# BLOOD PLASMA PROTEIN REGENERATION AS INFLUENCED BY INFECTION, DIGESTIVE DISTURBANCES, THYROID, AND FOOD PROTEINS

## A Deficiency State Related to Protein Depletion*

By S. C. MADDEN, M.D., P. M. WINSLOW, J. W. HOWLAND, Ph.D., and G. H. WHIPPLE, M.D.

*(From the Department of Pathology, The University of Rochester School of Medicine and Dentistry, Rochester, N. Y.)*

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Our belief that plasma proteins participate actively in the complex internal protein metabolism of the body should be adequate reason for our continued interest in blood plasma protein regeneration. It appears that the plasma can contribute protein readily to body tissues or body stores, but only in small amounts and with difficulty can the body contribute protein to the blood plasma—for example in fasting. The term "dynamic equilibrium" has been used to express the ebb and flow between plasma, organ, and tissue proteins. When reserve stores are exhausted the blood plasma production depends almost wholly upon food factors coming into the body from the intestine (16, 13, 9).

*Reserve stores* under ordinary conditions are adequate to tide the body over any emergency call for new formed plasma proteins. A study of the reserve stores is in progress and it is probable that the stores of materials from which plasma proteins can be fabricated are distinct from the stores of hemoglobin building material.

*Infection* can inhibit the formation of hemoglobin in anemia (17) and in like fashion it can inhibit the formation of plasma protein (Tables 3 and 3-*a* and Chart A below) during plasmapheresis. The mechanism of this interesting reaction is discussed below.

* We are indebted to Eli Lilly and Company for valuable materials used in these experiments.

A *deficiency state* which may result fatally can be produced in these dogs by long continued plasma depletion combined with low protein intake even when the accessory diet factors, salts and vitamins, are adequately represented in the diet. It is in a sense an exhaustion of the protein stores, perhaps an injury of the essential intracellular protein matrix of the body cells and a disturbance of the protein-forming mechanism. At any rate, the A/G ratio falls, resistance to infection is greatly reduced, and the output of new plasma protein drops to very low levels.

The method of these experiments is simply stated, although not always simply executed. By daily bleeding and return of washed red cells suspended in a modified Locke's solution (plasmapheresis) the normal plasma protein level in dogs (5 to 7 per cent) is reduced to about 4 per cent. Here it is maintained fairly constantly over long periods of time and presumably the hypoproteinemia acts as a strong stimulus for the regeneration of plasma protein. The protein of the diet consumed is credited with production of the plasma protein removed except for the *reserve store* of plasma protein related to the previous dietary history and necessarily removed in the initial weeks before a constant output is displayed. These depleted and standardized dogs then react with considerable uniformity to various proteins and are to be considered as biological test machines by which the investigator may evaluate the protein-building worth of various proteins, amino acids, and protein mixtures incorporated in the diet.

## *Methods*

In most respects the procedures used were the same as described in previous papers (8, 15, 13) from this laboratory. The dogs used had received the Laidlaw-Dunkin distemper prophylactic. They were kept in clean metabolism cages with water available at all times. Daily feedings, usually about 5 hours after plasmapheresis, were consumed voluntarily and well, except as noted in the clinical histories. Urine collections were strongly acidified with concentrated sulfuric acid and saved for weekly analysis. Despite precautions fecal contamination did occur and was removed as far as possible by filtration of the sample for analysis.

The various diets are detailed in the clinical histories. In general the basal ration was calculated to contain 0.6 to 1 gm. protein and 70 to 80 calories per kilo of body weight. Most rations included cane sugar or corn syrup (Karo Blue Label), Vitavose (Squibb), lard or cottonseed oil or butter fat (the supernatant and filtered fat from melted creamery butter), and bone ash. All diets contained

canned tomatoes, cod liver oil, and salt mixture (12)—without iron. The figures accepted for protein content of the various articles given in the diet are here given. Except as noted, the determinations were made in this laboratory by macro-Kjeldahl analysis and the protein calculated as 6.25 times the total nitrogen. Protein content: pork kidney, 16.2 per cent; pork liver, 20 per cent; canned salmon (total contents), 19 per cent; beef heart, 16 per cent; Vitavose, 8 per cent and 15 per cent;[1] canned tomato, 1.2 per cent; bran flakes (Post's), 13.2 per cent; boiled potato, 2.5 per cent; beef extract (Liebig), no protein but 9 per cent nitrogen;[2] serum, concentrated (Lilly), 86 per cent; yeast (Fleischmann), 13 per cent;[2] dog red blood cells, 33 per cent; soy bean meal, 40 per cent; thyroid powder, U.S.P. (Armour), 41.5 per cent; rice polishings and beef stomach digest (Lilly), 31.7 per cent. All amino acids used were obtained from Eastman Kodak Company; the specific ones were *l*-cystine, glycine, *d*-glutamic acid, tryptophane, and the dihydrochlorides of *dl*-lysine, *l*-histidine, and *d*-arginine. The rice polishings-beef stomach *digest* is the dried and defatted material obtained from acid digestion of equal parts of the fresh materials so concentrated that 1 gm. equals 1 gm. of each of the original substances. 1 gm. of the concentrated serum used contained the material obtained by alcoholic precipitation and drying of about 11 ml. of original serum. Liver extract (Lederle) "for intramuscular use" is said to contain in 1 ml. the active material from 100 gm. of fresh tissue. The soy bean meal used was light brown in color (7) and probably, though not certainly, of the same lot as that previously tested. We do not know how much heat was applied in the process of its preparation. All substances were fed as purchased with the following exceptions: the pork kidney and the beef heart were cooked in a double boiler and fed with the broth; the soy bean meal was cooked with water for 1 hour in a double boiler into a thick mush; pork liver was ground and fed raw; yeast was autoclaved for 15 minutes at 15 pounds steam pressure (256°F.); dog red blood cells were washed in modified Locke's solution, measured when packed by centrifuging, and coagulated with mild heat; potatoes were boiled, peeled, and ground. All the components of the daily diet were thoroughly mixed together before feeding. For production of the sterile abscesses, spirits of turpentine (0.8 ml.) was injected subcutaneously into the lateral thoracic regions, using aseptic precautions.

The procedure of plasmapheresis was carried on as described (8) and modified (13) in previous reports from this laboratory. The chemical methods used also were the same as previously outlined with the following exceptions. Selenium dioxide (in solution, selenous acid) was used as catalyst in place of cupric sulfate in the macro-Kjeldahl digestion mixture (10). Total digestion time was thereby reduced to 75 minutes and distillation was facilitated by lessened foaming. The digestion mixture used contained concentrated sulfuric acid, 20 ml.; potassium sulfate, 10 gm., and selenium, 0.1 gm. (2 cc. of a 7 per cent solution of selenium

[1] As given by manufacturer for different lots.

[2] Determinations made in Department of Vital Economics.

dioxide). Total nitrogen and albumin nitrogen of the plasma and total urinary nitrogen were all determined with this mixture. Half the quantity of selenium was used in determining plasma non-protein nitrogen, in which procedure trichloracetic acid was used to precipitate the protein (14). Nitrogen determinations on test food substances and plasma fractions were checked by the established method (using cupric sulfate as catalyst) and the results thus far obtained have indicated the shorter method (employing selenium) to be of equivalent accuracy.

