

BLOOD PLASMA PROTEIN GIVEN BY VEIN UTILIZED IN BODY METABOLISM

II. A DYNAMIC EQUILIBRIUM BETWEEN PLASMA AND TISSUE PROTEINS

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The statement that blood plasma proteins can be utilized freely in the body economy has an heretical flavor yet it seems difficult if not impossible to explain the experimental data given below on any other basis. Dogs receiving only sugar by mouth can be maintained practically in nitrogen equilibrium by suitable amounts of blood plasma given intravenously. We realize that these statements will be challenged but hope that the experimental data will be adequate to convince even the skeptic.

Body metabolism can undergo extraordinary adjustments under the stress of emergency and we see the body produce large amounts of new hemoglobin and red cells during anemia periods with the diet intake limited to sugar and inorganic salts of iron. More than 100 gm. new hemoglobin can be produced by the dog in a 2 week period and we must assume that the new hemoglobin comes from tissue protein breakdown and conservation. The details of this experiment have been reported (1) very recently. Furthermore, in anemia, during fasting periods alone, the dog is able to conserve products coming from tissue breakdown and produce a considerable amount of new hemoglobin (30-40 gm. per 2 week period). In this emergency the tissue or blood plasma protein must contribute to the formation of the much needed hemoglobin.

When the plasma proteins are depleted (plasmapheresis) in short experiments (4), a rapid appearance of new plasma protein (reserve) and a slow return of plasma protein concentration to normal will be

observed during fasting periods. Evidently this new plasma protein is produced in the body with zero protein intake and must come from reserve stores of plasma protein producing material or from breakdown and conservation of split products coming from tissue proteins. Both mechanisms may well be concerned.

Another important example of this "give and take" between various body proteins within the body was reported by Davis and Whipple (2). Dogs poisoned with chloroform may show a liver necrosis involving two-thirds of all the liver parenchyma yet they can regenerate the liver back to normal on an intake of sugar alone. This will all take place within 10–12 days and represents a very large production of highly specialized liver cell protein coming from other body protein and tissue cells or perhaps in part from plasma protein.

One need not be surprised therefore in reviewing the tabulated experiments below which indicate that body metabolism can utilize a surplus of plasma protein made available by intravenous injection with zero food protein by mouth. If this mechanism comes into play so promptly in an emergency we must admit as a possibility that it may take place to a limited degree during normal metabolism.

Fifty years ago it was believed (Voit and others) that the plasma proteins had much to do with the nutrition of tissue cells but with the discovery that the amino acids were absorbed from the intestine and carried throughout the body attention was focused on these "building stones." It was assumed purely on negative evidence that the plasma proteins were inert substances, having nothing to do with tissue nutrition. This may be the proper time to revise somewhat our conception of the plasma proteins, their usefulness and fate in the body.

One may inquire whether this introduced protein is broken down to amino acids before it is utilized in the body. This question cannot be answered as yet but some evidence is at hand to indicate that the breakdown does not carry to the end stage of the amino acids as the liver would probably deaminize some of this material and thereby increase the urea nitrogen in the urine. The urinary N does increase when the plasma protein is fed by mouth (Table 22). For the present we may imagine that the intravenously introduced plasma protein is broken down to "intermediates" before incorporation into the body tissues. The same thing is observed (6) when hemoglobin is injected

into the anemic dog. The animal conserves either dog, sheep, or goose hemoglobin up to 90–100 per cent and builds it up into new dog hemoglobin and red cells.

Methods

The two dogs used were maturing litter-mate male hounds with a normal adult weight of about 16 kg. Each experiment is divided into four periods: a fore-period of 2–5 days sugar feeding; then two 7 day periods of intravenous plasma injection; and finally a 5 day after-period of sugar feeding. The weight loss, urinary nitrogen, urinary protein, plasma protein, plasma albumin, plasma globulin, plasma non-protein nitrogen, and plasma and blood volumes were determined during each period.

