# RAIDING OF BODY TISSUE PROTEIN TO FORM PLASMA PROTEIN AND HEMOGLOBIN

## What Is Premortal Rise of Urinary Nitrogen?

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This title indicates our belief that under conditions of *protein fasting* the body will give up large amounts of protein from its organs and tissues to produce new hemoglobin and plasma protein when experimental anemia and hypoproteinemia (double depletion) are maintained by bleeding. Evidently in this emergency the body attempts to correct the anemia and hypoproteinemia at the expense of the body tissues—its circulating proteins have a priority over the tissue and organ proteins. In some dogs this raiding may progress actively up to the terminal fatal state but usually decreases in the final 2 or 3 weeks. As the body weight loss continues, individual organs and their component cells lose protein. This loss may be interpreted, in part at least, as a loss of enzymes or proenzymes. It is at least possible that the loss of cell enzymes may account for the diminished cell function (liver function decrease) and lessened blood protein production. There is no evidence of nitrogen waste due to tissue breakdown—rather all nitrogen is conserved and used frugally. This is further evidence that the exchange between tissue protein and blood protein is in fact a part of the protein "ebb and flow" between cells and plasma with no significant excess of protein breakdown and related nitrogen loss (11).

Reserve stores of easily removed protein are readily demonstrated in *hypoproteinemia* alone (7). Once these reserves are removed the remaining body proteins do not contribute readily to the production of new plasma protein which depends largely upon food protein or protein-building material (including amino acids) for its production under these conditions.

Reserve protein stores are readily demonstrated when the dog is continuously bled to establish an anemia but the iron reserves are important. The production of new hemoglobin in anemia may be controlled by the available iron or by protein intake (4). When one attempts to produce anemia with liberal intake of iron and very low protein intake, there is a rapid loss of plasma by simple bleeding which soon leads to double depletion (*anemia plus hypoproteinemia*). If one attempts to replace the plasma removed in the daily bleeding it soon becomes apparent that this added plasma protein can be used to make new hemoglobin (10) and the state of anemia is lessened by just so much as the returned plasma protein is turned over into new hemoglobin. Eventually the use of isotopic labelled plasma protein (3) will help to clear some of these obscure points.

The condition of anemia and hypoproteinemia (double depletion) is a severe strain upon the dog and cannot be tolerated indefinitely as is true for anemia alone and hypoproteinemia alone, either one of which can be continued for years. Perhaps the factor of appetite is of major importance and frequently lack of appetite terminates the double depletion experiments.

Dogs show considerable variation in their capacity to tolerate this state of double depletion on a very low protein diet, some showing 30 per cent body weight loss in 6 weeks and others tolerating 8 weeks with but 13 per cent weight loss. Usually the output of blood proteins and related bleeding is largest in dogs which suffer the greatest weight loss.

## EXPERIMENTAL OBSERVATIONS

The experiments tabulated below show considerable variations between individual dogs kept on a uniform regime. Weight loss runs from a maximum of 5 per cent per week to a minimum of 1.6 per cent per week. Output of total blood proteins ranges from a maximum of 66 to a minimum of 39 gm. per week, there being a definite correlation between weight loss and protein output. For every kilogram weight loss some dogs will produce 49 gm. blood protein or again 60, 100, or even 140 gm. Liver parenchyma and skeletal muscle for example contain 19 to 21 gm. per cent protein.

