# DIETARY PROTEINS

# Their Function in Health and Disease

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An imbalance in protein stores results from insufficient or poor quality dietary protein, reduced caloric intake, or malnourishment from disease. This imbalance is characterized by a shift in the distribution of blood and tissue proteins; some are greatly reduced, others are even increased. The pattern of enzyme systems is altered markedly in the liver, less so in some other tissues. A shift in metabolism, involving all nutrients, is associated with loss of tissue proteins. Malnourishment can lead to an increase in anabolic processes, so that nitrogen retention and tissue regeneration are more efficient in the depleted than in the normal animal. The protein stores can be regenerated or maintained by feeding the proper mixture of amino acids either orally or intravenously. Regeneration to a normal balance is most rapid in the presence of a pattern of amino acids such as is represented by whole egg proteins. Nitrogen balance is an integration of gains and losses from different tissues and is a function of many variables such as the dietary protein, protein stores, and endocrine balance.

A CONTINUOUS TURNOVER of nitrogen takes place in the body of an animal, a turnover that is part of ntegrated catabolic and anabolic processes (2, 17, 22, 26). The catabolic processes supply energy and some intermediary compounds essential for anabolism, one set of processes being correlated with the other. Depletion in tissue nitrogen occurs when tissue catabolism exceeds anabolism. The function of dietary proteins is to supply the nitrogen and essential amino acids (20) in proper amounts and kinds to meet the demands of anabolism.

## Catabolism vs. Anabolism

The compounds of nitrogen, the end products of catabolism, are illustrated in Figure 1 (6). These data were obtained while feeding a protein-free diet to dogs, first during a control period, then during a period when the tissue proteins were in a depleted state, and finally for a short time after the tissue proteins had been repleted.

These nitrogen compounds were all derived from catabolism of constituents of the tissues, a basic catabolism which may be called endogenous (13). The excretion of urea nitrogen decreases and increases with the magnitude of the so-called labile protein stores of the animal. The minimum excretion of urea nitrogen is reached, however, when only a portion of the protein reserves of the animal have been depleted. This reduction in excretion could be the result, in part, of fluid shifts sweeping excess urea from the body, but it does represent the minimum tissue catabolism of the depleted state. The excretion of creatinine, being mostly independent of the magnitude of the protein stores, may represent the end product of a more conservative mechanism of endogenous metabolism.

The dietary proteins must supply the essential amino acids to meet the demands of this basic catabolism, the demands of anabolic processes associated with maintenance and growth. The

white bar over $A$ in Figure 2 records the excretion of urinary nitrogen in dogs fed a protein-free diet. This excretion is one measure of the magnitude of the basic tissue catabolism in these animals. The bar with slanted lines over $A$ illustrates data obtained in these same dogs fed sufficient egg white protein to maintain nitrogen equilibrium. Under such conditions the nitrogen intake equals the nitrogen excretion and the animal neither loses nor gains nitrogen. The data over $A$ can be interpreted to mean that the feeding of egg white protein did not alter the basic endogenous catabolism, possibly even reducing it. Reduction in excretion of urinary nitrogen has been reported during the feeding of egg white and various proteins supplemented with methionine to rats and to dogs (4, 23). In fact, the feeding of methionine in a protein-free diet will reduce the excretion of urea nitrogen in these animals, a reduction which illustrates the marked effect this amino acid has upon tissue metabolism.
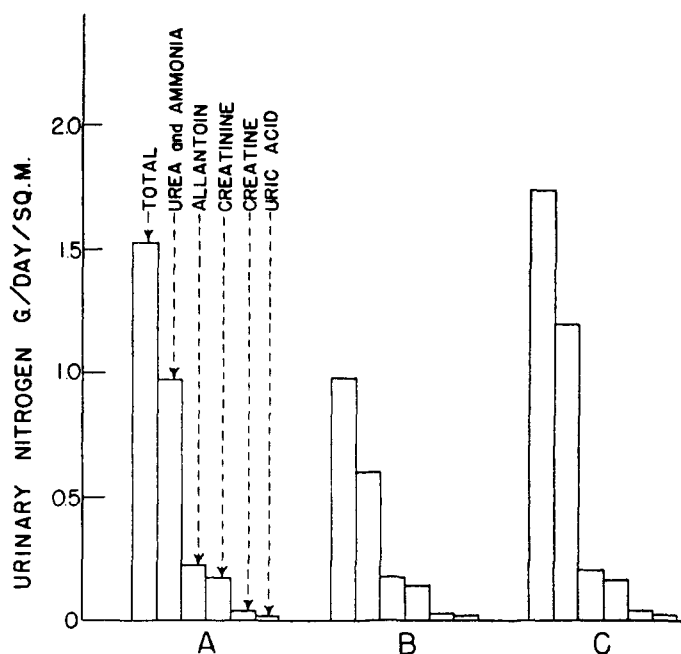
Figure 1. Average excretion of urinary nitrogen in two dogs fed protein-free diets