Since all plasma nitrogen determinations were made on samples from the pooled blood from each bleeding, in which samples saturated sodium citrate solution (1 part to 100 parts blood) was the only anticoagulant, *all figures pertaining to circulating plasma nitrogen are low.* When equal portions of the same blood are treated with isotonic sodium oxalate, 1.4 per cent (1 part to 5 parts blood), instead of saturated sodium citrate, it is found that the citrated plasma volume is from 3 per cent to 16 per cent greater than the oxalated, tending to vary directly as the oxalate hematocrits over the range from 40 per cent to 60 per cent. This indicated dilution by hypertonic sodium citrate and the known physiological dilution occurring during large bleedings both operate to produce lower concentrations of elements in the pooled plasma than existed in the animal body. Moreover, the shift of protein from plasma to corpuscles thought to occur upon addition of hyperosmotic solutions to blood *in vitro* (2) would tend further to reduce the plasma protein concentration as given. *The figures for total grams of protein removed remain unaffected.*

## EXPERIMENTAL OBSERVATIONS

In the following tables and clinical histories are recorded the complete observations made on two dogs. In all instances, except the "initial sample" at the start of each experiment, the figures are those of weekly totals or averages. A plasmapheresis was performed usually on 6 of the 7 days of each week, although sometimes this procedure on only 5 of the 7 days sufficed to maintain the average blood plasma concentration between 3.80 and 4.30 per cent for the period. During weeks of fasting or of sugar feeding, exchanges were less frequent, and in the experiments with chemical inflammation higher averages were maintained to insure against possible severe depressions of the plasma protein level. As defined in this laboratory, the *potency ratio* of a given substance means the number of units of protein in this substance which are associated with the production of one unit of plasma protein. Obviously the lower the ratio the higher the potency.

In calculating nitrogen balance the weekly loss of nitrogen in feces of 2.7 gm. is based on a determination made during the previous work (13) on these dogs. Large factors in producing the total negative balance for the period of observation are the nitrogen lag or carry over from the higher intake level of kennel diet and the nitrogen in the *reserve store* of plasma protein removed.

In Tables 1 and 1-*a* are shown 25 weeks of experimentation on a dog (34-152) used successfully in a previously reported test period (13) of 21 weeks. During this former period the basal output averaged close to 12 gm. plasma protein per week and the reserve store, after 26 days on a basal diet followed by 1 week of dextrose feeding, amounted to only 10 gm. plasma protein. The first weeks in the present experiment offer a conspicuous contrast. Careful study of Table 1 indicates that the true basal output per week is 14 gm. plasma protein. It is obvious that this dog has a large *reserve store* of plasma protein-producing materials (56 gm. plasma protein above basal removed in the first 5 weeks). It requires 6 more weeks to remove all of this reserve store. In an attempt to explain this observation we may argue that the dog, on account of the experience with plasmapheresis the year previously, had developed greater capacity to conserve and retain tenaciously this reserve store of protein building material. This hypothesis will be tested in other animals.

The potency ratios for the raw and cooked kidney (Table 1) obviously cannot be determined accurately, as the reserve store has not been completely exhausted and contributes an uncertain amount to the protein output during such periods.

*Amino acids* if given in proper mixtures should be well utilized to form plasma protein. *Lysine* is well represented in the plasma protein, but given alone with this basal ration it is inert. *Lysine* when given with histidine and arginine (3) does have a slight effect (Table 1, period 19) but only 3.7 gm. plasma protein are produced above the basal control level.

Some observations on milk production in rats (5) suggested a trial of the combination of amino acids represented in glutathione. There does appear to be a definite increase in plasma protein output during and for 3 weeks following the feeding of cystine, glutamic acid, and glycine. Above an estimated basal production of 14 gm. per week

TABLE 1

*Blood Plasma Depletion and Regeneration*
*Kidney Retested. Influence of Certain Amino Acids*

Dog 34-152.

| Period 7 days | Diet Kidney basal + supplement | Protein intake Total for 7 days | Plasma protein removed Total for 7 days | | | Protein removed above basal* | Blood plasma Average concentration | |
|---|---|---|---|---|---|---|---|---|
| | | | Albumin | Globulin | Total | | Total protein | A/G ratio |
| | | *gm.* | *gm.* | *gm.* | *gm.* | *gm.* | *per cent* | |
| | Kennel | | | | | | 6.50 | 1.3 |
| 1 | Dextrose, 420 gm. | 0 | 4.6 | 3.5 | 8.1 | | 6.26 | 1.3 |
| 2 | Basal | 64 | 20.0 | 17.9 | 37.9 | | 5.07 | 1.1 |
| 3 | Basal | 64 | 11.2 | 11.0 | 22.2 | 56.3 | 4.35 | 1.0 |
| 4 | Basal | 64 | 13.0 | 12.8 | 25.8 | | 4.29 | 1.0 |
| 5 | Basal | 64 | 9.0 | 9.3 | 18.3 | | 4.13 | 1.0 |
| 6 | Basal + kidney (raw) 400+ gm. | 129+ | 15.7 | 12.9 | 28.6 | 24.3 | 4.12 | 1.3 |
| 7 | Basal | 64 | 10.8 | 9.2 | 20.0 | | 4.24 | 1.2 |
| 8 | Basal | 64 | 9.3 | 8.4 | 17.7 | | 4.02 | 1.1 |
| 9 | Basal + kidney (cooked) 350 gm. | 121 | 10.7 | 11.2 | 21.9 | 12.4 | 4.14 | 1.0 |
| 10 | Basal | 64 | 7.8 | 7.9 | 15.7 | | 4.04 | 1.0 |
| 11 | Basal | 64 | 8.4 | 8.4 | 16.8 | | 4.07 | 1.0 |
| 12 | Basal + lysine, 7 gm. | 64 | 6.5 | 6.2 | 12.7 | | 4.02 | 1.1 |
| 13 | Basal | 64 | 6.2 | 6.7 | 12.9 | | 4.09 | 0.9 |
| 14 | Basal + cystine, 7 gm., glutamic acid 8.4 gm., and glycine, 42. gm. | 64 | 7.9 | 7.9 | 15.8 | 7.1 | 4.20 | 1.0 |
| 15 | Basal | 64 | 8.4 | 7.4 | 15.8 | | 4.17 | 1.1 |
| 16 | Basal | 64 | 8.3 | 7.9 | 16.2 | | 4.14 | 1.0 |
| 17 | Basal | 64 | 7.4 | 7.9 | 15.3 | | 4.14 | 0.9 |
| 18 | Basal | 64 | 6.5 | 6.7 | 13.2 | | 4.19 | 1.0 |
| 19 | Basal + histidine, 7 gm., lysine, 7 gm., and arginine, 2.8 gm. | 64 | 8.0 | 8.6 | 16.6 | 3.7 | 4.20 | 0.9 |
| 20 | Basal | 64 | 7.0 | 7.7 | 14.7 | | 4.09 | 0.9 |
| 21 | Basal | 64 | 7.0 | 7.4 | 14.4 | | 4.23 | 0.9 |
| 22 | Fasting | 0 | 4.5 | 6.1 | 10.6 | | 4.23 | 0.7 |
| 23 | Basal | 64 | 5.4 | 8.9 | 14.3 | | 4.20 | 0.6 |
| 24 | Basal + rice polishing-beef stomach digest, 350 gm. | 175 | 7.8 | 11.7 | 19.5 | 14.1 | 4.29 | 0.7 |
| 25 | Basal | 63 | 8.2 | 14.4 | 22.6 | | 4.08 | 0.6 |

* Estimated basal output equivalent to 14 gm. plasma protein per week.