TABLE 1

*Prolonged Blood Protein Depletion—Death*

Dog 43-400

| Period | Weight | Protein intake | | Food consumption | Plasma volume | Protein output | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Hemoglobin | | Plasma protein | |
| | | Type | Weekly | | | Level | Output per wk. | Level | Output per wk |
| *1 wk.* | *kg.* | | *gm.* | *per cent* | *cc.* | *gm. per cent* | *gm.* | *gm. per cent* | *gm.* |
| 1 | 21.8 | Basal + squash | 33 | 95 | 918 | 13.5 | 98.3 | 4.9 | 26.0 |
| 2 | 20.9 | Basal + squash | 29 | 82 | 928 | 11.8 | 52.3 | 4.4 | 17.9 |
| 3 | 20.0 | Basal + salmon 75 | 28 | 81 | 816 | 11.0 | 37.5 | 4.6 | 11.7 |
| 4 | 19.3 | Basal + salmon 75 | 120 | 87 | 966 | 11.0 | 35.8 | 4.5 | 12.0 |
| 5 | — | Basal + salmon 75 | 114 | 91 | — | 8.0 | 33.5 | 5.2 | 15.4 |
| 6 | 17.6 | Basal + salmon 40 | 83 | 80 | 992 | 8.0 | 39.6 | 5.0 | 23.4 |
| 7 | 16.8 | Basal + squash | 47 | 94 | 930 | 8.2 | 42.4 | 4.7 | 23.2 |
| 8 | 16.0 | Basal + squash | 38 | 80 | 886 | 6.5 | 33.0 | 4.2 | 17.2 |
| 9 | 15.2 | Basal + squash | 23 | 77 | 882 | 6.5 | 1.3 | 4.1 | 0 |
| 10 | 13.6 | Basal + squash | 17 | 79 | 774 | 7.3 | 19.9 | 4.2 | 10.5 |
| 11 | 12.9 | Basal + squash | 15 | 65 | 714 | 7.3 | 1.5 | 3.9 | 0 |
| Totals | | | 547 | | | | 395 | | 157 |

8.9 kg. = 41 per cent body weight *loss*—3.7 per cent per wk.

Table 1 (dog 43-400) shows the longest double depletion experiment (11 weeks), the largest total per cent weight loss (41 per cent or 3.7 per cent per week), and a production of 62 gm. blood protein per kilo weight loss. Rather more protein than usual was contained in the "protein-free" diet (periods 1 to 10) to improve food ingestion (salmon meat and squash) but the weekly protein intake was quite low. Death occurred 4 days after termination of experiment in spite of abundant food protein intake during the 4 days.

Autopsy showed an interstitial pneumonia which is all too frequent in this type of experiment and may cause death within 2 or 3 days. If this interstitial pneumonia had developed a week or so earlier in a less acute form, there would have been a considerable increase in urinary nitrogen in the week or so preceding death. It is easy to overlook this type of pneumonia as the lungs in gross are not conspicuously abnormal—in fact in this very case (Table 1—dog 43-400) the lungs were passed in gross as normal.

It is noted that the body proteins were raided up to the last week to support blood protein levels. There was steady weight loss and a weekly total blood protein removal or loss of about 50 gm. During the last 3 weeks the total blood protein production was about 40 gm. for the 3 weeks including some hemoglobin not removed.

*Experimental History*—Table 1.

Dog 43-400. Pointer female adult. Kept in laboratory kennels for 6 months under close observation and optimum dietary regime. Nov. 15, 1944—Blood protein depletion begun. Blood volume 1364 cc. Plasma protein 5.3 gm. per cent. Prolonged depletion experiment (Table 1).

Periods 1 to 4. Daily diet of *protein-free* basal biscuit 450 gm., yeast 3 gm., liver extract powder 2 gm., canned squash 200 gm., reduced iron 600 mg. Period 2. A/G ratio 1.5. Nov. 29—Periods 3 to 7, canned salmon added to diet to stimulate appetite, beginning with 75 gm., decreasing to 40 gm. Period 6. A/G ratio 0.80. Period 7. Canned salmon omitted from basal diet. Jan. 9, 1946—Choline chloride 300 mg. and 20 gm. dextrose added to diet. Total choline given is 700 mg. daily. Period 9. A/G ratio 0.65. Jan. 26—*Experiment is terminated* because of extreme weight loss. Daily diet of table scraps 1200 gm., pig liver 200 gm., yeast 5 gm. Food consumption 72 and 76 per cent for 2 days. A/G ratio 0.67. Jan. 28—Kennel diet 800 gm., pig liver 300 gm., salmon bread 300 gm., Klim 20 gm., yeast 5 gm. Food consumption 60 per cent. P.M. fresh milk 400 cc., canned salmon 100 gm., consumption 100 per cent. Jan. 30—found dead.