Handling of Animals.—Each of the dogs was first vaccinated against distemper. Two or more intravenous injections of donor's plasma were given to accustom the animal to the procedure. The dog was weighed daily in the morning before anything was done. 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. 2 hours before this and each subsequent catheterization 50 gm. of dextrose in 300 cc. of water was administered by stomach tube to favor a diuresis which would wash out residual nitrogenous waste products. Each succeeding day of the experiment the dog received this dose of sugar and water by stomach tube, usually between 2 and 3 o'clock in the afternoon. On alternate days 15 gm. of kaolin were added to the dextrose solution in an attempt to prevent diarrhea. When the experiment was over the dogs were placed on kennel diet and were not used again until they had regained their former weight, usually periods of 6–8 weeks being required.

Collection and Analysis of Urine Samples.—The urine was collected daily around 4 p.m., this being the time of the stated catheterizations. The metabolism cage was kept in the laboratory under close observation throughout the day and urine voided was put aside in a separate bottle containing 5 cc. of toluol. A wire screen was placed beneath the floor of the metabolism cage to catch any hair or thick excreta. In this way contamination occurred only when the dog defecated or vomited and urinated before the cage contamination was detected and cleaned out. The few times that this did occur were at night. Care was taken to wash down the sides of the cage with distilled water. The daily urinary output was diluted to a known volume (usually 500 cc.) and analysed for nitrogen and protein. Nitrogen determinations were run in duplicate by the macro-Kjeldahl method. Results that did not check within 1 part in 60 were repeated; this reduces the probable error to less than 1 per cent. A modification of the gravimetric method of Folin and Denis (3) was used to determine urinary protein excretion. One-tenth of the diluted urine was boiled for 15 minutes with 5 cc. of 5 per cent acetic acid in a 100 cc. centrifuge tube, centrifugalized at 2,500 R.P.M. for 5 minutes or longer, the supernatant fluid poured off, the precipitate stirred up with 50 cc. of 0.5 per cent

acetic acid, again centrifuged and the supernatant fluid discarded; the precipitate stirred up again in 50 cc. of 50 per cent alcohol and centrifuged for the third time. The supernatant fluid was again discarded, and the centrifuge tube stoppered and set aside for the next day's analysis which was added directly to the precipitate in the tube. At the end of each period (5-7 days) the precipitate was carefully transferred to a Kjeldahl flask and its nitrogen determined by the macro-method. The amount of the precipitate was so small by the usual gravimetric method that it is the belief of the authors that this modification not only facilitates the determination but also increases its accuracy. The prescribed method of Folin and Denis was used in the first experiment, and it was only with the greatest care that one could get anything like satisfactory checks, for the usual daily precipitate was weighed in tenths of a milligram. At no time during this first experiment was the precipitate heavy enough to give a reading by the Esbach method.

Supplemental Injections and Feedings.—During the periods of plasma injection a donor was bled 500 cc. into a flask containing 5 cc. of saturated sodium citrate, done usually in the morning. The blood was centrifugalized for 35 minutes at 3,000 R.P.M. in 100 cc. centrifuge tubes, the plasma drawn off with suction, measured, warmed to 40-45°C., and given by stomach tube or injected into the jugular vein as indicated in each experiment, approximately 15 minutes being required for the plasma to run into the vein. Sometimes during the injection of the last 100 cc. the dog exhibited jerky movements of the extremities but these could be controlled by stopping the flow of plasma for a minute or so. A noticeable acceleration and decrease in the volume of the pulse usually preceded these jerky movements. These jerky movements, and sometimes retching, were most frequently encountered during the first two or three injections; thereafter the dog seemed to tolerate the procedure better. Occasionally, in spite of all precautions, the dog vomited during the latter part of the injection or soon after it was over. When the dogs were returned to their cages in a few minutes they appeared perfectly normal in all respects.