TABLE 1-*a*

*Weight, Nitrogen Balance, and Blood Findings*

Dog 34-152.

| Period 7 days | Diet Kidney basal + supplement | Weight | N intake | N in plasma removed | Urinary N | Negative N balance | R.B.C. hemato-crit | Plasma volume |
|---|---|---|---|---|---|---|---|---|
| | | *kg.* | *gm.* | *gm.* | *gm.* | *gm.* | *per cent* | *cc.* |
| | Kennel | 15.1 | | | | | 51.5 | 652 |
| 1 | Dextrose, 420 gm. | 14.5 | 0.0 | 1.3 | 9.7 | 13.7 | 49.8 | 631 |
| 2 | Basal | 13.9 | 10.2 | 6.1 | 12.4 | 11.0 | 45.3 | — |
| 3 | Basal | 14.0 | 10.2 | 3.6 | 10.9 | 7.0 | 43.8 | 588 |
| 4 | Basal | 14.0 | 10.2 | 4.1 | 10.5 | 7.1 | 49.5 | 636 |
| 5 | Basal | 14.0 | 10.2 | 2.9 | 11.0 | 6.4 | 49.9 | 614 |
| 6 | Basal + kidney, 400+ gm. | 14.1 | 20.6+ | 4.6 | 10.1 | +3.2 | 51.2 | 704 |
| 7 | Basal | 14.2 | 10.2 | 3.2 | 8.1 | 3.8 | 51.2 | 664 |
| 8 | Basal | 14.0 | 10.2 | 2.8 | 9.2 | 4.5 | 51.3 | 572 |
| 9 | Basal + kidney, 350 gm. | 14.1 | 19.3 | 3.5 | 10.6 | +2.5 | 50.4 | 622 |
| 10 | Basal | 14.1 | 10.2 | 2.5 | 9.5 | 4.5 | 48.3 | — |
| 11 | Basal | 14.3 | 10.2 | 2.7 | 6.7 | 1.9 | 48.5 | 590 |
| 12 | Basal + lysine, 7 gm. | 14.1 | 11.5 | 2.0 | 8.8 | 2.0 | 49.1 | 555 |
| 13 | Basal | 14.2 | 10.2 | 2.1 | 7.2 | 1.8 | 51.4 | 582 |
| 14 | Basal + cystine, 7 gm., glutamic acid, 8.4 gm., glycine, 4.2 gm. | 14.5 | 12.6 | 2.5 | 6.2 | +1.2 | 51.8 | 553 |
| 15 | Basal | 14.5 | 10.2 | 2.5 | 7.7 | 2.7 | 51.7 | 589 |
| 16 | Basal | 14.8 | 10.2 | 2.6 | 5.8 | 0.9 | 51.3 | 625 |
| 17 | Basal | 14.8 | 10.2 | 2.5 | 8.0 | 3.0 | 51.9 | 555 |
| 18 | Basal | 14.9 | 10.2 | 2.1 | 8.0 | 2.6 | 52.5 | 571 |
| 19 | Basal + histidine, 7 gm., lysine, 7 gm., arginine, 2.8 gm. | 14.9 | 14.3 | 2.7 | 11.2 | 2.3 | 52.0 | 589 |
| 20 | Basal | 15.0 | 10.2 | 2.4 | 8.1 | 3.0 | 52.4 | — |
| 21 | Basal | 15.1 | 10.2 | 2.3 | 9.4 | 4.2 | 54.3 | 620 |
| 22 | Fasting | 14.3 | 0.0 | 1.7 | 9.8 | 14.2 | 54.8 | 460 |
| 23 | Basal | 14.4 | 10.2 | 2.3 | 8.1 | 2.9 | 53.4 | 573 |
| 24 | Basal + rice polishings-stomach digest, 350 gm. | 14.5 | 28.0 | 3.1 | 13.1 | +9.1 | 52.1 | 587 |
| 25 | Basal | 14.7 | 10.1 | 3.6 | 10.9 | 7.1 | 50.6 | — |

the *plasma protein output* referable to this amino acid supplement (period 14) is 7.1 gm. What fluctuations occurred in plasma volume and protein concentration favored a slight increase in the mass of circulating plasma protein. The animal gained more than 0.5 kg. in weight during this 4 weeks' period and displayed a *decrease* in

urinary nitrogen. There was a positive nitrogen balance about equivalent to that noted after feeding 350 gm. cooked kidney. This all speaks for utilization of these amino acids in protein metabolism. In contrast, during the week of histidine, lysine, and arginine feeding (period 19) the urinary nitrogen *increased* almost to the amount of the excess nitrogen intake.

The complication of spoon feeding was introduced in the 20th period because the dog refused its food. A period of fasting did not improve the appetite, although at all times the animal readily ate the diet when spoon fed. The fasting output (period 22) was apparently higher than usual, but on deducting 5.7 gm. accounted for by the conspicuous shrinkage of plasma volume, the usual output is noted.

A digest of beef stomach and rice polishings (period 24, Table 1) displays an expected capacity for plasma protein production. Despite the fact that the experiment was terminated after 5 days of the second after period, a fairly high potency ratio (7.9) was already indicated. Too much weight cannot be given to this test of the rice polishings digest, as the subsequent acute fatal infection may have been developing during this period.

We note a sharp drop in the A/G ratio during the last four periods (Table 1). This drop frequently indicates trouble. Apparently the capacity to form albumin is more sensitive to harmful factors than is the capacity to produce globulin. During the last week of life a rapid sedimentation rate of the red cells was noted as a further index of trouble.

The total *negative nitrogen balance* for the 25 weeks amounts to 90.6 gm. There is little weight loss. Obviously there has been a serious depletion of the essential protein matrix of the body cells, and this may well be an important influence in lowering resistance to bacterial infection. The red cell hematocrit showed no significant fall at any time, excluding anemia as a possible cause of lowered resistance in spite of all the bleeding.

*Clinical History.*—Dog 34-152 (Tables 1 and 1-*a*). An adult male mongrel hound weighing 15.1 kg. had been previously tested over a period of 21 weeks (13) and had subsequently rested on kennel diet for 16 weeks. The dog was fasted during the 1st week except for the daily administration of 60 gm. dextrose in 200 ml. water by stomach tube. The basal daily diet then provided was the same

as that used during the previous test period (13), except for a deduction of 10 gm. sugar and the addition of 7 gm. lard. It contained 50 gm. (raw weight) cooked pork kidney (8.1 gm. protein); 25 gm. canned tomato (0.3 gm. protein); 5 gm. Vitavose (0.4 to 0.75 gm. protein); 120 gm. cane sugar; 15 gm. cod liver oil; 37 gm. lard; 10 gm. butter fat; 5 gm. bone ash; 1 gm. salt mixture. This diet furnished about 75 calories per kilo body weight daily and had a bulk of 200 ml. The daily diet was voluntarily consumed 100 per cent until the middle of the 19th week. From then on much or all of the diet had to be spoon fed. This procedure met with no resistance and the food was swallowed 100 per cent. Except for some decrease in spontaneous activity, no change in the animal's clinical condition was detected at this time. With hope of stimulating the appetite and in view of a gradual weight gain, 10 gm. cod liver oil was deducted from the daily diet during the last 6 weeks. On one occasion only, the day before the end of the last recorded week, an estimated 15 per cent of the day's diet was regurgitated. 2 days later a rapid *sedimentation rate* of the blood drawn for sampling was noted. The plasma protein level on this day was 3.87 per cent. Outspoken signs of illness were absent at this time, although the dog did appear below par. Then followed a progressively rapid downhill course through irritability, stupor, and convulsions to death on the 5th day after the end of the 25th week.

*Autopsy* disclosed acute vegetative endocarditis; focal acute myocarditis with abscess formation; acute suppurative nephritis; infected thrombus partially occluding left common iliac artery; bronchopneumonia; splenic infarct; cerebral hemorrhage; hemosiderin deposits in lymph nodes, spleen, liver, and bone marrow.