Autopsy showed the usual atrophy of tissue and organs associated with this severe weight loss. In general the organs show no pathology except the lungs which in histological sections show a widespread, diffuse, acute *interstitial pneumonia.* This is adequate cause of death. Lowered resistance due to protein depletion is surely a very important factor.

Table 2 shows two experiments on the same dog (41–53), a rest interval of 4 months intervening. These two experiments are technically excellent as the dog ate almost all of the basal diet (very low protein content). The only nitrogen in the diet was contained in the yeast and liver extracts. The *raiding of body protein* continued throughout every week up to the last weeks when about 40 gm. of blood protein were removed in each experiment. In the first experiment the weight loss was 4 per cent per week and in the second experiment, 5 per cent per week. This dog was over-at weight the beginning of each experiment. Production of blood protein was respectively 61 gm. per kilo weight loss and 48 gm. per kilo weight loss.

The removal of over 100 gm. blood protein in the first week in each experiment represents in part a mobile reserve including some circulating hemoglobin as indicated by a fall in the hemoglobin levels. This dog seems to favor plasma protein production

TABLE 2

*Prolonged Blood Protein Depletion*

Dog 41-53

| Period | Weight | Protein intake | | Food consumption | Plasma volume | Protein output | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Hemoglobin | | Plasma protein | |
| | | Type | Weekly | | | Level | Output per wk. | Level | Output per wk. |
| *1 wk.* | *kg.* | | *gm.* | *per cent* | *cc.* | *gm. per cent* | *gm.* | *gm. per cent* | *gm.* |
| 1 | 24.0 | Basal | 19 | 100 | 949 | 12.7 | 104.3 | 5.7 | 30.6 |
| 2 | 22.0 | Basal | 17 | 91 | 1115 | 10.8 | 47.8 | 5.2 | 23.4 |
| 3 | 21.0 | Basal | 15 | 89 | 1089 | 8.2 | 37.8 | 5.4 | 24.0 |
| 4 | 19.7 | Basal | 18 | 97 | 1035 | 7.2 | 20.5 | 5.0 | 16.1 |
| 5 | 18.6 | Basal | 16 | 94 | 964 | 6.3 | 24.3 | 5.5 | 19.7 |
| 6 | 18.0 | Basal | 19 | 100 | 1075 | 6.3 | 15.0 | 5.2 | 10.1 |
| 7 | 17.2 | Basal | 19 | 100 | 970 | 5.5 | 25.3 | 4.9 | 15.5 |
| Totals.................. | | | 123 | | | | 275 | | 139 |
| 6.8 kg. = 28 per cent body weight *loss*—4 per cent per wk. | | | | | | | | | |
| 1 | 27.5 | Basal | 17 | 92 | 1222 | 10.1 | 106.0 | 5.5 | 31.0 |
| 2 | 24.0 | Basal | 14 | 72 | 1141 | 9.1 | 61.6 | 5.3 | 29.1 |
| 3 | 22.4 | Basal | 15 | 79 | 1179 | 6.4 | 45.6 | 5.4 | 28.5 |
| 4 | 21.0 | Basal | 29 | 100 | 1235 | 5.9 | 9.2 | 5.4 | 7.5 |
| 5 | 20.3 | Basal | 29 | 100 | 1260 | 7.3 | 22.6 | 5.5 | 15.7 |
| 6 | 19.3 | Basal | 23 | 90 | 1100 | 5.9 | 24.0 | 5.0 | 15.6 |
| Totals.................. | | | 127 | | | | 269 | | 127 |
| 8.2 kg. = 30 per cent body weight *loss*—5 per cent per wk. | | | | | | | | | |

and maintains a 5 per cent plasma protein level while the hemoglobin levels are reduced to 5 to 6 gm. per cent. This in contrast to Tables 1 and 3 where the depletion is limited by plasma protein levels (4 per cent) while hemoglobin levels are 7 to 10 gm. per cent. It did not appear safe to carry these experiments further because of about 30 per cent weight loss.

