PARENTERAL PLASMA PROTEIN MAINTAINS NITROGEN EQUILIBRIUM OVER LONG PERIODS

By ROGER TERRY, M.D., WILLIAM E. SANDROCK, M.D., ROBERT E. NYE, JR., M.D., AND G. H. WHIPPLE, M.D.

(*From the Department of Pathology, The University of Rochester School of Medicine and Dentistry, Rochester, New York*)

(Received for publication, February 28, 1948)

The main thesis of this paper is the "dynamic equilibrium" between body protein and plasma protein. A steady state or balance exists between the body protein stores, protein wear and tear, and protein production. Protein production includes the plasma proteins which probably represent the largest fraction of new formed protein. Body protein reserve stores are largely intracellular. We believe the plasma proteins are the means of a fluid interchange between reserve stores and the organ cells in which protein is produced, modified, and utilized in the body economy. The term "protein pool" suggests this fluid exchange within the body.

Earlier experiments in this laboratory showed that plasma protein as plasma as the sole source of protein given by vein could maintain nitrogen equilibrium for 2 to 4 weeks (3, 11, 13). The last report (3) showed evidence of some "intoxication" which developed in two of these experiments. An attempt to reproduce this "intoxication" in the experiments tabulated below was not successful, and we are inclined to explain the "intoxications" previously described as due to contaminated plasma or vitamin deficiency or both. The experiments given below are quite satisfactory and diet deficiencies were guarded against. The plasma was handled with more complete asepsis so we conclude that "intoxication" as described (3) is not an inevitable part of the experiment when large volumes of dog plasma are given parenterally.

In several experiments in other series at various intervals the dog's circulating plasma was fractionated and various elements estimated after the parenteral injection of plasma had been started—*e.g.*, fibrinogen, globulins, albumins using coagulation, chemical methods, and electrophoretic technique. No uniform deviation from the normal plasma protein pattern was observed. Depleted dogs have a tendency to show some increase in globulins at the expense of albumins. It seems to us that the behavior of *fibrinogen* is a very good illustration of the fact that long continued plasma given over months does not modify significantly the level of any plasma protein in the circulation. Fibrinogen is a very special protein used in the body for the production of blood clots. Yet obviously it is used in the body economy presumably within cells to supply special cell needs (other than coagulation) since otherwise the fibrinogen

Downloaded from http://rupress.org/jem/article-pdf/87/6/547/1183855/547.pdf by guest on 22 June 2021

would accumulate. This is true of all blood plasma proteins, the evidence being conclusive that all these various plasma proteins do serve the purpose of body cell protein replacement.

There have been differences of opinion (4–6) relative to the type of experiment here described. In certain experiments (6) there was evidence of primary nitrogen retention and subsequent nitrogen excess excretion. We believe this reaction was due to the citrate used to collect plasma and that the use of heparin will give the response tabulated below. Some writers have criticized our early experiments as being too short—that the plasma proteins were simply tucked away as inert protein in body tissues to be released in the after period. These objections are surely met by continued experiments of 2 to 3 months in which the dog was maintained in nitrogen and weight balance and in a state of lively well being all this time.

These experiments are not easy and call for meticulous technique, dogs conditioned to unusual food mixtures (very low protein diet), freedom from infection, and attention to all diet factors. Peritoneal absorption of plasma is rapid and in some instances less disturbing to the dog than intravenous injection. It obviates some rapid blood volume fluctuations.

Methods

Plasma was obtained from a colony of 10 large healthy donor dogs maintained on kitchen scraps to which a tablespoon of powdered lextron (Eli Lilly and Co.) was occasionally added. (Lextron is a liver extract plus iron.) Blood was drawn into sterile flasks containing sufficient heparin to prevent clotting. The blood was poured into sterile rubber-capped 100 cc. centrifuge tubes and spun at high speed for 20 to 30 minutes. The plasma was suctioned off into a sterile flask, and after thorough mixing an aliquot was taken for Kjeldahl analysis. The remainder was placed in a sterile gravity injection bottle and the plasma was administered immediately in a single intravenous or intraperitoneal injection each day. The time for administration varied from 10 to 20 minutes.

The two recipient dogs were adult healthy mongrel females under observation in the experimental colony for years. (See experimental histories below for details.) During the experiment the dogs were kept in galvanized iron metabolism cages in the laboratory under constant supervision, with access to water at all times. Before the initial dose of plasma was given in each experiment, the red cells of the recipient dog were cross-matched with the serum or plasma of each donor dog to exclude isoagglutinins and isohemolysins (18).