All donors were large, healthy dogs, weighing 25 kg. or more, and a sufficient number were used so that no donor was bled over three times during any 2 week period. The blood hematocrit of the donor was determined at each bleeding and, if found to be below normal, supplemental liver feeding or a rest period was instituted. Emphasis is placed on these points for, in calculating the daily nitrogen intake, we have assumed that all donors' plasma contains not less than 6 gm. of protein per 100 cc. At no time did the donors show the slightest ill effect of these repeated fairly large bleedings and they always ate all of their liberal allowance of kennel diet. It is for these reasons that we feel justified in assuming that the donors' plasma contained not less than 6 per cent protein, a figure about 0.5 per cent lower than the average of several actual determinations.

EXPERIMENTAL OBSERVATIONS

During the plasma depletion experiments in the preceding paper, a large surplus of normal dog plasma was available. Recent experi-

ments (5) in the laboratory had interested us in renal thresholds for hemoglobin which are very distinct and measurable values. It was thought that possibly a plasma protein renal threshold might be demonstrated if the concentration of plasma protein was pushed up to very high levels. To our surprise we found that although large amounts of plasma protein were given intravenously several days each week there was practically no escape by way of the urine which showed only traces of protein. The plasma protein level could not be pushed up more than 50 per cent—the plasma protein was being removed from the circulation and not by way of the urine. Obviously this called for a study of the nitrogen intake and output to understand what was happening.

Preliminary Experiment, Dog 32-130.—Between November 14, 1932 and January 20, 1933, this dog received 3,875 cc. of normal dog plasma containing approximately 227 gm. protein. Injections of 150–250 cc. citrated plasma were given intravenously several times a week. Kennel diet of hospital table scraps was eaten throughout the entire period. The dog was immature and as it grew gained weight from 10 kg. to 14.5 kg. A litter mate as control reached the same weight on January 20. The dog was normal throughout. Total plasma protein at start was 6.32 per cent. December 1, plasma protein = 7.37 per cent. December 31, plasma protein = 7.65 per cent. January 6, plasma protein = 7.04 per cent. The urine was followed closely for protein and at times showed a strong trace, again a faint trace, and often was negative for protein. Obviously there was no significant urinary escape of these plasma proteins nor were the values for blood plasma protein concentration much above normal.

Table 21 gives in summary three experiments on the same dog (32-131) with suitable rest periods of 6–8 weeks between to permit of complete return to normal weight. The table shows the experiments in the order in which they were done but perhaps we may best consider the last or control experiment first. The dog received only 50 gm. dextrose and water by stomach tube daily. There is a steady loss in weight and a uniform negative nitrogen balance of 1.8–2.1 gm. daily. There is the usual concentration of plasma volume from 850 cc. to 450 cc. observed in fasting dogs. There is little change in values for total proteins in the plasma but the albumin-globulin ratio does change. At the start the dog shows a somewhat unusual A/G ratio 1.0 which subsequently falls to A/G 0.76 with a rise in globulin values. These figures are given in the *clinical history* of Dog 32-131 below.

The figures for total circulating plasma protein obviously show a considerable loss (48 per cent) from 49 gm. to 25 gm.

When we contrast this control experiment (Dog 32-131) with the first experiment, Table 21, there are many significant differences. During the 2 week period of plasma injection the dog receives 179 gm.

TABLE 21

Plasma Protein Given by Vein Utilized in Body.—Sugar Control
Dog 32-131.

Experimental periods	Days	Injected plasma N, daily average	Urinary N, daily average	Negative N balance, daily average	Urinary N as protein N, daily average	Total blood plasma protein	Circulating plasma protein period end	Weight at period end
First Plasma protein by vein—sugar by stomach tube								
		gm.	gm.	gm.	gm.	per cent	gm.	kg.
Fore-period.	5	0	1.649	1.649	0.0	5.25	33.0	15.44
Plasma protein 81 gm.	7	1.86	2.165	0.305	0.011	6.94	47.3	14.78
“ “ 98 “	7	2.33	1.476	+0.854	0.038	7.01	44.7	13.92
After-period.	5	0	1.770	1.770	0.011	6.06	42.2	13.40
Second Plasma protein by vein—sugar and fat feeding								
Fore-period.	5	0	2.499	2.499	0.003	5.94	42.2	17.38
Plasma protein 112 gm.	7	2.565	2.446	+0.119	0.052	7.62	64.2	17.28
“ “ 103 “	7	2.366	2.436	0.070	0.021	8.46	59.3	16.31
After-period.	5	0	2.606	2.606	0.003	7.33	51.9	15.75
Third Sugar alone by stomach tube								
Control.	5	0	1.826	1.826	0	6.77	48.9	17.90
“	7	0	2.105	2.105	0	6.17	40.7	16.67
“	7	0	1.897	1.897	0	5.98	37.8	15.57
“	5	0	—	—	0	5.62	25.3	15.17