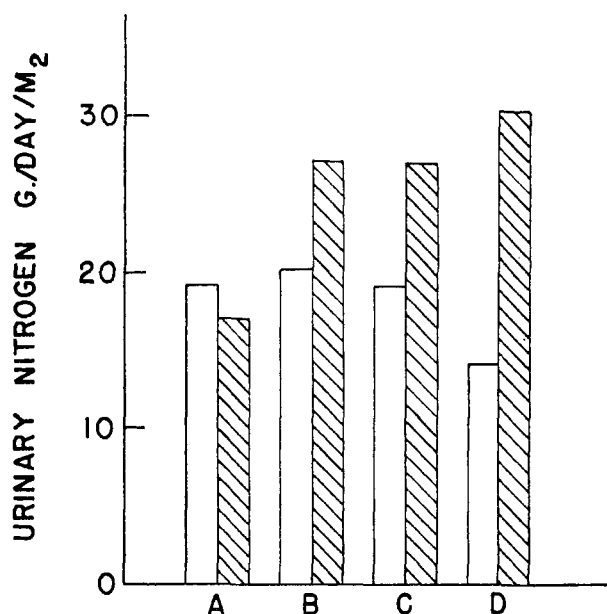A. Control. B. Protein-depleted state. C. Repleted state. Data from (6)



Figure 2. Excretion of urinary nitrogen in dogs

White bars. Average excretion in 6 dogs fed protein-free diet. Slanted lines. Excretion of dogs fed sufficient dietary nitrogen to maintain nitrogen equilibrium. A, egg white; B, dehydrated fat-extracted beef protein; C, casein; D, wheat gluten

Dietary nitrogen can, therefore, reduce the excretion of urea nitrogen possibly through a reduction in the so-called endogenous tissue catabolism. The data over A could also be interpreted to mean that all of the amino acids supplied by egg white were utilized for anabolic purposes.

B, C, and D in Figure 2 represent data obtained while first a protein-free diet (white bar) and then a protein (bar with slanted lines) were fed, the proteins being dehydrated beef, casein, and wheat gluten, respectively. Again, sufficient of each of the proteins was fed to maintain approximate nitrogen equilibrium. The increase in excretion of urinary nitrogen while protein was fed may be said to be the result of exogenous catabolism of amino acids which could not be utilized for anabolic purposes. Thus only a portion of the pattern of amino acids in these proteins is available for synthesis, smaller in wheat gluten than in casein or dehydrated beef.

It is possible to design a diet to limit the excretion of nitrogenous wastes to the minimum required by the basic catabolism of the body. Protein sources such as eggs, mixtures of casein and lactalbumin, mixtures of cereal proteins, and milk proteins can be selected, which will keep catabolism to this basic minimum. The dietary nitrogen intake can be limited to the amount needed to meet the demands of anabolism, so that an excess does not increase urinary nitrogen. Such a diet would be of interest, for example, in problems involving excretion (kidney damage) or in situations where the demands for water must be limited. The obligatory demands of the body for water, for example, increase with the rise in excretion of nitrogenous wastes.

## Excess Tissue Catabolism

Nutritional errors, such as a low caloric intake, deficiency in dietary nitrogen, lack of certain vitamins, or starvation will increase loss of tissue nitrogen. A similar loss may be associated with illness. The concept of a dynamic state of proteins, of an "ebb and flow" between various tissues (26), is consistent with the finding that the several compartments of the body lose or gain nitrogen at different rates. Total circulating plasma albumin and $\gamma$-globulins, for example, decrease rapidly while the $\alpha$-globulins remain unchanged or may even increase in animals fed a low nitrogen diet (9). Blood proteins can take priority over tissue proteins as an animal is depleted (27), while cytoplasmic proteins of the gut and liver are catabolized more rapidly than those of other tissues of the body (1, 12, 14).