During the course of the experiment 8665 ml. of red blood cells were withdrawn and 10,092 ml. were returned suspended in modified Locke's solution containing a total of 541 gm. glucose. Weekly, non-protein nitrogen determinations varied from 15 to 25 mg. per cent during the entire period. The first supplement of kidney in period 6 was an accident and the amount of raw kidney was a little in excess of 400 gm. The second supplement of kidney was cooked and mixed with the basal ration over the 7 day period. The final supplement in Tables 1 and 1-*a* was a digest of beef stomach and rice polishings described under Methods.

Tables 2 and 2-*a* present the results of satisfactory tests over a continuous period of 26 weeks. When these observations were begun the dog (33-11) had been on kennel diet for 16 weeks following a previously reported experiment (13) of only 6 weeks' duration. The initial level of blood plasma protein concentration (6.09 gm. per cent) was 0.75 gm. per cent higher than that of the previous experiment, and the reserve store of plasma protein building material was just 4 times greater (34.1 gm.). It appears that both animals acquired larger protein reserves and higher plasma protein concentration levels following plasmapheresis experiments done 4 months previously. On

TABLE 2

*Blood Plasma Depletion and Regeneration*
*Dried Serum, Yeast, Thyroid, Hemoglobin Decreasingly Potent*
*Iron or Intramuscular Liver Extract Inert*

Dog 33-11.

| Period 7 days | Diet Salmon and kidney basals + supplements | Protein intake Total for 7 days | Plasma protein removed Total for 7 days | | | Protein removed above basal* | Potency ratio Protein intake to protein output | Blood plasma Average concentration | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Albumin | Globulin | Total | | | Total protein | A/G ratio |
| | | *gm.* | *gm.* | *gm.* | *gm.* | *gm.* | | *per cent* | |
| | Kennel | | | | | | | 6.09 | — |
| 1 | Dextrose, 350 gm. | 0 | 4.4 | 3.3 | 7.7 | | | 5.80 | 1.5 |
| 2 | Kidney basal | 64 | 14.0 | 8.4 | 22.4 | | | 5.03 | 1.7 |
| 3 | Kidney basal | 64 | 12.5 | 9.0 | 21.5 | | | 4.20 | 1.4 |
| 4 | Kidney basal | 64 | 9.9 | 8.6 | 18.5 | 34.1 | | 4.44 | 1.1 |
| 5 | Salmon basal A | 47 | 6.7 | 7.1 | 13.8 | | | 4.01 | 0.9 |
| 6 | Salmon basal A | 47 | 4.8 | 5.1 | 9.9 | | | 3.98 | 0.9 |
| 7 | Salmon basal B | 74 | 6.7 | 5.3 | 12.0 | | | 4.25 | 1.2 |
| 8 | Salmon basal B + yeast, 450 gm. | 133 | 9.9 | 8.1 | 18.0 | 13.4 | 4.4 | 4.21 | 1.2 |
| 9 | Salmon basal B | 74 | 10.2 | 7.7 | 17.9 | | | 4.09 | 1.3 |
| 10 | Salmon basal B | 74 | 7.7 | 5.8 | 13.5 | | | 3.93 | 1.3 |
| 11 | Dextrose, 350 gm. + iron, 1.4 gm. | 0 | 5.0 | 4.8 | 9.8 | | | 4.13 | 1.1 |
| 12 | Salmon basal B | 74 | 5.1 | 5.3 | 10.4 | | | 4.00 | 1.0 |
| 13 | Salmon B + liver extract (parenteral) | 74 | 6.1 | 6.4 | 12.5 | | | 4.01 | 1.0 |
| 14 | Salmon basal B | 74 | 6.3 | 5.9 | 12.2 | | | 4.21 | 1.1 |
| 15 | Salmon B+ red blood cells, 218 gm. | 146 | 9.3 | 8.1 | 17.4 | 7.1 | 10.1 | 4.14 | 1.2 |
| 16 | Salmon basal B | 74 | 7.5 | 6.2 | 13.7 | | | 4.06 | 1.3 |
| 17 | Salmon basal B + iron, 1.4 gm. | 74 | 6.8 | 5.6 | 12.4 | | | 4.13 | 1.2 |
| 18 | Salmon basal B | 74 | 5.5 | 5.4 | 10.9 | | | 4.07 | 1.0 |
| 19 | Salmon B + dried serum, 35 gm. | 104 | 8.8 | 7.9 | 16.7 | 8.5 | 3.5— | 4.10 | 1.1 |
| 20 | Salmon basal B | 63± | 8.1 | 7.7 | 15.8 | | | 4.16 | 1.1 |
| 21 | Kidney basal | 64 | 2.8 | 2.8 | 5.6 | | | 3.80 | 1.0 |
| 22 | Kidney basal | 64 | 5.3 | 6.0 | 11.3 | | | 3.98 | 1.0 |
| 23 | Salmon B + soy bean, 175 gm. | 144 | 8.8 | 9.4 | 18.2 | 5.8 | 12.1 | 4.13 | 0.9 |
| 24 | Salmon basal B | 74 | 5.7 | 5.9 | 11.6 | | | 3.95 | 1.0 |
| 25 | Salmon basal B + thyroid, 70 gm. | 103 | 7.3 | 8.1 | 15.4 | 5.5 | 5.3 | 4.32 | 0.9 |
| 26 | Salmon basal B | 74 | 6.7 | 7.4 | 14.1 | | | 4.01 | 0.9 |

* Estimated basal output equivalent to 12 gm. plasma protein per week.

TABLE 2-*a*

*Weight, Nitrogen Balance, and Blood Findings*

Dog 33-11.