Blood for analysis was placed in graduated centrifuge tubes, using 1 cc. of a 1.4 per cent solution of sodium oxalate as an anticoagulant. This was centrifuged at high speed for 30 minutes and the total nitrogen in the plasma determined by macro Kjeldahl analysis using 1 cc. samples of plasma in triplicate. The usual factor 6.25 was used to convert to grams of protein. Because hematuria was encountered early in the first experiment, the procedure of catheterization at the end of each period was discontinued and the periods were terminated simply by changing collection bottles containing 5 cc. of toluene as a preservative every 48 hours. The total urinary nitrogen was determined by macro Kjeldahl analysis using 2 cc. aliquots in triplicate. Urea and ammonia nitrogen were determined simultaneously by aeration following digestion with urease using 2 cc. samples in duplicate. The amount of protein nitrogen in the urine was determined by precipitation of the protein in 10 cc. of urine by 90 cc.

Downloaded from http://rupress.org/jem/article-pdf/87/6/547/1183855/547.pdf by guest on 22 June 2021

of 5 per cent trichloracetic acid. In the first half of the first experiment, the filtrate was analyzed to determine the non-protein nitrogen content of the urine, and the urinary protein nitrogen arrived at by difference. Because of the possibility of magnification of small errors by this procedure, all subsequent determinations represent total nitrogen analyses of the urinary protein precipitated and collected on filter paper.

The dogs received only 50 gm. of glucose dissolved in 200 cc. water by stomach tube daily except during the periods when they received a very low protein diet as indicated in the tables.

The *very low protein diet* contained 0.012 per cent nitrogen and was made as follows: Sucrose 2600 gm., bone ash 78 gm., salt mixture 78 gm., liver powder, (Lilly B_1) 26 gm., powdered yeast (type 200-B) 26 gm. The above ingredients were thoroughly mixed, and then the following was added in portions, mixing thoroughly with each addition: Mazola oil 235 gm., cod liver oil 50 gm., lard (melted) 490 gm. Final mixing was done until the mass was homogenenous. The salt mixture was Wesson's modification of the Osborne-Mendel mixture (17).

Whenever the dogs received this low protein diet, 25 mg. of nicotinic acid and 200 mg. of choline were added daily as a vitamin supplement. The total N content of the casein used was determined accurately by macro Kjeldahl analysis (13.1 per cent). The figures in the tables represent the calculated nitrogen intake from all possible sources including casein, vitamins, and low protein diet. All sources of nitrogen are accounted in the Summary Table.

Special vitamin mixture—each 10 cc. daily dose contained: Vitamin A 5,000 U.S.P. units, thiamin hydrochloride 6.0 mg., riboflavin 6.0 mg., pyridoxine hydrochloride 5.0 mg., calcium pantothenate 5.0 mg., nicotinamide 50.0 mg., inisotol 200.0 mg., paraminobenzoic acid 50.0 mg., ascorbic acid 50.0 mg., 2-methyl-1,4-naphthoquinone 1.0 mg., vitamin D 500 U.S.P. units, rice polish concentrate 1.0 mg., linoleic acid 500.0 mg., choline chloride or citrate 400.0 mg., distilled natural tocopherols 50.0 mg. The emulsifying agent was pectin (supplied to this laboratory by Eli Lilly and Co.). Kjeldahl analysis showed this material to contain 74 mg. N in each daily dose. Whenever this mixture was added to the glucose solution for administration by stomach tube, the extra nitrogen appears in the tables under Diet, Total N

EXPERIMENTAL OBSERVATIONS

Tables 1 and 1-concluded give all the experimental data relating to the longest experiment with intravenous injections of normal dog plasma. Following a fasting period of 2 days, *dog 43-346* was given only 50 gm. of glucose by stomach tube daily for 10 days. This allowed the nitrogen equilibrium to reach a base line level. The urinary protein nitrogen in the first two periods represents a gross hematuria caused by catheterization which promptly disappeared when this procedure was discontinued.

The base line total urinary nitrogen (periods 2 and 3 of Table 1) is about 7 gm. per 4 days. During periods 4, 5, and 6 the total urinary nitrogen is below 6 gm. per 4 day period in spite of plasma protein parenterally (8.5 to 9.8 gm. per period). This represents a positive urinary nitrogen balance of 10.5 gm., due in part perhaps to a repletion of reserve protein stores. In period 9 we note a urinary nitrogen of 12.4 gm. but about 2 gm. belong to period 8 due to urinary carry over.

The non-protein diet replacing the sugar brings the urinary nitrogen back to its base line fasting level or below and there is a strong positive N balance. *Weight gain* is observed (periods 11 to 17). The urea and ammonia fraction of

Downloaded from http://rupress.org/jem/article-pdf/87/6/547/1183855/547.pdf by guest on 22 June 2021

the urinary nitrogen (Table 1) shows no unusual change and is compatible with a frugal metabolism of body protein. Some of the irregularities of the urinary nitrogen figures are due to residual urine because catheterization was contraindicated.