plasma protein and not more than 1 per cent of this escapes in the urine. About 10 gm. remains in the circulation or 5 per cent. If we compare this with the *control fall* in plasma protein of about 20 gm. we may say that another 10 per cent should be added making approximately 15 per cent protein which is accounted for. Therefore about 85 per cent of the injected protein is used up in the body metabolism and we note a *positive urinary nitrogen balance* between protein N intake and urinary N output. If we include the fecal N loss (about 0.8 gm.

daily) and the loss due to bleeding (blood volume and plasma protein determination) the total nitrogen balance is slightly negative and during this 2 week period there is a loss of 1.5 kg. body weight—compare with loss of 2.3 kg. in sugar control period. There is no significant change in the albumin-globulin ratio but a moderate rise in plasma volume and circulating plasma protein.

The second experiment, Table 21, shows a similar reaction with 215 gm. plasma protein injected and a slight positive nitrogen balance between protein N injected and urinary nitrogen. The dog consumed 885 calories daily of fat and sugar during the 1st week and 590 calories daily in the 2nd week. During this 2-week period there was loss of weight of 1.0 kg., most of this in the 2nd week—compare with the control on sugar alone of 2.3 kg. body weight loss. The plasma volume remains unchanged as does the albumin-globulin ratio. There is a gain of about 10 gm. circulating plasma protein.

Clinical Summary

Dog 32-131—See Table 21, first experiment.

March 3. Weight 17.2 kg., young mongrel hound. Plasma protein = 5.6%; albumin = 3.61%; globulin = 1.99%; N.P.N. = 20 mg.; plasma volume = 762 cc.; blood volume = 1,552 cc.; red cell hematocrit = 50%.

March 5-7. All food withheld. Weight fell to 16.3 kg.

March 8. Daily 50 gm. glucose + 300 cc. water by stomach tube. Catheterization to start metabolism experiment.

March 12. Plasma protein = 5.25%; albumin = 3.54%; globulin = 1.71%. Catheterization. Plasma injection begun.

March 14. Plasma volume = 629 cc.; blood volume = 1,234 cc.; red cell hematocrit = 49%.

March 19. Plasma protein = 6.94%; albumin = 3.98%; globulin = 2.96%; N.P.N. = 18 mg. Catheterization.

March 20. Plasma volume = 685 cc.; blood volume = 1,161 cc.; red cell hematocrit = 41%.

March 26. Plasma protein = 7.01%; albumin = 4.28%; globulin = 2.73%; N.P.N. = 24 mg. Catheterization.

March 27. Plasma volume = 637 cc.; blood volume = 1,043 cc.; red cell hematocrit = 38%.

March 31. Plasma protein = 6.06%; albumin = 3.89%; globulin = 2.17%; N.P.N. = 16 mg.; plasma volume = 696 cc.; blood volume = 1,209 cc.; red cell hematocrit = 41%. Final catheterization. No fecal contamination of urine during entire period. Dog put on kennel diet.

Second experiment—Table 21.

May 26. Weight 19.1 kg. Plasma protein = 6.51%; albumin = 4.10%; globulin = 2.41%; N.P.N. = 23 mg.; plasma volume = 730 cc.; blood volume = 1,551 cc.; hematocrit = 52%.

May 25–27. All food withheld; weight fell to 18.8 kg.