| Period 7 days | Diet | Weight | N intake | N in plasma removed | Urinary N | Negative N balance | R.B.C. hematocrit | Plasma volume |
|---|---|---|---|---|---|---|---|---|
| | | *kg.* | *gm.* | *gm.* | *gm.* | *gm.* | *per cent* | *cc.* |
| | Kennel | 12.5 | | | | | 56.8 | 383 |
| 1 | Dextrose, 350 gm. | 11.9 | 0.0 | 1.2 | 18.7 | 22.6 | 53.4 | 396 |
| 2 | Kidney basal | 11.3 | 10.2 | 3.5 | 15.2 | 11.2 | 46.3 | 396 |
| 3 | Kidney basal | 11.4 | 10.2 | 3.4 | 10.6 | 6.5 | 44.5 | 411 |
| 4 | Kidney basal | 11.5 | 10.2 | 3.0 | 11.0 | 6.5 | 48.9 | 426 |
| 5 | Salmon basal A | 11.6 | 7.5 | 2.2 | 10.5 | 7.9 | 49.6 | 444 |
| 6 | Salmon basal A | 11.6 | 7.5 | 1.6 | 9.4 | 6.2 | 46.2 | 367 |
| 7 | Salmon basal B | 11.5 | 11.8 | 1.9 | 10.2 | 3.0 | 49.6 | 387 |
| 8 | Salmon basal B + yeast, 450 gm. | 11.6 | 21.3 | 2.9 | 13.4 | +2.3 | 50.0 | 419 |
| 9 | Salmon basal B | 11.6 | 11.8 | 2.9 | 11.1 | 4.9 | 49.1 | 402 |
| 10 | Salmon basal B | 11.6 | 11.8 | 2.2 | 8.9 | 2.0 | 49.0 | 395 |
| 11 | Dextrose, 350 gm. + iron, 1.4 gm. | 11.1 | 0.0 | 1.6 | 7.0 | 11.3 | 48.8 | 353 |
| 12 | Salmon basal B | 11.1 | 11.8 | 1.7 | 7.9 | 0.5 | 47.2 | 391 |
| 13 | Salmon B + liver extract (parenteral) | 11.2 | 11.8 | 2.0 | 9.8 | 2.7 | 49.6 | 344 |
| 14 | Salmon basal B | 11.3 | 11.8 | 1.9 | 9.4 | 2.2 | 49.7 | 409 |
| 15 | Salmon B + red blood cells, 200 cc. | 11.4 | 23.4 | 2.8 | 13.6 | +4.3 | 49.4 | — |
| 16 | Salmon basal B | 11.4 | 11.8 | 2.2 | 9.1 | 2.2 | 52.2 | 414 |
| 17 | Salmon basal B + iron, 1.4 gm. | 11.5 | 11.8 | 2.0 | 9.3 | 2.2 | 52.4 | 376 |
| 18 | Salmon basal B | 11.5 | 11.8 | 1.7 | 8.7 | 1.3 | 51.8 | 360 |
| 19 | Salmon B + dried serum, 35 gm. | 11.7 | 16.6 | 2.7 | 9.7 | +1.5 | 51.5 | 389 |
| 20 | Salmon basal B | 11.6 | 10.1 | 2.5 | 6.6 | 1.7 | 52.7 | 372 |
| 21 | Kidney basal | 11.8 | 10.2 | 0.9 | 7.0 | 0.4 | 51.1 | 445 |
| 22 | Kidney basal | 11.7 | 10.2 | 1.8 | 7.9 | 2.2 | 51.9 | 358 |
| 23 | Salmon B + soy bean, 175 gm. | 11.9 | 23.0 | 2.9 | 13.0 | +4.4 | 53.1 | 396 |
| 24 | Salmon basal B | 12.2 | 11.8 | 1.9 | 9.7 | 2.5 | 52.8 | 409 |
| 25 | Salmon basal B + thyroid, 70 gm. | 11.9 | 17.1* | 2.5 | 12.5 | 0.6 | 52.6 | 345 |
| 26 | Salmon basal B | 11.8 | 11.8 | 2.3 | 9.6 | 2.8 | 54.0 | 390 |

* Includes nitrogen in 6 gm. of beef extract.

the salmon basal diet the estimated basal protein output equaled that on the kidney basal (about 12 gm.) but the salmon is less efficient with its 16 per cent larger protein content.

The autoclaved *yeast* was readily consumed by the dog and produced none of the gastro-intestinal disturbance noted with the non-autoclaved material (13). Each 4.4 gm. of yeast protein resulted in the production of 1 gm. of plasma protein. The carry over into the week after feeding was equal to the output of the feeding week.

Two attempts to get further information on a possible relationship of oral *iron* to plasma protein regeneration tend to deny a direct relationship. A previous test (15) adding 2 gm. ferric citrate (360 mg. iron) daily to a vegetable protein diet was accompanied by an excess protein output of 14.4 gm. but this was associated with anemia. This quantity is too large to be accounted for by any experimental variation such as blood volume fluctuation. In the present experiments during one period 1.15 gm. ferric citrate scales (200 mg. iron) in solution were given with 50 gm. dextrose daily by stomach tube (period 11, Table 2). The output of 9.8 gm. exceeds the expected fasting output but little, and the following week on basal diet yields less than the basal output. When the same amount of ferric citrate was added to the basal diet during period 17, the result was negative.

A clinical observation reported to us (by Dr. D. J. Stephens) occasioned the trial of intramuscular liver extract. No effect on plasma protein regeneration was indicated. Oral liver extract enabled protein production in proportion to its protein content (15); the product for intramuscular use contained no protein.

Proteins in *red blood cells* (about 95 per cent hemoglobin) have a low potency ratio of 10. Hemoglobin when fed to anemic dogs has a potency ratio of 10, that is, one must feed about 100 gm. hemoglobin to produce 10 gm. new hemoglobin in red cells (19).

Dried *serum*, 1 gm. of which is obtained by alcoholic precipitation from about 11 ml. original serum, demonstrated excellent capacity for plasma protein production (potency ratio 3.5) despite some gastric disturbance, which in the following week resulted in the loss by vomiting of an amount estimated to equal 1 day's diet. A depression in plasma regeneration to a fasting level (period 21, Table 2) followed this upset and may have been due in part to the gastro-intestinal

disturbance. The change from salmon to kidney basal may have had an influence in the depression, or a slowly developing deficiency state may have become manifest.

*Soy bean meal* (period 23, Table 2) retested at this time showed the expected prompt effect without carry over, but the total plasma protein output was less than in a previous experiment (13). We have observed before that the potency ratio of a given food factor may remain unchanged or may show considerable change when tested with different basal diets. The soy bean meal with a kidney basal showed a potency ratio of 7.1 in contrast to this experiment with a salmon basal and potency ratio of 12.1. The soy bean feeding did favor a weight increase. A possible deficiency state may also have been a factor in this reaction.

Thyroid powder given in large doses (period 25, Table 2) will accelerate metabolism, and it was thought that such acceleration might increase or decrease plasma protein production, depending upon the site of action. As a matter of fact, there is no change in the picture and the protein of the thyroid powder is utilized just like any other protein, with a potency ratio of 5.3, indicating excellent utilization. It will be useful to know the reaction to thyroxin alone.

During periods 27 to 31, Table 3, Dog 33-11 was progressing into a serious deficiency state due to inadequate protein intake and a steady drain on the plasma protein. The basal diet no longer enabled the dog to produce 12 gm. plasma protein each week. The dog had a distaste for food necessitating spoon feeding with occasional regurgitation, and there developed superficial ulcers on the skin over the gluteal regions. There was considerable loss of hair but no weight loss. There was a definite fall in the A/G ratio.

The amino acid feeding (period 28, Table 3), gave no significant change in the plasma protein output, but this may be a false picture because of the deficiency state. The positive nitrogen balance suggests some utilization of some of these amino acids (compare period 14, Table 1-*a*).

Periods 37 and 39, Tables 3 and 3-*a*, present experiments on the effects of *inflammation* upon plasma protein regeneration. Several previous observations (13) have been made on the influence of unwanted bacterial infection arising in the course of an experiment.

This influence has been uniformly depressant to plasma protein formation. In the present observations local inflammation with abscess production and a certain degree of accompanying general intoxication has been produced by the chemical irritation of turpentine injected

TABLE 3

*Blood Plasma Depletion and Regeneration*
*Influence of Sterile Abscess*

Dog 33-11.