The second interval (Table 1-concluded) of sugar by mouth and parenteral plasma (periods 18 to 27) does not show as complete nitrogen conservation for these 40 days as was observed in the first comparable interval (periods 4 to 10). There is a negative urinary nitrogen balance of 4.3 gm.—the weight loss is the same as in periods 4 to 10. During this second interval there is a continuous albuminuria (total 6.1 gm. urinary protein N). Undetermined nitrogen remains unchanged. These factors are probably related (urinary protein and slight negative urinary nitrogen balance), and explain in part the less efficient protein conservation; also the protein stores are probably repleted in the presence of the hyperproteinemia of 10 to 11 gm. per cent. When the parenteral plasma protein is replaced by casein by mouth the proteinuria promptly

TABLE 1

Plasma Parenterally Plus Sugar by Mouth

Dog 43-346

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			8.77	5.91	67.4	1.40	1.46		9.6	−8.77
2			7.09	5.18	73.1	1.15	0.36			−7.09
3*			3.62	2.81	77.4	0.81	0	8.17	8.6	−3.62
4		9.79	5.98	4.77	79.6	1.21	0			3.81
5		8.57	5.51	4.45	80.8	1.06	0	9.90		3.06
6		9.56	5.91	4.79	81.1	1.12	0	10.40	8.2	3.65
7		7.88	7.63	6.36	86.4	1.16	0.11			0.25
8		9.65	7.17	5.75	80.2	0.86	0.56		8.1	2.48
9		10.16	12.41	9.46	76.1	1.17	1.81			−2.25
10*		4.47	5.06	3.72	73.5	0.51	0.83	9.99	7.6	−0.59
Non-protein diet begins; sugar ends										
11	0.15	8.60	7.49	4.93	66.0	1.02	1.34			1.26
12	0.15	7.43	6.53	4.82	73.7	1.15	0.56	10.20	8.1	1.05
13	0.15	9.97	5.42	4.20	77.5	0.90	0.32	9.34	8.4	4.70
14	0.15	8.95	6.49	4.76	73.3	1.37	0.36	10.00		2.61
15	0.15	9.69	7.35	5.67	77.2	1.08	0.60		8.4	2.49
16	0.15	9.41	6.11	4.66	76.3	1.04	0.41	9.85	9.0	3.45
17*	0.08	4.21	4.11	3.29	80.0				8.9	0.25

* 2 day periods.

Downloaded from http://rupress.org/jem/article-pdf/87/6/547/1183855/547.pdf by guest on 22 June 2021

TABLE 1—*Concluded*

Plasma Injections Plus Sugar by Mouth
Proteinuria Disappears When Plasma Injections Cease

Dog 43-346

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.*
18		9.17	8.24	6.89	83.6	1.17	0.18	9.86	8.5	0.93
19		8.56	10.00	8.14	81.4	1.36	0.50	10.35		−1.44
20		9.43	7.98	7.03	88.1	0.67	0.28	10.54	8.3	1.45
21		8.50	10.05	8.18	81.4	1.18	0.69	10.67		−1.55
22		8.69	8.41	6.94	82.5	0.99	0.48	11.46	7.8	0.28
23		8.44	9.95	7.77	78.0	1.28	0.90	9.26		−1.51
24		8.27	8.06	6.71	83.2	0.88	0.47			0.21
25		8.52	8.34	6.75	81.0	0.79	0.80	11.00	7.4	0.18
26		8.34	10.65	8.19	76.8	1.50	1.16	11.24		−2.31
27		8.38	8.89	7.11	80.0	1.08	0.70			−0.51
Casein plus non-protein diet begin, plasma injections end										
28	6.00		6.66	5.16	77.5	1.20	0.30	8.22	7.9	−0.66
29	6.00		7.30	5.88	80.6	1.42	trace	7.32		−1.30
30	6.00		4.23	3.02	71.3	1.21	0	7.84	7.8	1.77

Total figures given under Experimental observations.

clears, indicating no renal injury, and the urinary nitrogen decreases. There is a suggestion here that the utilization of parenteral plasma protein has a ceiling beyond which the body cannot use as efficiently this available plasma protein. The renal threshold for plasma protein is probably a part of this limitation of internal protein metabolism.

The total *volume of plasma* injected was 19,173 cc. in 92 days, averaging 208 cc. per day. During periods 4, 5, 7, and 8, the plasma was given intraperitoneally without reaction. Slight diarrhea occurred on only one day, which did not duplicate the troublesome diarrhea reported in previous experiments in this laboratory (13).

The total amount of *nitrogen injected* in this experiment (Table 1, periods 4 to 27 inclusive) was 204.63 gm. averaging 2.22 gm. per day. The total urinary output of nitrogen during this 92 day period was 183.54 gm. averaging 2.0 gm. per day. If we assume 0.2 gm. N is lost daily in the feces, the dog would still be in positive nitrogen equilibrium for the over-all period. (See Summary Table.)