The differential loss of tissue proteins together with shifts in water balance is illustrated by the data recorded in Table I.

These data illustrate the decrease in plasma proteins and plasma volume associated with depletion; repletion restores the balance between the two. Repletion with casein hydrolyzate caused an unusual rise in plasma globulin. Chow (8) has presented data to show that casein hydrolyzate stimulates production of both plasma albumin and globulin in depleted dogs, whereas lactalbumin favors regeneration of albumin. He suggests the presence of a "directive substance" in casein which influences anabolic processes. Thus the type of protein fed affects the characteristics of the shift from the imbalance of depletion toward a new balance of repletion and growth.

An increase in extracellular fluid is

## Table I. Average Data on Effects of Depletion and Repletion on Plasma Proteins

Six dogs used for repletion with lactalbumin hydrolyzate and five dogs for repletion with casein hydrolyzate. Each dog received 0.35 gram of hydrolyzate nitrogen per day per kg. of body weight for 30 days.

| | Plasma Protein, G., % | Plasma Volume, Ml. | Total[a] Circulating Albumin, G. | Total[a] Circulating Globulin, G. |
|---|---|---|---|---|
| | | Lactalbumin Hydrolyzate | | |
| Control | 6.45 | 459 | 17.9 | 11.5 |
| Depleted | 4.53 | 405 | 6.8 | 11.2 |
| Repleted | 5.92 | 499 | 18.1 | 11.3 |
| | | Casein Hydrolyzate | | |
| Control | 6.14 | 495 | 17.9 | 12.5 |
| Depleted | 4.39 | 456 | 7.8 | 12.2 |
| Repleted | 6.06 | 566 | 18.6 | 16.0 |

[a] Determined by salt fractionation method.

## Table II. Data Obtained from Control and Protein-Depleted Rats

| | Body Wt., G. | Organ Wt., G. | Organ/ Body Wt. | Water, % | Protein, % Dry Wt. |
|---|---|---|---|---|---|
| | | | Liver | | |
| Control | 462 | 14.54 | 3.15 | 71.8 | 58.4 |
| Depleted | 202 | 11.36 | 5.26 | 65.4 | 31.7 |
| | | | Heart | | |
| Control | 462 | 1.26 | 0.27 | 81.1 | 70.1 |
| Depleted | 202 | 0.64 | 0.39 | 78.5 | 71.3 |

associated with the loss in water-holding capacity of plasma. A nutritional edema of this type, together with its correction by feeding casein hydrolyzate, is illustrated in Figure 3 (6). The rise in extracellular fluid is probably associated with loss of water from all tissues of the body. This does not mean, however, that the percentage of water in these tissues is reduced. Even the percentage of water and protein in the blood may remain within normal ranges in depleted animals. Usually, however, there is a reduction in percentage of protein in the plasma (hypoproteinemia) and in a tissue like the liver, but not necessarily in the heart (see Table II).

Total heart water and protein are reduced, however, because of the reduction in size of this organ, although the loss in weight for the heart is not as great as for the body as a whole. Some of the protein space of the liver may be considered to be displaced by fat, since fatty livers are produced in depleted animals which are fed a relatively high caloric intake.

A reduction in plasma albumin and γ-globulin without a significant change in other globulin fractions in the depleted animal has been described (9). This imbalance is characterized still further by other changes in nitrogenous constituents. Macek (15), for example, reported a decrease in dialyzable plasma peptides associated with a reduction in dietary protein intake. On the other hand, as the protein stores of the animal were reduced, there was a progressive increase in concentration of plasma peptides soluble in picric acid. Similarly, Mesier (16) found an increase in polypeptides soluble in sulfosalicylic acid, this polypeptide fraction contributing to a high polarographic activity of the plasma. All these changes were reversed by repletion. Crossley et al. (11) reported an increase in the sulfosalicylic acid–soluble fraction and in polarographic activity of plasma in animals suffering debilitating diseases. These data can be interpreted to mean that excessive tissue catabolism is identified with a rise in polypeptide fragments of the plasma proteins.

