume 2077 cc.; red cell hematocrit 56 per cent. Feb. 26–28, all food withheld. Mar. 1, weight 23.1 kg.; plasma protein 6.04 per cent; albumin 4.75 per cent; globulin 1.29 per cent; N.P.N. 28 mg.; plasma volume 901 cc.; blood volume 1956 cc.; red cell hematocrit 54 per cent; daily 80 gm. glucose by stomach tube. Catheterization. Mar. 6, catheterization. Horse plasma injection, intraperitoneally, begun. Marked urticarial reaction 30 min. after initial injection. Vomited 3 hours after injection. Mar. 11, retched after injection, no vomitus. Urine contaminated by feces. Mar. 12, last plasma injection. Mar. 13, catheterization. Mar. 17, catheterization. Mar. 21, final catheterization. Dog normal and returned to kennel.

Dog hemoglobin given intraperitoneally (Dog 33-178, Table 22) proves to be a very interesting experiment. About 125 gm. of dog hemoglobin (or a total of about 20 gm. of N) was given into the peritoneum during 11 days. During the two periods of injection and the after period an excess of urinary N amounting to about 17 gm. was found. This accounts for practically all the injected hemoglobin. We note only a trivial fall in the red cell hematocrit as contrasted with other like experiments without hemoglobin injections. We may argue

therefore that a little of the injected hemoglobin was utilized to keep up the high level of the red cell hematocrit. This would seem to preclude any possibility that the body could use the *globin* from hemoglobin as it does injected dog plasma proteins in other experiments.

One significant observation deserves comment (Table 22-*a*, Dog 33-178). The A/G ratio changes conspicuously and falls to 0.62 and 0.55 while the globulin fraction shows a great increase. It is suggested that this may be due to the *globin* from the hemoglobin after the pigment radicle has been split off. It would appear in the globulin fraction by the method used. The A/G ratio promptly comes back to normal when the hemoglobin injections are discontinued as presumably the globin was broken down and discarded as excess urinary N. During this period the plasma showed bile pigmentation but not any red color. The same type of change is noted when the hemoglobin is given by vein (Table 21-*a*, third period) but it is not as conspicuous.

This apparent increase in globulin and total plasma proteins may be due to the *globin* from injected hemoglobin, explaining the high values for total circulating plasma protein.

Clinical History, Dog 33-178.—See Tables 22 and 22-*a*, fourth experiment. Feb. 5, weight 17.0 kg. Plasma protein 5.61 per cent; albumin 3.82 per cent; globulin 1.79 per cent; N.P.N. 21 mg. Feb. 5–7, all food withheld. Feb. 8, weight 15.9 kg., plasma protein 5.35 per cent; albumin 2.84 per cent; globulin 2.51 per cent; plasma volume 503 cc.; blood volume 1061 cc.; red cell hematocrit 52.0 per cent. Daily, 70 gm. dextrose plus 10 gm. kaolin, plus 300 cc. water, by stomach tube. Catheterization. Feb. 13, intraperitoneal hemoglobin injection started. Catheterization to start metabolism experiment. Feb. 14, hemoglobin in urine, gross ++; spectroscopically ++. Feb. 20, hemoglobin in urine, gross ?; spectroscopically +. Catheterization. Vomited immediately after injection. Appeared lethargic for following 18–20 hours. Feb. 23, hemoglobin in urine gross 0; spectroscopically +. Last hemoglobin injection. Feb. 24, catheterization. Feb. 28, catheterization. Dog returned to kennel.

DISCUSSION

When we found that new hemoglobin in large amounts could be produced in the anemic dog while fasting (1) and recalled the observation (2) that much new liver protein could be regenerated during protein fasting (chloroform poisoning and liver repair during sugar periods), when we observed that from a protein reserve plasma

proteins could be produced in large amounts above the basal output we used the term "dynamic equilibrium" to indicate an ebb and flow between various organ, plasma, and body proteins. We were tempted to picture these *protein reserves* as water spiders skittering about on the mill pond of body metabolism activated by the stimulus of the moment. It would appear that there are patterns for the actions of these water spiders and the pattern for plasma protein movement and construction is more limited than the pattern for hemoglobin protein.

To produce abundant plasma protein the dog must have suitable diet food factors or draw upon the reserve protein store and when this reserve is depleted so far as we can see the dog is almost wholly dependent upon food protein to make new plasma protein. There is evidence (Table 4, Paper I) that when the protein reserve is exhausted, during fasting periods the dog can produce little if any new plasma protein. This has a bearing upon the *nature of this reserve store* of protein building material for plasma protein. It would seem that the reserve store was not in the form of fixed and mature organ protein otherwise we would expect some plasma protein regeneration during fasting periods when body protein is being broken down. It is possible that this reserve store is in the form of an immature or pro protein material of large molecular form stored in various organs, for example the liver. These substances may be designated as "intermediates."

The possibilities are much more varied and our pattern of behavior for *hemoglobin* much more elaborate as the anemic dog can produce new hemoglobin not only from diet factors and from a reserve protein store but by conservation of protein waste products normally present during any fasting period (1). Foreign hemoglobins (sheep and goose) are promptly picked up and recast to form new dog hemoglobin in anemia, and hemoglobin destroyed in the anemic dog is promptly turned over into new hemoglobin and red cells.

There was a real reason why we wished to know whether the dog could utilize the globin from hemoglobin during periods of emergency. In the plasmapheresis experiments we return an excess of washed red cells because many of these introduced cells rapidly go to pieces in the circulation. The washing and other trauma presumably injure the red cells and shorten their life in the circulation. Could this excess

hemoglobin be utilized to make plasma protein or other body proteins? The evidence is against this possibility as given above.

Evidence that the dog cannot utilize foreign plasma protein and cannot utilize the globin from hemoglobin except to form new hemoglobin for red cells has been given. This evidence by contrast actually strengthens the case for the ready utilization of dog plasma protein when given parenterally during protein fasting. We should think of the plasma proteins as being continuously repleted from the food factors as they are called on for various protein needs within the body.

SUMMARY

Foreign plasma protein (horse) introduced parenterally into the protein fasting dog is not utilized in the body economy. Its fate appears to be disintegration and elimination as excess urinary nitrogen. This is totally different from the fate of dog plasma protein under similar conditions.

Dog hemoglobin given parenterally to the protein fasting dog is not utilized as is dog plasma protein to keep the animal in nitrogen equilibrium but the globin is largely broken down and discarded as excess urinary nitrogen. A small part of the injected hemoglobin is probably utilized to maintain the red cell concentration in the blood at high levels.

Dog plasma given parenterally in a protein fasting dog will maintain the dog in nitrogen equilibrium and there is no surplus nitrogen elimination in the after periods. It is apparent that the introduced plasma protein is utilized efficiently in body metabolism to replace or repair tissue protein. It is suggested that although this is an emergency reaction the same reactions may go on in normal internal metabolism. The observation that *foreign* plasma and dog hemoglobin cannot be utilized when given parenterally actually strengthens this last argument for a normal contribution from plasma proteins to body proteins.

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