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Protein metabolism as influenced by aging and some of the steroid hormones

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PROTEIN METABOLISM AS INFLUENCED BY AGING AND SOME OF THE STEROID HORMONES

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INTRODUCTION

Nutrition at any age is a porblem involving a great many subjects, varying from supply and processing to the biochemistry and final disposition of the elements of the human diet. The problem as it affects the aging individual has received comparatively little attention in relation to its importance, considering the increasing age of the population. Geriatrics has become to occupy a prominent place in the present day medical practice and the differences in nutrition, if any, in this group of patients deserve a careful evaluation in the medical literature.

Of the basic elements of nutrition, the problems associated with protein are apparently the most complex and least worked out in detail. In the preparation of this paper some of the recent literature concerned with the metabolism of protein has been reviewed. Attention has been paid to the gross over-all metabolism of protein, rather than to the details of the metabolic pathways of the individual amino acids.

The work which has shown the differences in protein metabolism noted in the aged individuals has been reviewed and will be discussed. The associated subject of the influence of the steroid hormones, particularly the sex steroids is also reviewed as there is some evidence that their deficiency may be related to the metabolic changes noted.

THE GENERAL SCHEME OF PROTEIN METABOLISM

The study of nitrogen balance has been well worked out and is well known. Most of present day knowledge of protein metabolism is derived from studies using this technique. It is simply a system of dietary book-keeping or metabolic balance sheet which balances nitrogen intake, against nitrogen excretion and can obviously give little information concerning the intermediate steps by which the nitrogen of the diet becomes the nitrogen of the excretion.

Nitrogen metabolism has in the past been thought of in terms of exogenous and endogenous sources. This division has been shown to be entirely artificial and has been replaced by the concept of a "metabolic pool". (Sprinson, 1949). The constituents of the diet enter this pool after absorption and their pathway of metabolism is then indistinguishable from that of the same elements which are derived from the body substance. Sprinson defines the metabolic pool of the body as that mixture of compounds, derived either from the diet or the breakdown of tissues which the animal (or organ or cell) employ for the synthesis of tissue constituents. The nitrogenous compounds of the metabolic pool constitute the nitrogen pool.

This pool of nitrgenous compounds is in a dynamic equilibrium with the proteins of the body tissue. Practically, it may be thought of as a pool of amino acids which are in equilibrium with the protein molecules of the body tissue. As these amino acids are mobilized and modified to be incorporated into the protein molecules of the cellular substance or deaminated and degraded to produce energy, they are replaced by the amino acids of the diet or of breakdown of other tissue protein molecules.

This principle of dynamic equilibrium has been well established and studied by means of isotopically labelled amino acids (Sprinson, 1949). From this study has come the recognition of the varying lability of the body proteins. Schoenheimer, in 1942, performed experiments in which rats were fed isotopically labelled amino acids. The N15 use to label the amino acids was then recovered from the plasma, liver, kidney, intestines and erythrocytes. The concentration of the isotopic nitrogen in the various organs are shown in the table. Those proteins from which the higher concentrations of labelled nitrogen were recovered, are obviously the ones which have the higher rate of turn-over or shorter half-life. Similar work has not been carried out on humans, but in general the rates of turn-over in the various organs are thought to be roughly comparable (Sprinson, 1949).

These proteins with the shorter half-life are the labile proteins of the body and serve as the protein "stores of the body". Capable of incorporating extra amino acids into molecules when the dietary intake is relatively great, they can readily release amino acids as they are needed to replace structural protein or to produce energy. It can be seen now that a dynamic

Protein Fraction	Compound Administered			
	l-Leucine Atom Percent	d-Leucine Atom Percent	Glycine Atom Percent	d-l Tyrosine Atom Percent
Total Plasma Protein	1.65	1.15	1.77	1.45
Fibrinogen	1.79	1.06	2.35	
Euglobulin	1.51	0.89	1.82	
Pseudoglobulin	1.78	1.20	1.76	
Albumin	1.72	1.04	1.67	
Liver Protein	0.93	0.89	1.40	1.95
Kidney Protein	1.36	0.97		
Intestinal Tract Protein	1.48	0.76	0.97	
Erythrocytes	0.29	0.28	0.44	0.45
Hemin	0.14			
Probable Error of Calculated Values	0.04	0.04	0.06	0.15

equilibrium exists between the different proteins of the body. It is well known that an increase in protein intake of the individual will cause a transient positive nitrogen balance, followed by re-establishment by nitrogen balance at a higher level of intake and excretion. Conversely, when the nitrogen intake is decreased there is an initial negative nitrogen balance with a subsequent establishment of equilibrium at a lower level of intake and excretion (White, 1952). This "metabolic lag" is apparently taken up by the labile protein stores of the body, high levels of intake causing storage within their molecules and low levels depleting these stores to supply energy and components for structural protein in the less active body mass. In prolonged starvations Sprinson has shown that even ~~pneumonia~~ ammonia nitrogen may be used in protein synthesis and that as the protein stores are depleted the more inert proteins are called upon to supply amino acids for metabolism.