| Period 7 days | Diet | Protein intake Total for 7 days | Plasma protein removed Total for 7 days | | | Protein output Per cent of protein intake | Protein output Per cent of estimated basal output* | Blood plasma Average concentration | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Albumin | Globulin | Total | | | Total protein | A/G ratio |
| | | *gm.* | *gm.* | *gm.* | *gm.* | | | *per cent* | |
| 27 | Salmon basal B | 74 | 4.2 | 5.0 | 9.2 | | | 3.81 | 0.8 |
| 28 | Salmon B + tryptophane, 7 gm., cystine, 7 gm., glutamic acid, 8.4 gm., glycine, 2.8 gm. | 72 | 3.0 | 3.9 | 6.9 | | | 3.89 | 0.7 |
| 29 | Salmon basal B | 58± | 2.8 | 3.6 | 6.4 | | | 3.79 | 0.8 |
| 30 | Potato-bran | 68 | 1.4 | 1.7 | 3.1 | | | 3.85 | 0.8 |
| 31 | Potato-heart | 41 | 2.3 | 3.2 | 5.5 | | | 4.16 | 0.7 |
| | Raw liver A | 86 | | | | | | | |
| 32 | Raw liver A | 150 | 2.3 | 3.0 | 5.3 | | | 4.39 | 0.8 |
| 33 | Raw liver A | 150 | 5.5 | 6.3 | 11.8 | | | 4.69 | 0.9 |
| 34 | Raw liver A | 150 | 11.7 | 12.8 | 24.5 | | | 4.70 | 0.9 |
| 35 | Raw liver B | 115 | 10.9 | 13.5 | 24.4 | 21.2 | 101.0 | 4.46 | 0.8 |
| 36 | Raw liver B | 115 | 10.4 | 13.9 | 24.3 | 21.1 | 100.5 | 4.27 | 0.7 |
| 37† | Basal + turpentine abscess | 78 | 1.8 | 3.0 | 4.8 | 6.2 | 29.5 | 4.06 | 0.6 |
| 38‡ | Raw liver B | 129 | 9.2 | 14.8 | 24.0 | 18.6 | 88.6 | 4.33 | 0.6 |
| | Raw liver C | | | | | | | | |
| 39 | C + turpentine abscesses | 108 | 3.0 | 5.3 | 8.3 | 7.7 | 36.7 | 4.46 | 0.6 |
| 40 | Raw liver C | 107 | 9.1 | 14.8 | 23.9 | 22.3 | 106.2 | 4.54 | 0.6 |

* Estimated basal output on raw liver B diet equivalent to 21 per cent of intake.

† Period of 5 days.

‡ Period of 8 days.

subcutaneously. Systemic reaction is limited usually to the first 3 days after the injection of the turpentine and is marked by a mild reduction in activity and appetite and a sharp leucocytosis and fever. The urinary nitrogen is increased. With the walling off of the in-

flammatory area the general reaction subsides and uncomplicated healing begins immediately with the evacuation of the abscess on the 4th or 5th day. This limited, acute, controlled, inflammatory process affords a very satisfactory test period. Observations on two such periods (37 and 39) are listed in the tables (3 and 3-*a*).

TABLE 3-*a*

*Weight, Nitrogen Balance, and Blood Findings*

Dog 33-11.

| Period 7 days | Diet | Weight | N intake | N in plasma removed | Urinary N | Negative N balance | R.B.C. hematocrit | Plasma volume |
|---|---|---|---|---|---|---|---|---|
| | | *kg.* | *gm.* | *gm.* | *gm.* | *gm.* | *per cent* | *ml.* |
| 27 | Salmon basal B | 11.9 | 11.8 | 1.5 | 9.0 | 1.4 | 54.7 | 376 |
| 28 | Salmon B + tryptophane, 7 gm., cystine, 7 gm., glutamic acid, 8.4 gm., glycine, 2.8 gm. | 11.9 | 14.9 | 1.1 | 5.7 | +5.4 | 52.7 | 381 |
| 29 | Salmon basal B | 11.6 | 9.3± | 1.0 | 7.2 | 1.6± | 51.5 | 380 |
| 30 | Potato-bran | 11.2 | 10.9 | 0.5 | 7.9 | 0.2 | 48.2 | 424 |
| 31 | Potato-heart Raw liver A | 11.4 | 20.4 | 0.9 | 8.7 | +8.1 | 46.2 | 388 |
| 32 | Raw liver A | 11.6 | 25.0 | 0.9 | 10.5 | +10.9 | 50.9 | 510 |
| 33 | Raw liver A | 11.7 | 25.0 | 1.9 | 12.9 | +7.5 | 54.9 | — |
| 34 | Raw liver A | 11.8 | 25.0 | 3.9 | 14.2 | +4.2 | 52.9 | — |
| 35 | Raw liver B | 12.0 | 18.4 | 3.9 | 13.1 | 1.3 | 52.8 | — |
| 36 | Raw liver B | 12.1 | 18.4 | 3.9 | 8.8 | +3.0 | 52.2 | — |
| 37* | B + turpentine abscess | 12.4 | 12.5 | 0.8 | | | 48.7 | — |
| 38† | Raw liver B Raw liver C | 12.3 | 20.6 | 3.8 | 19.7‡ | +3.1§ | 49.6 | — |
| 39 | C + turpentine abscesses | 12.4 | 17.3 | 1.3 | 13.7 | 1.4§ | 53.5 | — |
| 40 | Raw liver C | 12.2 | 17.1 | 3.8 | 12.0 | 1.4 | 52.5 | — |

* Period of 5 days.

† Period of 8 days.

‡ Period 37 plus period 38.

§ Include abscess nitrogen, a total of approximately 1.7 gm. for the 2 periods (4).

The *raw liver diet* was chosen to favor a fairly large basal output of plasma protein and to be highly tempting to the dog during the periods of the experimental inflammation. The 37th period shows a marked depression of plasma protein regeneration and a conspicuous decline in the average blood plasma protein concentration accompanying

the production of one turpentine abscess. The figures indicate a drop in protein output to 29 per cent of the output of the previous (36th) week but a part of this drop is due to a decreased food intake. In the second abscess period (39th period) the process was prolonged to a full week by the production of two consecutive abscesses (Clinical

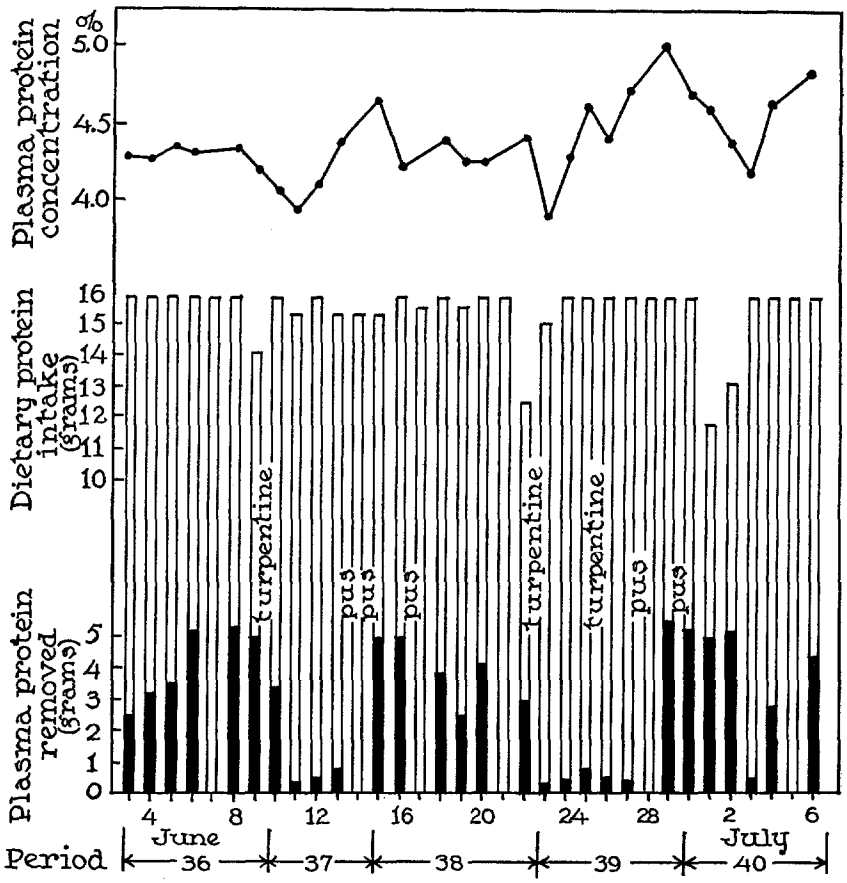


CHART A. Plasma protein production depressed by turpentine abscess.