May 28. Daily 50 gm. glucose with 300 cc. water by stomach tube. 15 gm. kaolin on alternate days. Catheterization to start experiment. Plasma protein = 6.33%; albumin = 4.03%; globulin = 2.30%; N.P.N. = 25 mg.; plasma volume = 736 cc.; blood volume = 1,446 cc.; red cell hematocrit = 48%.

June 2. Plasma protein = 5.94%; albumin = 3.18%; globulin = 2.76%; N.P.N. = 17 mg.; plasma volume = 710 cc.; blood volume = 1,420 cc.; red cell hematocrit = 50%.

June 2–7. 30 gm. mayonnaise + 20 cc. cod liver oil in addition to glucose.

June 9. Plasma protein = 7.62%; albumin = 4.68%; globulin = 2.94%; N.P.N. = 15 mg.; plasma volume = 842 cc.; blood volume = 1,414 cc.; hematocrit = 40%. Catheterized.

June 10. 25 gm. lard.

June 11–16. 50 cc. cotton seed oil by stomach tube; retained every day except June 15.

June 12. Gross contamination of urine with feces.

June 16. Plasma protein = 8.46%; albumin = 4.88%; globulin = 3.58%; N.P.N. = 18 mg.; plasma volume = 701 cc.; blood volume = 1,287 cc.; red cell hematocrit = 44%. Catheterized.

June 21. Plasma protein = 7.33%; N.P.N. = 19 mg.; plasma volume = 708 cc.; blood volume = 1,235 cc.; red cell hematocrit = 40%. Final catheterization. Placed on kennel diet.

Third experiment—Table 21.

August 2. Weight 19.5 kg. Plasma protein = 6.40%; albumin = 3.04%; globulin = 3.36%; N.P.N. = 16 mg.; plasma volume = 869 cc.; blood volume = 1,675 cc.; hematocrit = 48%. Total fasting.

August 3. Total fasting.

August 4. Plasma protein = 6.20%; albumin = 3.15%; globulin = 3.05%; N.P.N. = 9 mg.; blood volume = 1,858 cc.; plasma volume = 1,010 cc.; hematocrit = 46%. Catheterized at 11:00 a.m. to start experiment.

August 5–25. 50 gm. of glucose by stomach tube with 300 cc. of water. 15 gm. of kaolin added on alternate days.

August 9. Plasma protein = 6.77%; albumin = 2.90%; globulin = 3.87%; N.P.N. = 8 mg.; blood volume = 1,413 cc.; plasma volume = 722 cc.; hematocrit = 49%. Catheterized at 11:00 a.m.

August 16. Plasma protein = 6.17%; albumin = 2.60%; globulin = 3.57%; N.P.N. = 13 mg.; blood volume = 1,220 cc.; plasma volume = 660 cc.; red cell hematocrit = 46%. Catheterized at 11:00 a.m.

August 23. Plasma protein = 5.98%; albumin = 2.44%; globulin = 3.54%;

N.P.N. = 8 mg.; blood volume = 1,078 cc.; plasma volume = 632 cc.; hematocrit = 41%. Catheterized at 11:00 a.m.
 August 25. Weight 15.2 kg. Plasma protein = 5.62%; albumin = 2.52%; globulin = 3.10%; N.P.N. = 9 mg.; blood volume = 792 cc.; plasma volume = 452 cc.; hematocrit = 43%.

TABLE 22
Plasma Protein Given by Vein Utilized in Body.—Control Plasma by Mouth
 Dog 32-130.