The proteinuria noted after the 14th day of plasma injections is a phenomenon to be discussed in detail in a subsequent paper. Suffice it to say

Downloaded from http://rupress.org/jem/article-pdf/87/6/547/1183855/547.pdf by guest on 22 June 2021

here that protein appears in the urine quite regularly in our dogs whenever the protein stores are repleted and the circulating plasma protein concentration is greater than 9.5 gm. per cent. Dogs may show some individual variations in the appearance and the severity of the proteinuria. This proteinuria disappeared quite promptly when the plasma injections were discontinued, indicating lack of kidney injury.

During the intervals when the entire caloric intake of the dog was represented by only plasma and glucose, she lost weight at a rate closely approximating the expected weight loss if the extra calories needed for the dog's daily nutrition came from her own body fat. During the second part of Table 1, periods 11 to 17, the dog received adequate calories in the form of a very low protein diet (145 gm. daily of the mixture described under Methods). A gain in weight resulted.

The other two experiments were run simultaneously and the pooled plasma from each day's bleeding of the donor dogs was divided between the recipient dogs.

Dog 43-346 (Table 2) received a total of 18,090 cc. of plasma in 76 days averaging 238 cc. per day. The total amount of nitrogen injected in this experiment was 177.66 gm. averaging 2.34 gm. N per day. The total urinary output of N was 153.62 gm. averaging 2.02 gm. N per day. By assuming a

TABLE 2

Plasma Intravenously; Sugar by Mouth

Dog 43-346

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	7.89	6.29	79.8	1.60	0	6.79	10.8	−7.89
2		0	9.15	7.46	81.6	1.69	0	6.47	10.4	−9.15
3		0	5.93	4.30	72.6	1.63	0		10.1	−5.93
4		0	5.01	4.41	88.1	1.10	0	6.17	9.8	−5.01
5		7.54	4.74	3.55	74.9	1.15	0	7.18	9.9	2.80
6		7.90	3.75	2.76	73.7	0.99	0	8.70	9.9	4.15
7		6.49	4.87	3.84	78.8	1.03	0	9.27	9.8	1.62
8		7.54	6.02	4.76	79.0	1.26	0	9.07	9.6	1.52
9		7.24	6.17	5.01	81.2	1.16	Trace	9.57	9.6	1.08
10		8.01	3.74*	3.14	84.0	0.60	0	9.85	9.5	4.27
11		9.23	10.56	9.00	85.2	1.44	0.17	10.28	9.2	−1.33
12		9.56	9.62	7.78	80.9	1.39	0.45	9.98	9.0	−0.06
13	0.3	9.61	8.94	7.51	84.0	1.11	0.26	10.03	9.0	0.97
14	0.3	10.07	9.90	7.17	72.4	1.25	0.48	10.55	8.9	0.47

* Vomiting.

Downloaded from http://rupress.org/jem/article-pdf/87/6/547/1183855/547.pdf by guest on 22 June 2021

TABLE 2—*Concluded*

Plasma Intraperitoneally; Sugar Plus Vitamins by Mouth

Dog 43-346

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.*
15	0.3	7.57*	9.82	6.53	66.5	0.89	2.40‡	10.22	8.7	−1.95
16	0.3	9.80	8.03	6.57	81.8	1.07	0.39	10.10	8.7	2.07
17	0.3	11.07	7.89	6.42	81.4	1.04	0.43	10.30	8.9	3.48
18	0.3	12.33	8.09	6.84	84.6	0.73	0.52	10.40	8.8	4.64
19	0.3	10.31	9.29	7.33	78.9	0.97	0.99	11.02	8.5	1.32
20	0.3	10.91	10.29	7.52	73.1	0.98	1.79	10.98	8.3	0.92
21	0.3	10.07	12.38	9.08	73.4	1.19	2.11	10.75	8.2	−2.01
22	0.3	10.98	10.86	7.75	71.4	0.85	2.26	10.90	8.0	0.42
23	0.3	11.53	9.66	6.71	69.5	1.27	1.68	10.78	7.8	2.17
Plasma and sugar cease; casein plus non-protein diet begin										
24	8.00		10.37	7.68	74.1	1.28	1.41	10.10	8.0	−2.37
25	8.00		8.52	6.87	80.6	1.15	0.50	8.67	8.0	−0.52
26	8.00		8.25	7.00	84.6	1.13	0.12	7.38	7.8	−0.25

* Does not include 2.4 gm. injected into bladder.

‡ Plasma given by mistake into bladder.

total fecal nitrogen of 15.20 gm. for the same period, the dog again remained in nitrogen equilibrium. (See Summary Table.)

