Amino Acid Malnutrition

Edited by William H. Cole

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Library of Congress Catalog
Card Number: 53-753

LITHOPRINTED IN THE UNITED STATES OF AMERICA BY
CUSHING - MALLOY, INC., ANN ARBOR, MICHIGAN, 1957

AMINO
ACID
MALNUTRITION

RUTGERS UNIVERSITY
Bureau of Biological Research

THE ANNUAL CONFERENCES ON
PROTEIN METABOLISM

1952 *Protein Metabolism, Hormones and Growth*
80 pages

1953 *Some Conjugated Proteins*
73 pages

1954 *Serological Approaches to Studies of Protein Structure and Metabolism*
97 pages

1955 *Some Physiological Aspects and Consequences of Parasitism*
90 pages

1956 *Some Aspects of Amino Acid Supplementation*
85 pages

FOREWORD

As a regular feature of its program of studies on protein metabolism, the Bureau of Biological Research at Rutgers, The State University, has sponsored annual conferences on some aspect of that general theme since 1945. At each conference a small number of investigators, from four to eight, have been invited to present the results of their recent studies against the background of other contributions to the subject. Informality of presentation has been encouraged, and time has been allowed for free discussion of each report. The conferences have been open to all interested persons who registered for them. The average attendance has exceeded two hundred.

The first three conferences dealt with dietary proteins and protein derivatives, particularly therapeutic protein hydrolysates. The next four conferences considered caloric intake, certain amino acids, peptides, enzymes, vitamins, and minerals in relation to health and disease. The proceedings of the first seven conferences were distributed only to those who attended.

The next five conferences were concerned with protein metabolism, hormones and growth, conjugated proteins, serological approaches to protein structure and metabolism, physiological aspects and consequences of parasitism, and dietary amino acid supplementation. The proceedings of those conferences have been published to make the material available to an audience larger than the one in attendance. This policy is continued for the 1957 conference and will probably be followed for future conferences.

During recent years biologists and clinicians have been intensively studying the nutritive requirements of plants, animals, and man. Thousands of studies have been reported on plants, bacteria, fungi, protozoa, a few invertebrate animals, and many mammals. Perhaps the largest number has dealt with bacteria, the rat, the dog, and man. Emphasis has shifted from caloric intake to vitamins, to the quality and relative amounts of protein carbohydrate and fat required, to balances between them, and finally to detailed considerations of balances among the proteins and their constituent amino acids. Attention has also been given to the interacting roles of hormones, vitamins, and minerals.

In several studies on animals where the experimental conditions are more easily controlled than on humans, evidence has been secured that damage to the structure and function of the body from unknown causes may often be corrected by a specific modification or supplementation of the diet. Thus specific dietary constituents may act as therapeutic agents in preventing and curing diseases which were formerly not considered related to dietary intake. Manifestations of malnutrition may therefore be more widespread than are now recognized.

With the increasing attention given to human protein nutrition during recent years, some progress has been made in demonstrating that certain human disturbances and diseases may be cured by proper dietary supplements. For example, kwashiorkor is now recognized as one type of protein-deficiency disease, easily and quickly cured by adding milk to the diet. Detailed studies of this disease in Jamaican and Guatemalan children are presented in this volume. In other areas of the world where food is scarce, or where particular food habits interfere with adequate nutrition, several individuals and groups are making careful studies to determine the proper protein or amino acid supplementation required. For certain countries this may mean the formulation of an entirely new diet composed of materials available but not now used. Some of the work is being advised and supported in part by the United Nations through the Food and Agriculture Organization, the World Health Organization, the Children's Fund (UNICEF), and the Institute of Nutrition of Central America and Panama (INCAP). Private foundations are also providing financial aid to such studies, which promise the solution of one of the most difficult problems facing the world — i.e., inadequate nutrition. One of the first steps in this direction has been the authoritative survey of food production in eight countries of the world contrasted to that in the United States presented herewith.

Another frontier now possible of exploration by dietary tools is mental health. The rapid progress in the discovery and use of the so-called "wonder drugs of the nervous system" has not been accompanied by equal advances in the understanding of the central nervous system's metabolism. As an introduction to such knowledge, an original and critical summary of the amino acid and protein metabolism of the brain is included in this volume. Some outstanding differences from the metabolism of other tissues and organs are disclosed. Although no interpretation is yet possible, it is safe to assume that correlations between specific metabolic processes in the brain and aspects of behavior will be possible.

In addition to the subjects mentioned above, the Thirteenth Annual Conference on Protein Metabolism, devoted to Amino Acid Malnutrition, included an account of the repletion of protein-depleted animals as a background for human tests and a discussion of mixtures of pure amino acids as dietary supplements.

Acknowledgement is thankfully made to the authors of the papers for their assistance in preparing this book for prompt publication.