A great deal of work has been done to establish the level of nitrogen intake which meets the requirements of the human body. Rose, 1949-1950, determined the required amounts of each of the essential amino acids, using purified amino acid mixtures for the nitrogenous component of the diet. He was able to determine the metabolic role of each amino acid by removing it from the mixture and observing the nitrogen balance and clinical symptoms of the subject. The essential amino acids were thus recognized, that is, those amino acids without any one of which the body is unable to to maintain nitrogen balance regardless of the level of intake of nitrogen and calories. Furthermore, by successively reducing the dietary level of the particular amino acid under study Rose was able to determine the minimum daily requirement for each of these essential amino acids - that level of intake which just maintained the animal or human is nitrogen balance. Twice this amount, Rose suggested, might be the optimum daily intake.

The data shown in Rose's table was compiled on subjects receiving a diet of 55 calories per kilogram and a total nitrogen intake of 10 grams. The abundance of nitrogen is necessary in the diet for the synthesis of non-essential amino acids, as nitrogen balance could obviously not be maintained with the minimum levels of the eight essentials alone.

The minimum total nitrogen intake has not been accurately determined but the daily intake recommended by the National Research Council is 1 gram of protein per kilogram of body weight per day. This is about twice the amount required to keep healthy and young adults in nitrogen balance (Hoffmann, 1951). (Kountz, 1951). (Rose, 1949). It represents approximately 160 mg of nitrogen per kilogram of body weight. (Reifenstein, 1945).

Minimum and Recommended Intakes for Normal Men when Diet Furnishes Sufficient Nitrogen for Synthesis of Non-Essentials

Amino Acid	Minimum Daily Requirement	Recommended Daily Intake	Subjects Treated
	gm.%	gm.%	No.
L-Tryptophan	0.25	0.50	31
L-Phenylalanine	1.10	2.20	22
L-Lysine	0.80	1.60	27
L-Threonine	0.50	1.00	19
L-Valine	0.80	1.60	23
L-Methionine	1.10	2.20	13
L-Leucine	1.10	2.20	8
L-Isoleucine	0.70	1.40	8

CHANGES IN PROTEIN METABOLISM NOTED WITH AGING

Variation in protein requirement with increasing age has been reported by some workers. These studies have been based on the observation of nitrogen balance at different levels of intake. Whereas the nitrogen balance technique is an excellent guide in establishing the dietary requirement of the individual introgenous component as Rose did when the nitrogen intake was known to be adequate, there is some doubt as to whether it is a valid test in evaluating the adequacy of the entire protein intake. As was mentioned above, the subject's nitrogen balance depends upon the previous level of nitrogen intake. Any decrease in intake will be manifested by a negative balance, any increase by a positive balance. An equilibrium can be established at nearly any level of nitrogen intake, provided it is extended over a long enough period of time. The positive response to increased protein intake has been noted to be especially prolonged in older people, however (Kountz, 1947) and this may indicate a change in metabolism with age.

Other techniques have not been employed and should perhaps be investigated. Serum proteins are an unreliable guide as their level is jealously guarded by the body and are very late in their response to protein depletion (Pollack & Halpern, 1951, 1952). Tissue biopsy is quite accurate but involves technical difficulties (Pollack & Halpern, 1951). Techniques with isotopes as employed by Rose and Sam Pietro have not been employed in this field, but should offer much information.

Horwitt, 1953, cites a series of 31 patients during study on whom the nitrogen intake was cut from 11 grams per kilogram to 6.5 grams per kilogram per day. Practically all these individuals went into negative nitrogen balance as might be expected. During the next 3 months, however, these subjects returned to positive nitrogen balance and remained so for the succeeding 6months. No apparent differences were noted between the younger and older subjects. Horwitt did not give the ages of his subjects. As was previously stated, however, the amount required to maintain nitrogen equilibrium cannot necessarily be taken as an adequate daily diet as equilibrium can be established as such low levels of intake that over a prolonged period of time the subject might be placed in a precarious nutritional state (Pollack, 1951).