Studies similar to those made on blood have been started on the soluble liver proteins. Mesier (16) has shown that the total soluble proteins decrease upon depletion in body nitrogen. Most, if not all of the proteins of the liver are parts of enzyme systems. The literature is filled with records of reduced activity of enzyme systems in the protein-depleted

liver. Recently Wainio et al. (25) in these laboratories have studied the effect of protein depletion on a group of oxidative enzyme systems, a study which emphasizes the imbalance associated with loss of body nitrogen. They demonstrated that depletion does not reduce the activity of cytochrome oxidase per unit of liver nitrogen, possibly even increasing it, but does reduce the unit activities of other oxidases in the following order: xanthine oxidase > diphosphopyridine nucleotide cytochrome C reductase > uridase = succinic dehydrogenase > succinic oxidase > d-amino acid oxidase. Preliminary unpublished data (24) on the heart indicate that the unit activities of cytochrome oxidase, diphosphopyridine nucleotide cytochrome C reductase, and succinic dehydrogenase are not altered by depletion. Thus the heart compartment is not depleted in the same way as the liver compartment, differences which may reflect the special roles that each of these organs plays in the body of the animal.

Other examples of the effects of catabolism on tissue proteins could be given. Sufficient have been presented, however, to illustrate the development of an imbalance in tissue proteins as compared to normal with a probable reduction in the over-all basic catabolic activity. This depleted tissue, however, is poised for regeneration. Given a complete diet, nitrogen retention is superior in the depleted animal (5); the depleted system appears to be adapted toward regenerative processes. Under such conditions of an increased potential for growth in tissue nitrogen, the uptake and retention of $S^{35}$ DL-methionine are greater than normal (see Table III).

### Figure 3. Effect of depletion on extracellular fluid

Black bars, circulating plasma proteins. Slanted lines, plasma volume. White bars, extracellular fluid. A. Control period. B. Period of protein depletion. C. Period following repletion with casein hydrolyzate. Data from (6)
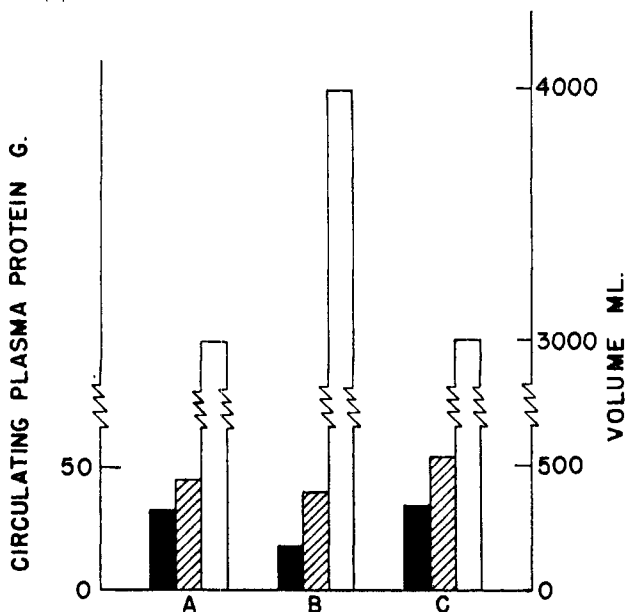
### Figure 4. Nitrogen balance

Over period of 30 days of feeding egg proteins (O) and wheat gluten (X) to different dogs which at beginning of feeding period were depleted in tissue proteins. Data from (5)
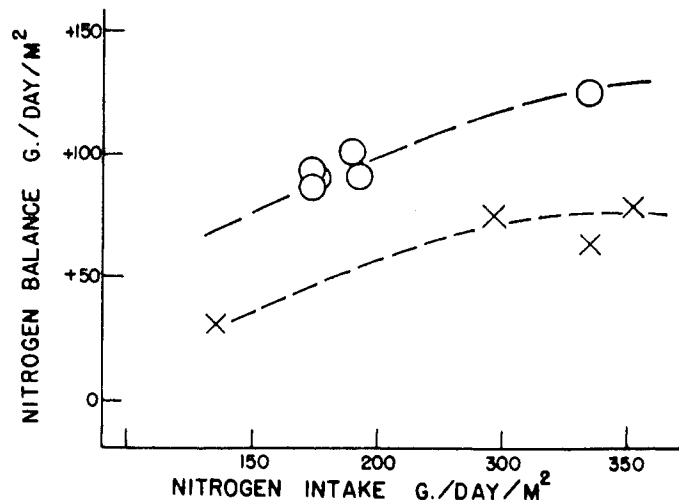
## Table III. Uptake and Retention of DL-Methionine