*Experimental History*—Table 2.

Dog 41-53. Coach male adult. Born January 1940. Continuous anemia history Feb. 22, 1943, to June 1, 1943. Plasma protein depletion begun. Daily diet of protein-free basal biscuit 450 gm., yeast 3 gm., liver extract powder 2 gm., reduced iron 600 mg. Blood volume 1190 cc., plasma volume 916 cc., weight 15.1 kilos. Plasma protein 5.1 gm. per cent. Regular double depletion experiments with interspersed recovery periods to Aug. 9, 1944.

Aug. 9—Prolonged depletion experiment, Table 2. Daily diet of protein-free basal biscuit 450 gm., yeast 3 gm., liver extract powder 2 gm., reduced iron 600 mg. Blood volume 1936 cc. Plasma protein 6.5 gm. per cent. Period 3. A/G ratio 1.6. Period 5. A/G ratio 1.6. Period 7. A/G ratio 2.2. Uneventful depletion history to Sept. 20, 1944. Dog in good condition other than weight loss. Regular double depletion experiments continued to Nov. 9, 1944. Recovery period to Mar. 15, 1945. Blood protein depletion begun. Beginning blood volume 2540 cc., plasma volume 1222 cc., plasma protein 5.8 gm. per cent. A/G ratio 2.0.

Second depletion experiment—Table 2.

Daily diet of protein-free basal biscuit 500 gm., yeast 3 gm., liver extract powder 2 gm., reduced iron 600 mg. Mar. 29—Period 3, biscuit reduced to 400 gm. daily, dextrose 40 gm. added to diet. A/G ratio 1.1. Period 4. A/G ratio 1.0. Period 5. A/G ratio 0.99. Apr. 20—Depletion experiment ends. A/G ratio 0.86. Dog in good condition other than weight loss.

TABLE 3

*Prolonged Blood Protein Depletion*

Dog 40-33

| Period | Weight | Protein intake | | Food consumption | Plasma volume | Protein output | | | | Total nitrogen weekly | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Hemoglobin | | Plasma protein | | | |
| | | Type | Weekly | | | Level | Output per wk. | Level | Output per wk. | Intake | Urinary output |
| *1 wk.* | *kg.* | | *gm.* | *per cent* | *cc.* | *gm. per cent* | *gm.* | *gm. per cent* | *gm.* | *gm.* | *gm.* |
| 1 | 23.8 | Fast | 0 | — | 1002 | 14.8 | 98.1 | 5.1 | 31.3 | — | 17.5 |
| 2 | 23.4 | Basal | 19 | 100 | 1199 | 10.0 | 43.4 | 4.4 | 18.1 | 3 | 14.3 |
| 3 | 23.3 | Basal | 19 | 100 | 1209 | 11.9 | 20.4 | 4.3 | 6.6 | 3 | 11.2 |
| 4 | 22.8 | Basal | 19 | 100 | 1140 | 11.5 | 42.9 | 4.3 | 13.8 | 3 | 9.2 |
| 5 | 22.7 | Basal | 18 | 96 | 1147 | 10.4 | 18.0 | 4.0 | 5.8 | 2.9 | 8.3 |
| 6 | 21.5 | Basal | 19 | 100 | 1116 | 9.2 | 16.2 | 4.1 | 6.1 | 3 | 7.5 |
| 7 | 21.4 | Basal | 14 | 75 | 1110 | 10.4 | 14.2 | 4.5 | 5.4 | 2.2 | 7.4 |
| 8 | 20.5 | Basal | 13 | 71 | 1065 | 10.9 | 23.6 | 4.2 | 7.4 | 2.1 | 8.9 |
| 9 | 19.5 | Basal | 12 | 68 | 1000 | 10.9 | 2.2 | 4.1 | 0 | 1.9 | 6.5 |
| Totals............. | | | 133 | | | | 335 | | 95 | 21.1 | 90.8 |

4.3 kg. — 18 per cent body weight *loss*—2 per cent per wk.