History, Dog 33-11). Depression in plasma protein regeneration was marked (36.5 per cent of that for the 36th week), but not as great as that during the first abscess and the average circulating plasma protein level was higher. This elevation in output and the further relative elevation of the 40th week may indicate accumulation of undepleted

protein and protein building materials during the 38th period, or may indicate a delayed output based on protein building materials accumulated during the abscess periods themselves. The urinary nitrogen was distinctly elevated during the second abscess period and remained high during the final week on account of the hemoglobinuria (Clinical History, Dog 33-11).

*Clinical History.*—Dog 33-11 (Tables 2 and 2-*a*, 3 and 3-*a*; Chart A). An adult female mongrel, born Nov. 25, 1932, in this laboratory, was raised on a salmon-bread-apricot diet. 2 months in the anemia colony were followed by 12 months on kennel diet. Its initiation to plasmapheresis continued only 5 weeks and has been reported (13). Some 16 weeks on kennel diet followed this depletion. Oct. 1, 1935, found the various initial determinations as given (Tables 2 and 2-*a*). During the 1st week 50 gm. dextrose in 150 ml. water were given daily by stomach tube. The kidney basal ration provided this dog was the same as that given Dog 34-152 (Table 1), except for reduction of cane sugar to 95 gm., lard to 30 gm., and cod liver oil to 10 gm. The salmon basal diets A and B were identical with the kidney basal ration, except for the substitution for the kidney of canned salmon, 20 gm. in A and 50 gm. in B. The latter daily diet contained 10.5 gm. protein and 926 calories. Weight was maintained and the diets were readily eaten. The yeast was weighed before autoclaving. In the 11th week 25 ml. of 4.6 per cent solution of ferric citrate scales (Merck) (17.4 per cent iron) were added to the dextrose given daily as in the 1st week, and in the 17th period the same quantity of iron was added to the basal diet. The liver extract was given intramuscularly, 1 ml. each day. The gastro-intestinal disturbances from the 19th to the 29th weeks, apparently related to the feeding, have been mentioned above. Beef extract tried in the 25th period was unsuccessful in overcoming dislike for the diet. Spoon feeding was largely successful, although, as indicated in the figures for protein intake, food was sometimes lost by regurgitation. The non-protein nitrogen varied from 12 to 24 mg. per cent in the first 25 weeks and from 23 to 32 mg. per cent in the following 15 weeks.

Upon changing in the 30th week to the potato-bran diet the dog voluntarily returned to 100 per cent food consumption for four days and then began to lose appetite. This diet contained 200 gm. boiled potato (5 gm. protein); 35 gm. bran flakes (4.6 gm. protein); 25 gm. tomato (0.3 gm. protein); 61 gm. corn syrup; 20 gm. cod liver oil; 20 gm. cottonseed oil; 1 gm. salt mixture. For 3 days in the 31st period 50 gm. beef heart (8.0 gm. protein) and 5 gm. Vitavose (0.75 gm. protein) were substituted in the above diet for the bran flakes, but still the response was poor.

From the 1st day the dog ate the *raw liver diet* avidly. Within 3 weeks the sores on the buttocks were completely healed and loss of hair appeared much reduced. The first liver diet consisted of 100 gm. raw liver (20 gm. protein); 10 gm. Vitavose; 50 gm. tomato; 94 gm. cane sugar; 20 gm. cod liver oil; 20 gm. cotton-

seed oil; 15 gm. bone ash; 1 gm. salt mixture. The B modification reduced the liver to 75 gm. and increased the sugar to 102 gm., maintaining a total caloric value of 903. The C modification maintained the same protein kind and quantity but reduced the caloric value to 800, and arrested the gradual weight increase. The animal maintained a good clinical condition despite the abscess production, although during these periods it frequently left a small amount of its diet and rarely regurgitated a small quantity.

To produce a sterile abscess turpentine was injected on the right side on the last day of the 36th period. Leucocyte count was 12,000 before injection and 56,000 2 days after. Rectal temperature was 41.3°C. ½ hour following plasmapheresis on the 1st day of the 37th period, but fell to 38.9°C. within 4 hours. The remaining 4 days of the period were without fever or plasmapheresis. On the 3rd day the local swelling was fluctuant and on the 4th day the abscess was drained by incision. 80 ml. of sanguineous seropurulent material were obtained. The leucocyte count dropped to 14,800 on this day. Culture of the abscess material produced no bacterial growth. The following day similar but thinner fluid, about 35 ml., was removed. Turpentine was evident in all this material, as well as in 17 ml. similar material aspirated 2 days later after the wound had closed. On this day, the 2nd of the 38th period, the leucocyte count rose to 39,400 and the temperature climbed to 40.2°C. shortly after plasmapheresis but dropped to 38° less than 4 hours later. During the balance of the period the leucocyte count averaged 25,000 and the rectal temperature did not exceed 38.2°C.

A second abscess was produced with turpentine on the left side on the last day of this period (period 38). Fluctuation in the abscess appeared on the 3rd day of the period and another turpentine injection was made on the right side. On the 5th day the second abscess was drained, yielding 77 ml. seropurulent material and on the 7th day 82 ml. of material was obtained from the third abscess. The leucocyte count reached 53,400 on the 5th day and the temperature 39.8°C. Both of these abscesses healed without further accumulation of fluid, and the leucocyte count gradually dropped to 11,200 by the last day of the 40th period. During the last period transitory pyrexia as high as 40.1°C. would sometimes appear after plasmapheresis, without any recognizable general disturbance.

On the 2nd day of the last period the red cells (131 ml.) prepared for injection were accidentally much overheated, then subsequently cooled to body temperature and injected. Hemoglobinuria was noted within 1 hour and few hours later slight icterus of sclerae and mucous membranes could be detected. No general disturbance in the behavior of the dog was recognized at any time, although part of the diet was refused for 2 days.

The dog was continued on the same diet for 14 days following the discontinuance of plasmapheresis at the close of the 40th period. On the 1st day the blood plasma protein concentration was 4.45 gm. per 100 ml. plasma; the 4th day it had risen to 4.68, the 8th day to 4.80, and the 14th day to 5.87.

## DISCUSSION

*Infection* and its influence on plasma protein production invite discussion. The "sterile abscess" due to turpentine gives the complete clinical picture of a bacterial abscess—inflammation, fever, leucocytosis, localized pus formation, and an increased urinary nitrogen. Moreover, it can be promptly terminated on the 3rd or 4th day, with subsequent rapid healing. Chart A shows that the sterile abscess causes a diminution in the production of new plasma protein during the abscess week. A small part of this initial reaction can be explained by extravasation at the site of the abscess and in the first abscess period the slight decrease in protein intake will explain a small part. The larger part is yet to be explained. We might argue that materials which would go to form the plasma proteins were deviated to repair the body tissues whose injury released the customarily noted (4) excess urinary nitrogen. One might also argue that the protein-forming mechanism (in the liver?) is disturbed by the abscess intoxication slowing up plasma protein formation. In the anemic dog this last thesis has been shown to obtain for the inhibition of hemoglobin formation caused by a sterile abscess (17).