Kountz (1951 & 1947), using subjects over the age of 80 years found he was unable to maintain nitrogen balance on diet containing less than 1 gram to 1.2 grams of protein per kilogram of body weight per day. Kountz had 27 elderly subjects in his study, 13 women and 14 men. Food was analyzed daily for nitrogen contents as was the subjects 24 hour urine output and stools. Results are shown in a table below.
The subjects were all offered a diet averaging 2020 to 2040 calories per day, 17-18% protein, 34-36% fat and 46-48% carbohydrates. In order to permit dietary adjustments patients were put on this diet 3 weeks prior to the test period.

The reason for this apparent increase in protein requirement with age

The Incidence of Nitrogen Balance Under Various Protein Intakes

Protein Ingested Per Kg. Body Weight in Grams	Types of Nitrogen Balance						Total No. Patients in Each Group
	Negative		Equilibrium		Positive		
	No.Pts.	%	No.Pts.	%	No.Pts.	%	
1.0-1.2	5	83.5	1	16.5	0	0	6
1.2-1.4	3	42.8	2	28.6	2	28.6	7
1.4-1.6	2	20.0	3	30.0	5	50.0	10
1.6-1.8					1		1
1.8-2.0	1						3
	11	40.8	8	29.6	8	29.6	27

is not clear at present. Some increase in fecal nitrogen in those subjects in negative nitrogen balance was noted by Kountz but this was far too small an amount to account for the negative nitrogen balances. Apparently then it is not a problem was decreased absorption. Chronic malnutrition seems to be a characteristic of old age and indeed attention has been called to the fact that malnutrition may stimulate and accelerate aging and that the processes of aging may be retarded by good nutrition. (MacBryde, 1953). MacBryde has also called attention to the way in which endocrine disfunction syndromes clinically mimmick aging, such as pituitary cachexia, Cushing's syndrome and progeria. Because of this possible relationship the role of some of the steroid hormones are next discussed.

THE INFLUENCE OF SOME OF THE STEROID HORMONES

Reifenstein, in 1942, reported a large series of patients with varying types of metabolic disorders in whom different hormones had been used therapeutically. Noting that some of these hormones produced a strong positive nitrogen balance he rated the hormones in order of their protein and metabolic activity. Whereas little or no affect was produced by androstenedione, diethylstilbesterol, pregnenalone, or progesterone, Reifenstein showed that a marked to moderate degree of protein anabolic acitivity was produced by estradiolbenzoate, estradiol diproprionate, methyltestosterone and testosterone proprionate. He further stated that these compounds were of clinical value for protein anabolism. This protein anabolic effect of these hormones has been confirmed by subsequent writers using varying methods of study (Abels, 1944; Ackermann, 1954; Bogdonoff, 1954; Cooper, 1954; Kountz, 1951; Pearson, 1954; and West, 1951).

Abels administered testosterone to two normal males and three males with gastric carcinoma. All were fed constant diets of 5 grams carbohydrate, 1.3 grams protein and 1.5 grams fat per kilogram body weight. Nitrogen balance and plasma protein levels were determined, during and after the administration of 50 mg testosterone proprionate to four of the subjects and 90 mg testosterone to one of the normal subjects. He found that despite the fact that during the administration of the steroid the total nitrogen retention was equivalent to 201 to 276 grams of protein. The total amounts of serum albumin and globulin decreased in all instances. Abels concluded that the steroid must either cause the production of tissue protein from circulating protein or that it inhibits the production of serum protein.

West in 1951 injected testosterone intravenously in four normal young men. Single doses of 150 to 250 mg of testosterone caused significant and prolonged retention of nitrogen in all cases. The nitrogen retention reached a peak in 2 days and continued at decreasing levels for 8-10 days. The maximum retention of nitrogen was 2.8 grams. Smaller doses of testosterone did not produce any significant change in nitrogen balance and West concluded that the effect was dependent upon achieving a high enough blood level of the hormone.

Cooper and associates in 1951 compiled a series of 30 cases of spinal cord injury with paraplegia, a condition in which severe nitrogen loss is well known. 15 cases were used as controls and the other 15 were treated with 50-100 mg of testosterone proprionate intramuscularly, per day. In all cases treated nitrogen loss was markedly diminished as compared to the untreated controls. It is also noted that decubitus ulcers in the untreated controls in the acute phase of spinal cord injury was 60%. There were no decubiti ~~incidents~~ in the treated group.