Table 3 (dog 40-33) is a very satisfactory experiment in which the weight loss is low (2 per cent per week) and the output of blood protein per kilo weight loss is 100 gm. This dog was much overweight at the start of the experiment. The raiding of body protein goes on through the 8th week but there is no evidence of production of blood proteins in the 9th week. Food consumption is excellent and the amount of "protein" in the basal diet is minimal. The total urinary nitrogen figures are significant and for a dog of this weight show maximal conservation of all nitrogenous elements. Slight differences from week to week are explained by lack of catheterization to terminate each 7 day period. The danger of infection in these dogs is adequate reason for this omission.

*Experimental History*—Table 3.

Dog 40-33. Bull, male adult. Born August, 1940. Continuous anemia history Oct. 27, 1942, to June 2, 1943. Regular anemia experiments. June 2–Plasma protein depletion begun. Blood volume 1285 cc., plasma volume 966 cc., weight 16.9 kilos. Daily diet of protein-free basal biscuit 450 gm., yeast 3 gm., liver extract powder 2 gm., reduced iron 600 mg. July 1, 1943, to Aug. 3, 1945—Regular double depletion experiments with interspersed recovery periods.

Aug. 3—Long depletion experiment (Table 3).

Period 1—Fast 1 week, blood protein depletion begun. Blood volume 1769 cc. Plasma protein 5.1 gm. per cent. Period 2. Daily diet of protein-free basal biscuit 400 gm., yeast 3 gm., liver extract powder 2 gm. Period 3. A/G ratio 0.77. Period 5. A/G ratio 0.90 Slight edema around eyes. Plasma by vein (133 cc.—7.7 gm.) because of low plasma protein level (3.95 gm. per cent) and high hemoglobin level (10.4 gm. per cent). No unfavorable reaction. Sept. 16—Edema disappeared. Periods 6 to 9. A/G ratios 1.0, 0.94, 0.88. Oct. 8—Period 9, edema of both front feet. Several skin lesions on leg joints. A/G ratio 0.78. Oct. 9—Left all food. Edema more general. Slight bleeding of foot pads. Various small skin abrasions on feet. Experiment terminated. Daily mixed diet with gradual recovery.

TABLE 4

*Prolonged Blood Protein Depletion*

Dog 42-1380

| Period | Weight | Protein intake | | Food consumption | Plasma volume | Protein output | | | | Total nitrogen weekly | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Hemoglobin | | Plasma protein | | | |
| | | Type | Weekly | | | Level | Output per wk. | Level | Output per wk. | Intake | Urinary output |
| *1 wk.* | *kg.* | | *gm.* | *per cent* | *cc.* | *gm. per cent* | *gm.* | *gm. per cent* | *gm.* | *gm.* | *gm.* |
| 1 | 15.7 | Basal | 90 | 100 | 641 | 14.6 | 104.3 | 4.9 | 21.0 | 12.8 | 14.1 |
| 2 | 15.9 | Basal | 76 | 94 | 766 | 11.9 | 51.9 | 4.7 | 7.5 | 12.1 | 13.3 |
| 3 | 15.2 | Basal | 52 | 94 | 733 | 9.7 | 44.1 | 4.3 | 15.4 | 8.3 | 12.7 |
| 4 | 14.7 | Basal | 14 | 71 | 752 | 9.2 | 15.2 | 4.3 | 5.7 | 2.1 | 8.2 |
| 5 | 14.6 | Basal | 15 | 75 | 756 | 7.6 | 27.6 | 4.4 | 11.5 | 2.2 | 5.8 |
| 6 | 14.0 | Basal | 13 | 70 | 744 | 7.6 | 1.5 | 4.3 | 0 | 2.1 | 3.9 |
| 7 | 13.9 | Basal | 12 | 66 | 711 | 9.3 | 2.0 | 4.0 | 0 | 2.0 | 5.2 |
| 8 | 13.7 | Basal | 13 | 70 | 682 | 10.2 | 2.0 | 3.9 | 0 | 2.1 | 6.5 |
| Totals.................. | | | 285 | | | | 249 | | 61 | 43.7 | 29.7 |
| 2.0 kg. = 13 per cent body weight *loss*—1.6 per cent per wk. | | | | | | | | | | | |
| 9 | 13.7 | Egg + basal | 214 | 83 | 674 | 8.0 | 39.8 | 4.6 | 18.9 | 34.2 | — |
| 10 | 12.5 | Egg + basal | 90 | 35 | 664 | 8.0 | 1.7 | 5.0 | 0 | 14.4 | — |