Experimental periods	Days	Injected plasma N, daily average	Urinary N, daily average	Negative N balance, daily average	Urinary N as protein N, daily average	Total blood plasma protein	Circulating plasma protein period end	Weight at period end
First Plasma protein by vein—sugar by stomach tube								
		gm.	gm.	gm.	gm.	per cent	gm.	kg.
Fore-period.	2	0	1.718	1.718	0.0	7.33	45.2	13.86
Plasma protein 88 gm.	7	2.000	1.988	+0.012	0.022	7.33	46.0	13.18
“ “ 104 “	7	2.389	1.564	+0.825	0.005	8.60	52.2	12.49
After-period.	5	0	1.895	1.895	0.003	6.28	36.8	11.76
Second Plasma protein by vein—sugar and fat by mouth								
Fore-period.	5	0	2.684	2.684	0.001	5.77	33.5	13.91
Plasma protein 109 gm.	7	2.485	1.869	+0.616	0.001	7.52	47.3	13.95
“ “ 120 “	7	2.750	2.614	+0.136	0.010	9.74	68.8	13.16
After-period.	5	0	2.492	2.492	0.004	7.79	59.7	12.54
Third Plasma protein and sugar by mouth								
Fore-period.	5	0	2.212	2.212	0	5.55	35.3	14.32
Plasma protein 139 gm.	7	3.17*	3.130	+0.040	0	4.76	28.8	13.47
“ “ 125 “	7	2.85*	2.970	0.120	0	5.63	25.1	12.90
After-period.	5	0	1.970	1.970	0	5.52	31.6	12.33

* Plasma protein given by mouth.

Table 22 shows three experiments on the same dog (32-130) with 6-8 week rest intervals for complete weight recovery. This dog is slightly smaller and was given more plasma protein by vein so the results are even a bit more striking. During the first experiment the dog received 192 gm. plasma protein by vein and 50 gm. glucose with water by stomach tube. The loss of protein in the urine was less than 1 per cent. There was a positive nitrogen balance between the nitro-

gen given by vein and that eliminated in the urine. The fecal nitrogen was analysed and found to be 0.8 gm. per day and together with the routine bleeding for analyses gives a total negative nitrogen balance. The loss of body weight in these 2 weeks is 1.3 kg. and is to be compared with the third experiment on the same dog with *plasma protein by mouth* and a body weight loss of 1.4 kg.

The second experiment (Table 22) is even better as more protein is given by vein (229 gm.) and more calories are taken by mouth with a very small total weight loss of 0.7 kg. During the 1st week a daily caloric intake of 1,020 was attained (fat and sugar) but the dog refused some of this mixture in the 2nd week and consumed only 680 calories daily. There was no change in the albumin-globulin ratio but a small increase in plasma volume and total circulating plasma protein.

The third experiment (Table 22) is of considerable importance and gives a different type of control. The dog was fed plasma protein by stomach tube in the amounts tabulated in addition to 50 gm. glucose daily. The plasma protein by mouth exceeds somewhat that given by vein and the urinary nitrogen is definitely higher due to this protein by mouth. This suggests deamination by the liver. The loss of body weight (1.4 kg.) exceeds considerably the loss in the second plasma injection experiment on the same dog (0.7 kg.). Evidently the protein by vein is a little more completely utilized to form new protein in the body than the same protein given by mouth. The fecal nitrogen was analysed and found to be 0.8 gm. per day, the same amount recorded when the plasma was given by vein. The plasma volume fell from 700 cc. to 570 cc. or less and there was a change in the albumin-globulin ratio due to the limited food protein intake. The albumin-globulin ratio fell from 1.5 to 0.9 indicating probably a more rapid production of globulin and a loss of total proteins with the fall in the plasma volume.

Clinical Summary

Dog 32-130—See Table 22, first experiment.

Weight 15.3 kg., young mongrel hound.

January 21-23. All food withheld, weight fell to 14.1 kg.

January 24. Plasma protein = 6.63%; plasma volume = 700 cc.; blood volume = 1,385 cc.; hematocrit = 49%. Catheterized to begin experiment. 50 gm.

of glucose with 300 cc. of water given by stomach tube every day until end of experiment. 15 gm. kaolin on alternate days.

January 26–February 9. Plasma protein injections.

February 2. Plasma protein = 7.33%; albumin = 4.70%; globulin = 2.63%; N.P.N. = 22 mg.; plasma volume = 628 cc.; blood volume = 1,105 cc.; red cell hematocrit = 42%. Catheterized.

February 9. Plasma protein = 8.60%; albumin = 5.48%; globulin = 3.12%; N.P.N. = 27 mg.; plasma volume = 607 cc.; blood volume = 964 cc.; red cell hematocrit = 37%. Catheterized.