Dog 44-98 (Table 3) received 18,350 cc. of plasma averaging 241 cc. per day. The total nitrogen injected was 180.29 gm. averaging 2.37 gm. N per day. The total urinary output in this same period was 167.65 gm. N. If the fecal nitrogen is estimated at 15.2 gm., the total nitrogen output is 182.85 gm. Since this dog received 48.1 gm. of N from casein and vitamins during the last 6 periods of plasma injection, she was in strong positive nitrogen balance for the total period. (See Summary Table.)

Tables 2 and 3 give the results of *intravenous* injection and Tables 2- and 3-concluded show that *intraperitoneally* administered plasma was utilized equally well. After a 2 day fast, each dog was maintained for 16 days on 50 gm. of glucose daily by stomach tube. Dog 44-98 (Table 3) received a mixture of vitamins (see Methods) each day with the glucose. Dog 43-346 (Table 2) received no vitamins at the start of the experiment. During the 10th period, after 3 weeks of plasma intravenously, this dog began to vomit daily and to show signs of toxocity although no significant negative nitrogen balance was encountered. The experience of earlier workers (3) was not repeated—intoxication and marked loss of nitrogen.

Downloaded from http://rupress.org/jem/article-pdf/87/6/547/1183855/547.pdf by guest on 22 June 2021

Because dog 44-98 was simultaneously showing significant amounts of protein in her urine and seemed to be healtuy except for loss of weight, injectable vitamin B was added to the plasma and the special vitamin mixture was added to the daily glucose diet (dog 43-346) (see Experimental history, dog 43-346). The vomiting stopped almost immediately even though it seemed to be partly a conditioned reflex. From this point until period 18, Table 3, the dogs received identical treatment and appeared in excellent condition except for the obvious loss of weight.

During the next 6 periods dog 44-98 (Table 3-concluded, periods 18 to 23) received 200 mg. choline, 25 mg. nicotinic acid, and 140 gm. of the non-protein diet daily plus sufficient casein to give 2.0 gm. N per day in addition to the plasma. The dog gained weight on this regime and the amount of protein in the urine increased. Each dog had mild diarrhea on only 1 day during the intraperitoneal injections.

During periods 24 to 26 the proteinuria in each dog decreased rapidly and on the 12th day following the last plasma injection no trace of protein was found in the urine from either dog. There was also no evidence of wastage or escape of the parenterally administered protein in the form of excess urinary nitrogen during this after period.

At no time did the experimental dogs develop a significant diarrhea. Because no solid food was supplied except at the times indicated in the tables, the volume of feces was always small. Previous investigations in this laboratory

TABLE 3

Plasma Intravenously; Sugar Plus Vitamins by Mouth

Dog 44-98

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	0	4.95	3.65	73.7	1.30	0	6.70	9.0	−4.65
2	0.3	0	4.81	3.58	74.4	1.23	0	6.20	8.5	−4.51
3	0.3	0	3.68	2.53	68.8	1.15	0		8.3	−3.38
4	0.3	0	3.61	2.64	73.2	0.97	0	5.77	8.2	−3.31
5	0.3	7.40	3.46	2.36	68.2	1.10	0	6.88	8.2	4.24
6	0.3	8.15	3.22	2.10	65.2	1.12	0	8.59	8.1	4.23
7	0.3	6.71	4.84	3.71	76.6	1.33	0		8.2	2.17
8	0.3	7.88	5.39	4.18	77.6	1.21	0	8.70	8.0	2.79
9	0.3	7.46	5.80	4.18	72.1	1.62	0	9.40	7.9	1.96
10	0.3	8.53	6.02	4.65	77.3	0.52	0.85	9.82	7.9	2.81
11	0.3	9.47	7.72	4.82	65.0	0.84	1.76		7.7	2.35
12	0.3	9.27	8.12	5.58	68.7	1.17	1.37	9.79	7.6	1.45
13	0.3	9.64	7.73	5.82	75.3	0.50	1.41	10.19	7.4	2.21
14	0.3	10.20	8.43	5.79	68.7	0.79	1.85	10.11	7.4	2.07

Downloaded from http://rupress.org/jem/article-pdf/87/6/547/1183855/547.pdf by guest on 22 June 2021