Studies on the protein anabolic effects of hormones have been done on such a small number of subjects over such short periods of time and with such varying conditions of diet That ~~such~~ accurate conclusions are difficult to draw, however, it can be stated that all the studies have shown that some androgens have a protein anabolic effect. The mechanism by which they produce this effect is not yet known.

Estrogens have not been shown to have any pronounced effect in producing positive nitrogen balance (Reifenstein, 1942 & Kountz, 1951). Bogdonoff in 1954 reported a series of 6 subjects, 3 with and 3 without osteoporosis on whom nitrogen balance studies were carried out while under treatment with 2 mg of stilbesterol per day. The diet contained 16.8 grams of nitrogen or approximately 100 grams of protein per day. He allowed a 20 day control period during which time the patients became adjusted to the diet, followed by a 20 day treatment period in which the patients were given the 2 mg of stilbesterol per day and this in turn was followed by a 20 day recovery period. Throughout the entire period of 60 days all subjects were in positive nitrogen balance, probably due to the increased nitrogen intake. The level of positive nitrogen balance fell steadily through the period of 60 days and was apparently not influenced by the administration of the stilbesterol. Ackermann and associates, in 1953, reported a series of 6 elderly women to whom estrogens were administered in sufficient dosages to re-institute menstruation. The diet contained 1 gram of protein and 35 calories per ~~were~~ kilogram of body weight. All subjects were initially in positive nitrogen balance on this diet and remained so throughout the experiment but had decreasing levels of nitrogen retention. Estradiol benzoate was administered at the rate of 2 mg per week and no effect upon the rate of decline of nitrogen retention could be demonstrated.

Kountz, however, in his studies involving 60 aged individuals over a period of up to 6 yrs concluded that in combination with androgens, estrogens have a primary effect upon the nutrition of the body, the result of which was to lessen the degenerative state of the body tissues. He made no reference to what this effect upon nutrition was, however, and his nitrogen balance study showed no increased retention with the use of estrodiol benzoate.

McGavak and associates, in 1954, showed consistent improvement in nitrogen balance and nutritional state in elderly people with the use of androgens. In the study evaluating the use of four different entrogens McGavak and associates rated testosterone, dehydro-testosterone, methylandrostenediol, and androsterone in that order for their nitrogen retaining abilities. Again the series have been very small and periods of study short. This and other work (Ackermann, 1954; Korenchevsky, 1948, 1953; Kountz, 1953, 1951) has suggested that testosterone may be a useful adjunct in the nutrition of the aged individual.

Korenchevsky, 1953, studied the processes of aging and the effects of deficiencies of sex hormones on these processes in 221 normal and 111 castrated rats, a total of 332 male animals. The changes in weight and histological changes in various organs were recorded. He showed that many of the changes due to senescence were accelerated and mimmicked by lack of gonadal hormones. He also showed that the use of sex hormones was effective in delaying these changes, at least in so far as they were due to lack of gonad secretion. Kountz, in 1951, observed a group of 60 aged individuals for a period of up to 6 yrs and noted some of the characteristic changes in some of the body tissues associated with aging. Under the influence of estrogens and androgens these changes were in part reversed and the tissues "re-vitalized" - apparently restored to an appearance in function of an earlier period of life. Although these studies seemed to indicate that the sex hormones are able to delay or even reverse the processes of aging, Korenchevsky points out that loss of gonadal function is a secondary rather than a primary cause of aging and that the usefulness of the sex hormones will be always confined to correcting the secondary factor in aging.

The site of action of these hormones in protein metabolism is not known at present. As was mentioned before knowledge of protein metabolism has had to come from studies of nitrogen balance. With the present day methods of determining the size of amino acid pool, rate of turn-over and of tracing specific amino acids to particular tissues, a great deal of more information should soon be available.

CONCLUSIONS

1. Aged individuals have been shown to require higher intake of protein in the diet than that normally recommended. The daily protein intake for the aged individual is in the neighborhood of 1.4 grams per kilogram per day. The reason for this increased protein requirement with aging is not apparent at present.

2. Androgenic hormones, particularly testosterone and testosterone proprionate have been shown to have pronounced protein anabolic effects. The site of action of these hormones is unknown at present. Estrogens have been shown not to have any pronounced effect upon protein metabolism.

3. Testosterone and testosterone proprionate have been used successfully to improve the nitrogen balance in normal diseased and aged individuals. Inasmuch as loss of gonadal function is a factor in chronic protein malnutrition of aging, these hormones are a valuable adjunct to the therapeutic nutrition of the aged.

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