February 14. Plasma protein = 6.28%; albumin = 4.16%; globulin = 2.02%; N.P.N. = 22 mg.; plasma volume = 586 cc.; blood volume = 944 cc.; red cell hematocrit = 38%. Dog placed on kennel diet.

Second experiment—Table 22.

May 1. Initial weight 15.8 kg.

May 1–3. All food withheld. Weight fell to 15.0 kg.

May 3. Plasma protein = 5.63%; albumin = 3.87%; globulin = 1.76%; N.P.N. = 29 mg.; red cell hematocrit = 48%. Catheterization to start metabolism experiment. 50 gm. glucose in 300 cc. water given daily by stomach tube. 15 gm. kaolin added on alternate days.

May 9. Plasma protein = 5.77%; albumin = 3.61%; globulin = 2.16%; N.P.N. = 18 mg.; plasma volume = 581 cc.; blood volume = 1,039 cc.; red cell hematocrit = 44%.

May 9–13. 50 gm. lard daily.

May 14. Received no fat.

May 15. Received 100 gm. mayonnaise; 40 cc. cod liver oil; 100 gm. karo corn syrup.

May 16. Plasma protein = 7.52%; albumin = 4.51%; globulin = 3.01%; N.P.N. = 14 mg.; plasma volume = 629 cc.; blood volume = 1,220 cc.; red cell hematocrit = 45%. Received 20 gm. mayonnaise, 10 cc. cod liver oil, 20 gm. karo.

May 17. 75 cc. of cotton seed oil.

May 18–20. Received 35 cc. cotton seed oil daily with urinary contamination by vomitus on last day.

May 22. 40 cc. of cotton seed oil.

May 23. Plasma protein = 9.74%; albumin = 5.92%; globulin = 3.82%; N.P.N. = 26 mg.; plasma volume = 706 cc.; blood volume = 1,146 cc.; red cell hematocrit = 36%.

May 28. Plasma protein = 7.79%; albumin = 4.93%; globulin = 2.86%; N.P.N. = 21 mg.; plasma volume = 766 cc.; blood volume = 1,155 cc.; red cell hematocrit = 34%. Dog placed on kennel diet.

Third experiment—Table 22.

June 20. Weighs 16.6 kg. Plasma protein = 5.90%; albumin = 3.51%; globulin = 2.39%; N.P.N. = 20 mg.; blood volume = 1,482 cc.; plasma volume = 797 cc.

- June 20-22. All food withheld. Weight fell to 15.5 kg.
- June 23. Plasma protein = 5.51%; albumin = 3.36%; globulin = 2.15%; N.P.N. = 34 mg.; blood volume = 1,213 cc.; plasma volume = 684 cc.; hematocrit = 43%. 50 gm. dextrose, 3 gm. kaolin. Catheterized to begin metabolism experiment. 50 gm. glucose in 300 cc. water given daily by stomach tube with 15 gm. kaolin on alternate days.
- June 28. Plasma protein = 5.55%; albumin = 3.17%; globulin = 2.38%; N.P.N. = 26 mg.; plasma volume = 636 cc.; blood volume = 1,064 cc.; hematocrit = 39%. Catheterized.
- June 29-July 13. About 250 cc. normal blood plasma by stomach tube given daily.
- July 5. Plasma protein = 4.76%; albumin = 2.58%; globulin = 2.22%; N.P.N. = 12 mg. Catheterized at 4:00 p.m. Blood volume = 1,060 cc.; plasma volume = 585 cc.; hematocrit = 45%.
- July 12. Plasma protein = 5.63%; albumin = 3.37%; globulin = 2.37%; N.P.N. = 13 mg.; blood volume = 814 cc.; plasma volume = 445 cc.; hematocrit = 45%. Catheterized.
- July 17. Plasma protein = 5.52%; albumin = 2.66%; globulin = 2.91%; N.P.N. = 14 mg.; blood volume = 821 cc.; plasma volume = 574 cc.; hematocrit = 45%. Catheterized.