Table 4 presents a dog (42-1380) which is very resistant to the "double depletion" especially in the last three periods. The total weight loss is only 2 kilos in 8 weeks—a 13 per cent loss, or 1.6 per cent per week. The production of blood proteins per kilo weight loss is 155 gm. For the first 5 weeks the picture of blood protein production is about as usual but the weight loss is minimal. In the last 3 weeks there is no production of plasma protein and only a modest increase in hemoglobin levels from 7.6 gm. to 10.2 gm.—perhaps an increase in hemoglobin of about 29 gm. This dog was inert and obviously in a state which might soon lead to death. It was decided not to risk a longer depletion. It is interesting to note that this dog produced 155 gm. blood proteins per kilo weight loss— not far from the 21 per cent protein as found in normal liver and skeletal muscle. This dog was not overweight at the start of this experiment (Table 4). The urinary nitrogen approached an absolute minimum during periods 5 to 8. An egg protein supplement in periods 9 and 10 caused a prompt rise in blood proteins with removal of a considerable surplus (60 gm.).

*Experimental History*—Table 4.

Dog 42-1380. Tan and white hound, adult male. Kept in laboratory kennels for over 1 year under close observation. Routine plasmapheresis experiments followed by recovery period of 6 months.

Oct. 17, 1945—Blood volume 1575 cc., plasma protein 5.3 gm. per cent, A/G ratio 2.0. Period 1. Daily diet of protein-free basal biscuit 400 gm., salmon bread 75 gm. decreasing to 40 gm. Periods 2 and 3. Yeast 3 gm., liver extract powder 2 gm. Nov. 9—Period 4, all salmon bread omitted from daily diet. Synthetic vitamin mixture 5 cc. sugar mixture 100 gm. (see Methods, Paper I) added to yeast and liver extract powder. A/G ratio 1.7. Period 6. Sugar mixture decreased to 60 gm. Period 7. Sugar mixture decreased to 50 gm. A/G ratio 1.0. Period 8. A/G ratio 0.96. Periods 9 and 10. Daily diet of protein-free basal biscuit 350 gm., whole eggs 250 gm., yeast 3 gm., liver extract powder 2 gm. Biotin 5 mg. added to diet. A/G ratios 1.0 and 0.90. Dec. 19—Food consumption dropping. Dog appears very apathetic. No skin lesions. Dec. 20—Experiment terminated. Recovery on liberal protein diet.

Table 5 (dog 40-34) in a sense is a control experiment with no bleeding except samples during the 13 weeks. Weight loss amounts to 8.3 kilos or 34 per cent of weight, or 2.6 per cent per week. The loss of circulating proteins is considerable (278 gm.) about 190 gm. hemoglobin plus 23 gm. plasma protein plus 65 gm. samples which represents the calculated blood protein loss, 190 plus 23, as the hemoglobin dropped from 20 gm. per cent to 13.8 gm. per cent and the plasma protein from 6.5 to 5.2 per cent. Shrinkage of blood volume came into the calculation—a drop from 2168 cc. to 1396 cc. During this 91 day low protein diet period the great bulk of the red cells became obsolescent (life of red blood cells = 100 to 130 days) and must have been replaced. The urinary nitrogen fell to very low levels and remained minimal. The A/G ratio showed a tendency to fall below 1.0 as is usual in such experiments.