TABLE 3—*Concluded*

Plasma Intraperitoneally; Casein Plus Non-Protein Diet

Dog 44-98

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.*
15	0.3	10.82	9.50	6.10	64.2	1.06	2.34	10.08	7.2	1.62
16	0.3	10.29	9.41	5.62	59.8	1.11	2.68	10.06	7.2	1.18
17	0.3	11.70	9.60	5.79	60.3	1.10	2.71	10.33	7.2	2.40
Sugar ends; casein plus non-protein diet begin										
18	8.00	10.13	11.28	6.66	59.0	1.17	3.45	10.25	7.3	6.85
19	8.00	11.46	12.83	7.89	61.5	1.33	3.61	10.46	7.5	6.63
20	8.00	10.28	12.20	7.20	59.0	1.25	3.75	10.21	7.6	6.08
21	8.00	9.46	13.48	8.95	66.4	0.99	3.54	9.64	7.8	3.98
22	8.00	10.30	14.45	9.50	65.7	1.78	3.17	9.86	7.9	3.85
23	8.00	10.59	14.47	9.40	65.0	1.31	3.76	10.04	8.0	4.12
Plasma ends; casein and non-protein diet continue										
24	8.00		12.43	9.04	72.8	1.60	1.79	9.13	7.9	−4.43
25	8.00		9.99	8.45	84.6	1.09	0.45	7.46	7.9	—1.99
26	8.00		7.66	6.48	84.6	1.08	0.10	6.72	7.8	0.34

(13) have shown that the fecal nitrogen is relatively constant under these basal conditions and varies from 0.1 to 0.2 gm. N per day. Therefore, fecal nitrogen was not determined in these experiments and the figure of 0.2 gm. N per day was assumed as a generous estimate in calculating the nitrogen balance for each dog.

The same remarkable conservation of injected plasma protein nitrogen is shown in *Table 2* as in Table 1 (periods 5 to 7). The total urinary N is below the minimum fasting level, giving a fairly large positive N balance of 8.57 gm. During periods 8 to 18 the total urinary N rises slowly but remains much below the intake of plasma N. Proteinuria begins and the urinary N rises slowly, in part owing to this protein N (periods 12 to 14). The plasma protein levels have now reached 10.0 gm. per cent. Conditions remain unchanged through periods 15 to 19. Positive urinary nitrogen balance is maintained. Proteinuria increases sharply in periods 20 to 23 although the plasma protein levels are unchanged. The undetermined N and the urea and ammonia N are little changed. Positive urinary N balance continues. During all this time, periods 5 to 23, there has been 2 kilos weight loss, with continuing sugar by mouth and plasma parenterally. When casein by mouth plus non-protein diet replaces the plasma intake there is no change in weight and no gain in N balance.

Table 3 shows some differences on comparison with Tables 1 and 2. We note

Downloaded from http://rupress.org/jem/article-pdf/87/6/547/1183855/547.pdf by guest on 22 June 2021

the extreme N conservation of 8.47 gm. in periods 5 and 6 where the minimum fasting level is above the levels of these first two periods of plasma administration. Periods 7 to 10 show strong positive urinary N balance (9.73 gm.) but the urinary N is rising a little. Periods 11 to 14 show a severe proteinuria but a continuing positive urinary N balance. Periods 15 to 17 show even more proteinuria, but the non-protein N and the urea-ammonia N show little change. During this long interval (periods 5 to 17) there is only 1 kilo weight loss—sugar by mouth and plasma parenterally. This dog develops a strong proteinuria at an earlier date, which goes much beyond the levels recorded in Table 2 though the plasma protein levels are if anything less. Recovery from the proteinuria is equally prompt in both dogs when plasma injection is discontinued.

When casein plus non-protein diet replaces the sugar by mouth and plasma continues we note that the proteinuria increases but not the plasma protein levels. There is a strong positive N balance, the urea-ammonia fraction increases, and there is 0.8 kilo weight gain. In period 24 it could be claimed that there is some loss of stored protein but the amount is insignificant.

SUMMARY TABLE

(Refer also to Summary Table in following paper.)

Dog No.	Periods	Total days	Nitrogen intake			Nitrogen excreted in urine		Protein nitrogen in urine	Estimated fecal nitrogen	Positive nitrogen balance
			From plasma		From diet					
			Daily average	Total	Total	Daily average	Total			
			gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
43-346	4–27	92	2.22	204	1.00	2.00	184	13.36	18.4	2.6
43-346	5–23	76	2.34	178	2.96	2.02	154	13.96	15.2	18.8
44-98	5–17	52	2.27	118	3.95	1.71	89	14.97	10.4	22.6
44-98	18–23	24	2.58	62	48.1	3.29	79	21.28	4.8	26.3

Experimental History—Tables 1 and 1–concluded.

Dog 43-346, normal adult female mongrel used previously for hemoglobin studies. Dog was given regular kennel ration (kitchen scraps) for several months prior to the experiment. Traumatic hematuria encountered at the end of the first period precluded further catheterization. Urine from each period was tested for protein with heat and dilute acetic acid. As soon as proteinuria appeared (period 7), the amount of protein nitrogen was determined quantitatively. Diarrhea was present only 3 times and vomiting only once during the entire experiment. The dog gained weight whenever adequate calories were supplied and was in excellent general condition at the end of the experiment.

Experimental History—Tables 2 and 2–concluded.