DISCUSSION

There is great temptation to use these facts to speculate about the problems of edema and hypoproteinemia in human disease. Perhaps discretion has some merit and we may leave these observations to the clinical investigators to use as they see fit in the study of human material. Observations in liver disease should prove to be of unusual value.

It may be proper to inquire whether there is evidence that the plasma proteins are *rapidly* depleted by fasting and restored by heavy protein feeding. The first paper (Chart A) shows how promptly the low plasma protein level will be restored by liver feeding. Fasting usually does not modify the total protein concentration in the plasma and the albumin-globulin ratio may not be changed. But fasting does cause conspicuous shrinkage of the blood plasma volume and therefore the total amount of circulating protein may decrease to 70, to 60, or even to 50 per cent of normal and represents a considerable loss of plasma protein—relatively more than the loss of general tissue protein (weight loss). To bring evidence for a considerable degree of rapid fluctuation in plasma proteins directly referable to diet intake

or fasting is a possibility. It seems certain that over periods of several days the plasma protein in circulation can be decreased by fasting or increased by heavy protein feeding.

It will be noted that in all the plasma injection experiments (Tables 21 and 22 and clinical histories, Dogs 32-130 and 131) there is a fall in hematocrit of about 10 per cent and this represents a loss of about 100 gm. hemoglobin when we calculate for changes in blood volume. This is very close to the actual amount of red cells and hemoglobin removed during the course of the experiment for routine analysis of plasma protein, blood volume, and red cell hematocrit. Usually a dog would regenerate new hemoglobin and red cells promptly to make up this loss but this reaction does not follow. As a possible explanation we refer to evidence that blood transfusions may inhibit red cell regeneration. If simple plasma transfusion has this same effect it may have some bearing on a correct explanation of this phenomenon. At any rate it seems fair to say that none of the injected plasma protein is utilized to form new hemoglobin.

The albumin-globulin ratio is unchanged after a long series of normal plasma injections. This would indicate that the body uses both these proteins in about the same amounts as represented in the normal plasma—not using more albumin than globulin. When we observe that in forming new plasma protein especially on a low protein intake we may see a preponderance of globulin, this suggests that globulin is more easily formed than albumin rather than that albumin is used up more expeditiously.

This “dynamic equilibrium” may mean a tidal ebb and flow between tissue protein and plasma protein. One may embrace in this equilibrium the food proteins but there seems to be little question that food proteins contribute to tissue proteins and plasma proteins depending upon the immediate needs.

Without food protein both the plasma and tissue proteins are progressively depleted and it is of interest to note that the plasma proteins may be relatively more depleted than are the tissue proteins (refer to Table 21, fasting alone, Dog 32-131). With adequate or high protein intake both plasma and tissue protein are restored and again this change is most conspicuous in the plasma proteins (Chart A, Paper I). There is much evidence that plasma proteins are more labile substances than are tissue proteins.

SUMMARY

Large amounts of normal blood plasma can be given intravenously to normal dogs over several weeks without causing any significant escape by way of the urine. There appears to be no renal threshold for plasma protein even with high plasma protein concentration (9.7 per cent).

Dogs receiving sugar by mouth and plasma by vein can be kept practically in nitrogen equilibrium and it would seem that the injected protein must be utilized by the body. If this can happen in this emergency we may suspect that normally there is a certain amount of "give and take" between body protein and plasma protein.

Plasma protein *fed* by mouth under identical conditions shows the same general reaction as noted with plasma by vein but the urinary nitrogen is a little higher and suggests that the *injected* protein is utilized a little more completely to form new protein. The difference may be explained as due to deamination in the case of protein by mouth.

During fasting periods the blood plasma proteins are used up and the total circulating protein may even decrease to one-half the normal level. The plasma protein concentration changes but little and the significant change is a shrinkage of plasma volume.

All these facts point to a *dynamic equilibrium* between tissue protein and plasma protein depending upon the physiological needs of the moment. In the absence of food protein the body can use material coming from one body protein to fabricate badly needed protein material of different character.

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