*Experimental History*—Table 5.

Dog 40-34. Male bull adult. Born August, 1940. Continuous anemia history Apr. 20, 1942, to Feb. 9, 1943. Regular anemia experiments. Beginning weight 23.6 kilos. Blood volume 2170 cc., plasma volume 1014 cc. Feb. 9, 1943—Daily diet of protein-free basal biscuit 450 gm., yeast 3 gm., liver extract powder 2 gm., reduced iron 400 mg. Plasma protein depletion begun. Regular double depletion experiments with interspersed recovery periods, to Sept. 7, 1945. Blood volume 2168 cc., plasma volume 1002 cc. Plasma protein 6.5 gm. per cent.

Sept. 7—Protein depletion experiment by diet only (Table 5).

TABLE 5

*Low Protein Intake—No Bleeding*

Dog 40-34

| Period | Weight | Protein intake | | Food consumption | Plasma volume | Protein levels | | Total nitrogen weekly | |
|---|---|---|---|---|---|---|---|---|---|
| | | Type | Weekly | | | Hemoglobin | Plasma protein | Intake | Urinary output |
| *1 wk.* | *kg.* | | *gm.* | *per cent* | *cc.* | *gm. per cent* | *gm. per cent* | *gm.* | *gm.* |
| | 24.6 | | | | 1002 | 20.0 | 6.5 | | |
| 1 | 23.5 | Fast | 0 | — | 910 | 20.0 | 6.1 | 0 | 27.7 |
| 2 | 23.0 | Basal | 18 | 97 | 966 | 18.6 | 6.6 | 2.9 | 20.9 |
| 3 | 22.4 | Basal | 15 | 85 | 1006 | 17.1 | 5.5 | 2.4 | 13.0 |
| 4 | 21.7 | Basal | 17 | 93 | 1044 | 15.6 | 5.3 | 2.7 | 12.3 |
| 5 | 21.9 | Basal | 16 | 86 | 1044 | 15.1 | 5.4 | 2.6 | 12.4 |
| 6 | 20.8 | Basal | 15 | 85 | 966 | 14.8 | 5.3 | 2.4 | 11.8 |
| 7 | 20.1 | Basal | 16 | 86 | 957 | 14.7 | 5.3 | 2.6 | — |
| 8 | 19.9 | Basal | 11 | 100 | 932 | 14.5 | 5.2 | 1.8 | 10.1 |
| 9 | 19.2 | Basal | 10 | 93 | 923 | 13.0 | 4.7 | 1.6 | 9.1 |
| 10 | 18.1 | Basal | 9 | 57 | 832 | 14.5 | 5.0 | 1.4 | 8.3 |
| 11 | 17.8 | Basal | 10 | 51 | 838 | 14.5 | 5.1 | 1.6 | 7.5 |
| 12 | 17.2 | Basal | 11 | 54 | 836 | 13.7 | 5.0 | 1.6 | 10.6 |
| 13 | 16.3 | Basal | 11 | 56 | 806 | 13.8 | 5.2 | 1.6 | 7.0 |
| Totals.................... | | | 159 | | | 190* | 23* | 25 | 151 |
| 8.3 kg. = 34 per cent body weight *loss*—2.6 per cent per wk. | | | | | | | | | |
| 14 | 16.9 | Egg, basal | 152 | 72 | 842 | 12.2 | 5.4 | 24.3 | 8.66 |
| 15 | 16.8 | Egg, basal | 162 | 90 | 800 | 12.8 | 6.2 | — | — |

* Represents loss of circulating protein—see text.