*Reserve stores* of materials which can promptly be converted into plasma proteins have been demonstrated in dogs. The amount of such stores depends in part on the diets of the preceding weeks. It would seem (Tables 1 and 2) that dogs which have been through periods of depletion by plasmapheresis, tend to heap up *greater reserve stores* of plasma protein building materials during intervening rest periods. Where these reserve stores are located is of some interest, and there seems to be no reasonable doubt that a part at least is stored in the liver but the liver cannot possibly hold all such large reserve stores as demonstrated in some dogs. The reserve store may amount to more protein than is contained in the entire liver and we may suspect the muscles as another possible depot (1). Addis and associates (1) and Luck (11) have given evidence for liver storage of proteins related to diet.

*Albumin-globulin ratios* are much talked about but they scarcely deserve this attention. There is always the uncertainty about the

separation of albumin and globulin into distinct fractions. Some methods used will give gross inaccuracies. A favorite argument is that a drop in the A/G ratio indicates a decreased albumin production and that the particular tissue concerned in the injury reaction is therefore responsible for the manufacture of the albumin of the plasma. For example Dalla Volta (18) notes a fall in the A/G ratio after x-ray injury of the bone marrow and like many others argues that the albumin is formed in the red marrow. *Any considerable disturbance* of the normal state in the dog will show a drop in the A/G ratio and a diet rich in some grain proteins will likewise cause a drop in the A/G ratio (13). Of the plasma proteins the *fibrinogen* is most labile and can be raised or lowered by a great variety of body changes (6). Albumin appears to be more labile than globulin and certainly escapes from the circulation more readily than does the larger globulin molecule—a good example is nephrosis with escape of albumin in the urine. Certain proteins in the food favor albumin production—for example muscle, liver, kidney, soy bean meal. In the present unsatisfactory state of our knowledge too much weight cannot be placed safely upon the interesting fluctuations of the albumin and globulin in the plasma.

The *potency ratio* as used in our papers means the grams of protein fed which will yield 1 gm. of new plasma protein in these depleted dogs. Potency ratios may vary depending upon a number of factors. The basal diet is probably most important as all our basal rations contain some protein which might be capable of supplementing an added protein (*e.g.* liver) but the protein of another basal ration might not act favorably. Presumably the new plasma protein results from the assembly of many amino acids and other materials (in the liver?) and the amount and character of the amino acids coming from one basal diet might determine the reaction with the amino acids coming from some accessory food protein. It is probable also that these dogs can utilize relatively small amounts of added protein more efficiently than larger supplements. The potency ratio would then rise as larger amounts of the food protein were added. The caloric intake may also be a factor. On a given diet it is probable that some dogs can make plasma protein more efficiently than others just as some dogs can make hemoglobin in anemia more efficiently on a given diet as compared with certain other dogs. Similar variations apply to

endurance, speed of running, resistance to infection, and other individual qualities. Obviously the potency ratio must be interpreted cautiously with understanding of all the conditions of the experiment, realizing that apparently insignificant variables may cause sizable differences in reaction. To show the potency ratio of *liver* we refer to Table 4 illustrating relatively stable potency ratios in different dogs under a variety of conditions.

Raw liver was given in Table 3 and this potency ratio of 5.5 may mean a more complete utilization of the uncooked tissue. On the other hand this dog was emerging from a deficiency state and may have been utilizing this protein at an even greater pace than during other more normal periods.

TABLE 4

*Potency of Liver in Various Dietary Régimes*

| Diet | Reference | Dog | Weight of dog | Daily dietary intake | | | Potency ratio |
|---|---|---|---|---|---|---|---|
| | | | | Basal + liver | | Liver protein total | |
| | | | | Calories per kg. | Total protein per kg. | | |
| | | | *kg.* | | *gm.* | *gm.* | |
| Liver added to potato-bran basal... | (15) | 32-130 | 13.4 | 99 | 5.8 | 60.0 | 6.5 |
| Liver added to kidney basal..... | (13) | 34-152 | 13.2 | 90 | 1.7 | 14.3 | 6.4 |
| Liver sole article of diet..... | (8) | 32-30 | 8.6 | 45 | 7.0 | 60.0 | 6.6 |
| Liver 91 per cent of protein in diet.. | Table 3 | 33-11 | 12.0 | 75 | 1.4 | 15.0 | 5.5 |

*Gastro-intestinal disturbances* are obviously important in this type of experiment and this was to be expected. Fresh yeast causes diarrhea and intestinal disturbance without obvious clinical intoxication but with a great fall in the plasma protein output. There is a conspicuous difference in the utilization of autoclaved yeast, which is well digested and gives a potency ratio of about 4.4. The fresh yeast caused a fall of plasma protein production to the fasting level (Table 5, Reference 13). Likewise in Dog 33-11 (Table 2, period 21) there was some gastro-intestinal disturbance which was probably in part responsible for this fall in the plasma protein output to a fasting level.

*Amino acids* in plasma depletion experiments cannot fail to intrigue the investigator. Theoretically it should be possible with the proper

mixture of amino acids to influence profoundly the plasma protein production. Such good fortune as yet has not been attained by our few experiments. A combination of cystine, glutamic acid, and glycine by mouth does have a slight influence on new plasma protein production and shows a positive nitrogen balance in this dog (Table 1). Much more work in this field is badly needed.

*Iron* when given with a potato-bran basal diet (15) in the presence of a moderate anemia did appear to have a definite influence upon plasma protein regeneration. When iron is given with a salmon basal diet or during fasting with a normal hemoglobin concentration (Table 2) there is no effect upon the plasma protein production. We cannot give any adequate explanation for this difference in the action of iron but hope to report in more detail later.

## SUMMARY

When blood plasma proteins are depleted by bleeding, with return of washed red cells (plasmapheresis), it is possible to bring dogs to a steady state of low plasma protein in the circulation and a uniform plasma protein production on a basal diet. Such dogs become test subjects by which the effect of various factors on plasma protein regeneration can be measured.

Dogs previously the subjects of plasmapheresis, during long rest periods appear to increase their stores of plasma protein building materials and their blood plasma protein concentrations above former normal levels.

A sterile abscess (turpentine) induces a marked reduction in plasma protein regeneration in these test dogs consuming an ample basal diet. The sharp reduction during the initial 24 hours may in part reflect an extravasation of plasma protein into the injured tissue but there also appears to develop a true disturbance of the mechanism which produces plasma proteins.

Digestive disturbances interfere seriously with plasma protein production. Whereas large quantities of live yeast upset digestion and form no plasma protein, autoclaved yeast is well utilized, having a potency ratio of 4.4.

Amino acids have been tested inadequately. A mixture of cystine,

glutamic acid, and glycine does seem to have a definite effect upon protein metabolism and plasma protein production.

Iron, under the conditions of these experiments, does not influence the output of plasma proteins. Liver extract (parenteral) is also inert.

The proteins of *red blood cells* when added to the diet are poorly utilized for plasma protein formation and show a potency ratio of only 10.1.

Kidney protein added to the kidney basal diet shows a potency ratio of about 5 as compared with 4.6 for that basal diet.

A digest of beef stomach and rice polishings shows a potency ratio of about 7.9. Dried powdered serum shows a potency ratio of 3.5, which is much less than fresh serum (2.6).

Powdered thyroid fed in doses sufficient to accelerate body metabolism shows no distinct effect upon plasma protein production not attributable to the protein in the thyroid powder itself.

Long periods (25 to 30 weeks) of plasma depletion and basal diet intake remove much protein from body fluids and tissues. Associated with this protein depletion the dog loses its appetite and may vomit some food. There is loss of hair, a tendency to skin ulceration, and a distinct lowering of resistance to infection. The plasma protein output may fall to fasting levels in spite of food intake sufficient to maintain weight. We believe this condition to be a *deficiency state* related to severe depletion of the essential protein matrix of the body cells.

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