| | Liver | | | Heart | | | Kidney | | | Testis | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Av. | Min. | Max. | Av. | Min. | Max. | Av. | Min. | Max. | Av. | Min. | Max. |
| | Micrograms of Sulfur per Gram of Tissue | | | | | | | | | | | |
| Control | 16 | 14 | 18 | 7 | 6 | 8 | 16 | 13 | 17 | 4 | 3 | 4 |
| Protein-free | 37 | 34 | 39 | 13 | 12 | 15 | 29 | 26 | 32 | 12 | 10 | 15 |
| | Micrograms of Sulfur per Gram of Protein | | | | | | | | | | | |
| Control | 99 | 82 | 108 | 53 | | | | | | | | |
| Protein-free | 346 | 311 | 376 | 83 | | | | | | | | |

## Dietary Protein and Regeneration

Emphasis has been placed upon the anabolic function of dietary protein. The pattern of amino acids fed must, therefore, meet the various needs of the anabolic processes of the depleted compartments of the body (7). Data have been presented to demonstrate that the maximum rate of filling of these stores may vary with the source of dietary protein. Egg protein, for example, will produce a much greater positive nitrogen retention in a depleted animal than wheat gluten, no matter how much wheat gluten is fed (see Figure 4). Thus there is a limit to the anabolic function of each dietary protein. Increasing intake above that limit contributes to exogenous catabolism, increasing the excretion of waste products of nitrogen. The different optimum concentrations for regeneration illustrated in Figure 4 for egg proteins and wheat gluten may mean that the mechanisms of filling the tissue compartments differ between these two dietary proteins. Allison et al. (5) presented data which suggested that wheat gluten fed to depleted dogs resulted in a larger proportional rise in plasma proteins than when egg proteins were fed.

Because the protein stores of the various compartments are filled within a shifting dynamic state, it is not surprising to find that different dietary proteins fill some tissue stores more rapidly than others (4, 5). One of the most interesting examples of the effect of dietary protein upon regeneration has been presented by Robscheit-Robbins and Whipple (18, 19). They studied regeneration of blood proteins in dogs and found that it is possible to "control the blood protein output so that more plasma protein would be produced on one diet and more hemoglobin with another." This possibility of controlling the rate of filling of different compartments by diet deserves further study, especially in the development of special diets for regeneration.

Retention of nitrogen is also a function of caloric intake. Rosenthal and Allison (21) emphasized recently that there is an optimum caloric intake for maximum anabolism, and that even a slight reduction below this optimum increases tissue catabolism. They demonstrated, however, marked retention of nitrogen in a depleted dog at very low caloric intakes, even though continued feeding at these low caloric intakes did eventually lead to excessive tissue destruction. Allison et al. (3) emphasized also that nitrogen is retained in a normal dog at low caloric intakes and low nitrogen intakes, so that the rate of loss of nitrogen is reduced even though nitrogen balance cannot be attained. Recently Cox et al. (10) have shown retention of nitrogen in the rat at low caloric intakes, and consequently marked slowing of the rate of loss of body nitrogen measured in terms of body weight and tissue analysis. Thus it is possible, even with restricted caloric intakes, to slow up the process of depletion through feeding a limited intake of dietary protein.

## Summary

A basic catabolism, the end products of which are excreted as urinary nitrogen, is fundamental to protein metabolism: a catabolism which is low in the protein-depleted animal where tissue nitrogen is in most need of conservation. The excretion of urea nitrogen is correlated with the catabolism of labile tissue proteins as well as with dietary nitrogen not used for anabolism. Dietary protein must supply the essential amino acids to meet the demands of basic catabolism, the demands of anabolic processes associated with maintenance and growth.

A shift of water from tissues to extracellular spaces accompanies loss of protein, some tissues losing more water and protein than others. An imbalance of tissue proteins and of enzymatic activities is produced, more marked in some tissues than in others.

Retention of dietary nitrogen is maximum in depleted tissues, as are the uptake and retention of $S^{35}$ DL-methionine. In the construction of the best diet for regeneration, emphasis is placed upon the fact that maximum anabolism may vary with the type of dietary protein. Thus the rate of filling of protein stores even of different tissue compartments can vary with the pattern of amino acids fed.

Increased tissue catabolism is associated with lowered caloric intake. Even at very low caloric intakes, however, the rate of loss of body nitrogen may be reduced by dietary nitrogen.

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