Period 1. Fast for 1 week. Period 2. Daily diet of protein-free basal biscuit 450 gm., yeast 3 gm., liver extract powder 2 gm. A/G ratio 1.2. Period 5. Daily diet of protein-free basal biscuit 300 gm., sugar mixture 75 to 100 gm. (See Methods, Paper I), synthetic vitamin mixture 10 cc. A/G ratio 1.2. Nov. 23—Period 11, sugar mixture decreased to 50 gm. Period 12. Dextrose 30 gm. replaces sugar mixture in diet. A/G ratio 0.87. Dec. 7. Period 14, protein depletion experiment terminated. A/G ratio 0.94. Daily diet of protein-free basal biscuit 350 gm., whole eggs 200 gm., yeast 3 gm., liver extract powder 2 gm. Period 15. Basal biscuit decreased to 250 gm. A/G ratio 0.59. Dec. 21. Diet of table scraps and recovery period. Dog in good condition other than weight loss. A/G ratio 0.72. Rapid recovery.

## DISCUSSION

*Premortal rise in urinary nitrogen* in long term fasting experiments is accepted in textbooks (1) and frequently mentioned in the literature. It is recorded as due to "greater use of body protein for energy." When the arguments for the use of the term "premortal rise" are reviewed they are none too convincing. A good review is given by Howe and Hawk (5) but some of the best long term fasting experiments (117 days' fast in a dog with recovery—Howe, Mattill, and Hawk (6)) show no evidence whatever of anything like a "premortal rise." Evidently it is not uniformly observed.

Our experiments tabulated above are not pure fasting experiments but the drain on body protein is very severe and one might expect a premortal rise in urinary nitrogen toward the end of these experiments on doubly depleted dogs on a very low protein diet. If there is any tendency for the body to use and break down protein for energy or other purposes these experiments should favor such a state. It is clear that the body protein is used without any wastage or increase of urinary nitrogen as would be noted if the body protein in excess of normal was broken down before transfer and use. The body protein contributes to the circulating plasma protein and hemoglobin pool without any extra loss of urinary nitrogen—a type of "dynamic equilibrium" which we have investigated under other conditions.

We venture to suggest a terminal infection as a possible explanation of some of the observations associated with a "premortal rise" of urinary nitrogen at the end of long depletion experiments. These animals depleted by low protein diet or otherwise show depleted reserves of plasma proteins and are surely wide open to a variety of infections. Given an infection the amount of excess urinary nitrogen would be determined by the severity of the infection and modified by the magnitude of the protein reserve stores. Depleted stores mean decreased output of urinary nitrogen related to any injury as compared with the normal dog with normal reserve stored (9, 2, 8). In spite of severe depletion of stores a pneumonia would cause significant increase in urinary nitrogen.

Infection in such a dog may sweep through the system as a septicemia or appear as an interstitial pneumonia, and at autopsy could readily be overlooked (Table 1). We suggest that to exclude this possibility and establish a "premortal rise of urinary nitrogen," careful autopsy examination, culture of organs, and study of histological sections are essential.

## SUMMARY

Dogs with sustained anemia and hypoproteinemia due to bleeding and a continuing low protein or protein-free diet with abundant iron will continue to produce much new hemoglobin and plasma protein for many weeks.

The stimulus of double depletion (anemia and hypoproteinemia) leads to raiding of body and tissue protein to fill the demand for new hemoglobin and plasma protein. The blood proteins in these experiments take priority over

the organ and tissue proteins. This is another illustration of the "ebb and flow" or dynamic equilibrium between organ or tissue protein and blood proteins.

The average dog cannot tolerate this drain of double depletion for more than 7 to 11 weeks and during this time may lose 30 to 40 per cent of body weight. Some dogs are much more resistant to this raiding than others. Some dogs show a high blood protein output during every week up to the danger point. With the largest blood protein output one usually observes the most rapid weight loss.

For every kilogram of weight loss we observe 50 to 140 gm. blood protein output. The weekly blood protein production ranges from 40 to 66 gm.

These experiments make heavy demands on the body protein and we expected to record a "premortal rise" in urinary nitrogen. No such observations are noted, rather a most frugal use of all protein and minimum figures for urinary nitrogen.

We suspect that "premortal rise" in many experiments means a terminal infection with the related catabolism of tissue protein and high urinary nitrogen.

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