Dog 43-346 (Table 1) was given a rest period of 4½ months on a diet of kitchen scraps. Repeated qualitative tests during this period revealed no trace of protein in the urine. During the 10th period (Table 2) the dog vomited copious amounts of watery fluid subsequent to the tube feeding. This probably caused some dehydration and oliguria. Because catheterization was contraindicated, there must have been some carry over of urine into period 11 to

Downloaded from http://rupress.org/jem/article-pdf/87/6/547/1183855/547.pdf by guest on 22 June 2021

account for the abnormally low total urinary nitrogen in period 10 and the abnormally high values in period 11.

Following this the dog began to salivate and vomit after every tube feeding. She apparently became conditioned to the point where vomiting would occur whenever the cage door was opened. Coprophagy was noted and during the first half of period 12 she again had oliguria. Tube feeding of sugar was stopped and crystalline vitamin B complex (solu-B, Upjohn) was added to the plasma for 6 successive days. Vomiting ceased and the animal lost her appearance of toxicity. When the 50 gm. of glucose by stomach tube was resumed, 10 cc. of the special vitamin mixture was added daily and no further vomiting or symptoms of toxicity were noted during the remainder of the experiment. On the last day of period 15, the entire dose of plasma was inadvertently injected into the dog's bladder without ill effect. This is reflected in the high figure for protein in the urine for this period.

Again, because of incomplete emptying of the bladder at the end of the last period of plasma administration (period 23), the urinary nitrogen excretion during the next period, when the dog received casein and low protein diet, was high.

Dog was frisky and healthy at the end of experiment, showing only evidence of weight loss.

Experimental History—Tables 3 and 3–concluded.

Dog 44-98, an adult mongrel female previously used for hemoglobin studies. Fed for several months prior to this experiment on kitchen scraps. She was given 10 cc. of special vitamin mixture daily with the 50 gm. glucose by stomach tube; no protein in the urine during 2 day preliminary fast. Qualitative tests revealed no proteinuria until period 10. No vomiting or diarrhea was noted at any time. The dog remained in excellent health except for loss of weight throughout the period of 52 days while receiving plasma as the sole source of nitrogen (plus 0.074 gm. from the vitamin mixture daily). From period 18 through 23 she received adequate calories and nitrogen from the casein and non-protein diet in addition to the plasma intraperitoneally. Weight gain was only moderate and fell off slightly when the plasma was stopped. Dog in excellent general condition at end of experiment.

DISCUSSION

The plasma proteins introduced into the dogs evidently contributed to the various body tissues and organs and participated in the normal maintenance and body metabolism. Unlike protein by mouth there was no loss of nitrogen (12) and no evidence of any profound break down of the introduced plasma protein, but obviously this plasma protein must have been modified in some way to replace the worn out cell and tissue proteins. All these questions are capable of experimental approach and a beginning has been made by various workers (7, 10, 14). It will be of much interest to follow the distribution of the introduced proteins, and this can be done with labeled amino acids built into the plasma protein molecules.

Using heavy nitrogen (N^{15}) to label lysine (8) it was possible to produce in the dog labeled plasma proteins which in turn were given intravenously to a a normal dog. This labeled plasma protein did not long remain in the circulation,—about 50 per cent disappeared within 24 hours,—but the disappearance did not subsequently continue at this rapid rate. It was not possible to determine the concentration of the lysine proteins in various organs because of high dilutions and limitations of heavy nitrogen analysis. Possibly radio-carbon-

Downloaded from http://rupress.org/jem/article-pdf/87/6/547/1183855/547.pdf by guest on 22 June 2021

labeled lysine may enable us to study the distribution of this amino acid in the body. This work is in progress.

Plasma volumes change readily under the above experimental conditions but the capacity of adjustment is very great (2, 9, 15, 16). The hematocrit values fall during intravenous injection of plasma but the fluid and proteins of the plasma promptly come to a balance and the protein concentrations are not measurably changed when large amounts of plasma containing 6 gm. per cent plasma protein are given to a dog with hyperproteinemia (10 per cent protein). Samples taken at 3, 35, and 240 minutes showed remarkably close agreement in protein concentration. Observations on human beings (1) indicate that following plasma injections fluid may leave the blood stream at rates of 100 cc. per minute and plasma protein may be removed at rates of 4 to 6 gm. per minute.

As one studies the three long experiments described above a pattern emerges. Following a fast of 12 to 16 days the *urinary N reaches a minimum level.* The protein stores of the body must be definitely depleted and protein wear and tear and turnover are minimal. Now whole plasma given parenterally with sugar by mouth presents an extraordinary conservation of N and we note the urinary N *falls below the fasting level.* The urea-ammonia fraction shows no significant change. This remarkable N conservation continues for 8 to 12 days and presumably there is considerable repletion of body protein stores or reserves. The protein wear and tear and wastage is minimal. After this interval the urinary N rises a little, but nitrogen equilibrium is maintained. Proteinuria begins in 12 to 24 days and about this time the circulating plasma protein levels reach 9.5 to 10.5 gm. per cent. The degree of the proteinuria varies widely from 0.2 to 1.8 gm. in some dogs to 0.8 to 3.0 gm. of protein nitrogen per 4 day period. The animal is still in nitrogen equilibrium or positive balance, although the dog loses the urinary protein which does not enter into the internal body metabolism. There is no significant loss of urinary nitrogen when the plasma protein injections cease.