WILLIAM H. COLE
Chairman
Conference Committee

January 30, 1957

CONTENTS

LIST OF FIGURES

LIST OF TABLES

1

REPLETION OF DEPLETED PROTEIN RESERVES IN ANIMALS[1]

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Protein malnutrition is associated with a loss in protein reserves in the blood and soft tissues of the body *(1-4)*. These reserves are the tissue proteins which rise and fall with corresponding increases or decreases in the dietary protein intake, and they are believed to reach an optimum in the presence of an adequate quantity and a proper balance of dietary amino acids. Depletion in protein reserves has numerous striking effects upon the metabolism and welfare of an animal. Since many protein reserves are enzyme systems, protein malnutrition alters rates of reactions in intermediary metabolism. If a vitamin is part of the coenzyme, the alterations may also be characteristic of a vitamin deficiency. Data have been presented, for example, which demonstrate the reduced ability of the depleted animal to detoxify or oxidize organic radicals, a reduction which is also associated with riboflavin and pantothenate deficiencies *(5)*. In general, labile protein reserves are associated with numerous metabolic functions, being important, for example, to maintenance of water balance *(6)*, to formation of antibodise *(3, 7)*, to healing of wounds *(8)*, to mechanisms for oxidation and detoxication *(5)*, and to correction of dietary deficiencies associated with periods of restricted food intake *(4)*.

There is still some question, however, concerning the magnitude of development of the tissue protein reserves for optimum welfare of the animal and the effect of dietary proteins of various nutritive values upon this development. The following experiments were designed to answer these questions in part. Dogs were depleted in reserves by feeding a protein-free diet and the rate of repletion studied in animals fed casein or wheat gluten. The casein was also supplemented with methionine and wheat gluten with lysine to determine the effect of increasing the nutritive value of these proteins. The results of repletion in depleted animals were compared with some previous studies on the effect of the same dietary proteins upon growth in young dogs.

METHODS

Adult dogs were fed a protein-free diet which contained the vitamin, mineral, and caloric requirements estimated for this animal under

[1] These researches were supported in part by grants-in-aid from the National Cancer Institute and the New Jersey Heart Association.

TABLE I

DIET FED TO DOGS

Ingredient	Grams A	Grams B	Vitamins	mg/2400 gm agar diet
Protein	0	250	Thiamin	2.0
Sucrose	229	0	Riboflavin	7.6
Dextrose	387	366	Nicotinic acid	16.0
Dextrin	187	187	Calcium pantothenate	13.0
Lard	153	153	Pyridoxine	1.0
Salt mixture	17	17	Choline	1000
Agar	27	27	2-methylnaphthoquinone	0.0006
Water	1400	1400	Alpha tocopherol	30.0
			Biotin	0.6
			Folic acid	0.6
			Vitamin A	55,000 units
			Vitamin D	11,000 units

"normal" conditions *(9)*. This is illustrated by A in Table I. The diet was prepared as follows: about two-thirds of the water was added to a kettle, together with the agar, the mixture being heated until the agar dissolved. The lard was cut into chunks, added to the hot agar solution, and the heat removed. While the lard melted, the carbohydrate, protein (if it was to be added), and salts were mixed and then added to the agar solution. Water was supplied to make up the amount recorded in Table I. The liquid was stirred until it thickened, the vitamins included, and the diet poured into pans to gel and to be stored in the refrigerator. The dogs were fed the protein-free diet equivalent to 80 calories/day/kg of body weight for a period of four weeks. Then for 28 days the animals were fed a diet containing protein such as the one illustrated by B in Table I. Urine and feces were collected so that nitrogen balances could be calculated for the whole experiment. Urea and ammonia were determined by the Conway method as modified by Steinetz *(10)*. Blood volumes and extracellular fluid determinations *(11, 12)*, electrophoretic analyses for plasma protein *(13)*, and plasma aldolase determinations *(14)* were made periodically.

DEPLETION OF PROTEIN RESERVES

Depletion of protein reserves is usually accompanied by a reduction in catabolic activity as reflected by a fall in the excretion of urea nitrogen (see Table II). The greater the labile protein reserves the higher the initial excretion of urea, but the excretion drops to a low and fairly

TABLE II

AVERAGE DATA OBTAINED FROM A STUDY OF 16 DOGS FED A PROTEIN-FREE DIET FOR 4 WEEKS

Weeks Depletion	Urea Nitrogen	*Urinary* Amonia Nitrogen	Creatinine
		mg/day/kg body weight	
1	162	22.5	20.4
2	111	22.5	21.0
3	94	22.1	21.4
4	93	21.5	19.3

constant value in all depleted dogs. Possibly the magnitude of reduction in excretion of urea to this constant value is one estimate of the quantity of reserves available for utilization under conditions of stress. The constancy of excretion of urea after initial depletion of reserves and of creatinine may be interpreted to represent essential catabolic activities that are of endogenous origin *(15, 16)*.