It seems probable that after the body protein reserves are repleted the dog can use only about so much plasma protein. Beyond this point hyperproteinemia develops (10 gm. per cent) and a good deal of the introduced protein will escape. The urinary N rises somewhat, but a positive N urinary balance is usually maintained. Given necessary carbohydrate, fat, salts, and vitamins by mouth with the parenteral plasma protein we note that weight equilibrium and normal health can be maintained for 3 months and probably very much longer. It is suggested that there is a ceiling for the use of introduced plasma protein when the depleted protein reserves are restored. This ceiling is high enough to permit maintenance of body nitrogen balance.

SUMMARY

Given adequate amounts of homologous plasma intravenously or intraperitoneally a protein-fasting dog can be maintained in nitrogen equilibrium for

Downloaded from http://rupress.org/jem/article-pdf/87/6/547/1183855/547.pdf by guest on 22 June 2021

several months, indicating efficient utilization of all plasma proteins in body metabolism. There is no accumulation of any specific plasma protein in the circulation, indicating that even highly specialized globulins (fibrinogen and others) are capable of participation in the general protein turnover and metabolism within the body. This is a fluid exchange or a *dynamic equilibrium* in protein metabolism. This exchange takes place without significant loss of nitrogen.

Body weight is maintained when adequate calories are supplied (very low protein diet) during the plasma injection periods.

No periods of unexplained intoxication were noted in the long experiments described. Health and activity were quite normal.

Continued hyperproteinemia with repletion of body protein stores and plasma protein levels of 9.5 to 10.0 per cent after 15 to 25 days produced proteinuria. A renal threshold for blood plasma proteins is suggested.

These experiments suggest that after protein reserves are repleted there is a limit or ceiling for the use of the introduced plasma protein, but this limit permits a nitrogen balance to be maintained. This limitation to the use of introduced plasma protein is closely related to the proteinuria.

BIBLIOGRAPHY

1. Beattie, J., *Lancet*, 1942, **2**, 445.
2. Beattie, J., *Brit. Med. J.*, 1942, **1**, 459.
3. Daft, F. S., Robscheit-Robbins, F. S., and Whipple, G. H., *J. Biol. Chem.*, 1938, **123**, 87.
4. Elman, R., *Physiol. Rev.*, 1944, **24**, 372.
5. Elman, R., in Advances in Protein Chemistry, (M. L. Anson and J. T. Edsall, editors), New York, Academic Press, Inc., 1947, **3**, 269.
6. Elman, R., and Davey, H. W., *J. Exp. Med.*, 1943, **77**, 1.
7. Fine, I., and Seligman, A. M., *J. Clin. Inv.*, 1943, **22**, 285.
8. Fink, R. M., Enns, T., Kimball, C. P., Silberstein, H. E., Bale, W. F., Madden, S. C., and Whipple, G. H., *J. Exp. Med.*, 1944, **80**, 455.
9. Hayward, G. W., and Jordan, A., *Brit. Med. J.*, 1942, **1**, 462.
10. Heidelberger, M., Treffers, H. P., Schoenheimer, R., Ratner, S., and Rittenberg, D., *J. Biol. Chem.*, 1942, **144**, 555.
11. Holman, R. L., Mahoney, E. B., and Whipple, G. H., *J. Exp. Med.*, 1934, **59**, 269.
12. Howland, J. W., and Hawkins, W. B., *J. Biol. Chem.*, 1938, **123**, 99.
13. Pommerenke, W. T., Slavin, H. B., Kariher, D. H., and Whipple, G. H., *J. Exp. Med.*, 1935, **61**, 283.
14. Schoenheimer, R., Ratner, S., Rittenberg, D., and Heidelberger, M., *J.Biol. Chem.*, 1942, **144**, 545.
15. Sharpey-Schafer, E. P., and Wallace, J., *Lancet*, 1942, **1**, 699.
16. Shearburn, E. W., *Surg., Gynec. and Obst.*, 1942, **74**, 343.
17. Wesson, L. G., *Science*, 1932, **75**, 339.
18. Wright, A., *Proc. Soc. Exp. Biol. and Med.*, 1936, **34**, 440.

Downloaded from http://rupress.org/jem/article-pdf/87/6/547/1183855/547.pdf by guest on 22 June 2021