In the following studies on repletion, the response of the animals was estimated primarily in terms of plasma proteins and nitrogen balance, two variables which are used extensively to measure loss and gain in protein reserves. Under the experimental conditions, the serum albumin concentration decreased with loss in body nitrogen in a semilogarithmic manner as illustrated in Figure 1. Body nitrogen loss was calculated by assuming the over-all protein content of the dog to be approximately 16 per cent. Plasma globulin concentration, on the other hand, at first increased slightly in concentration with depletion in reserves, but then decreased to a subnormal concentration at a point of maximum reduction in body nitrogen. At this point of maximum loss, the animal is approaching a critical stage where repletion is difficult or even impossible. The initial rise in globulin concentration is the result of a fall in plasma volume, the total circulating globulins either remaining unchanged or actually decreasing with depletion in reserves. This tendency for certain globulins to be reduced in depleted animals is illustrated by the data in Table III. The fact that a globulin fraction does not decrease on depletion, however, does not mean that such a fraction is not labile and cannot be reduced. The total circulating γ globulins, for example, may be reduced upon depletion in protein reserves but under the stress of infection, which often passes through a colony, this fraction may increase. This variability in response of γ globulin is an example of the capacity of the body to draw upon reserves to synthesize specific proteins when metabolism shifts in that direction.

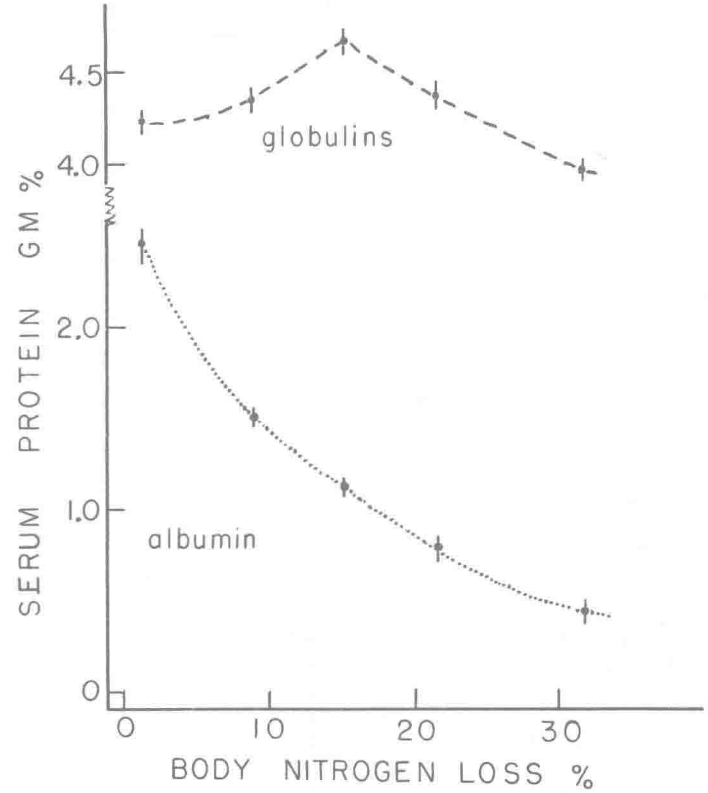

Figure 1. Grams per cent of serum albumin and serum globulin correlated with loss in body nitrogen in dogs fed a protein-free diet.

TABLE III

AVERAGE DATA OBTAINED FROM A STUDY OF 16 DOGS FED THE PROTEIN-FREE DIET OVER A PERIOD OF 28 DAYS

Weeks Depletion	Plasma Albumin	Plasma globuline					
		α_1	α_2	α_3	β_1	β_2	γ
				gm/kg body weight			
0	1.32	0.35	0.33	0.45	0.57	0.46	0.27
2	0.75	0.43	0.24	0.33	0.41	0.42	0.35
4	0.54	0.39	0.27	0.29	0.34	0.37	0.37

NUTRITIVE VALUES OF THE CASEIN AND WHEAT GLUTEN

Casein and wheat gluten were chosen as two dietary proteins to study the effects of nitrogen intake upon repletion of protein reserves because they represent a high and a low nutritive value, respectively, and yet both are deficient primarily in one, though different, essential amino acid. The nitrogen balance index of casein, for example, in the normal dog with full protein reserves was determined to be 0.74 *(17)*. Casein is deficient in sulfur amino acids for the dog so that adding methionine to casein raised the index to 1.0, equivalent to the value for egg proteins. The index for casein in the depleted dog was 0.84, a much higher value than in the normal animal, such augmentation in the utilization of dietary amino acids being common to the depleted animal. The index for wheat gluten was 0.44 in the normal dog but increased to 0.70 when the reserves were depleted. Wheat gluten is deficient primarily in lysine so that the addition of this amino acid in concentrations about equal to the amount found in casein raised the index in the normal dog to 0.73 *(17)*.

NITROGEN BALANCE DURING REPLETION

Feeding 0.2 gm casein nitrogen/day/kg of body weight to the depleted dogs for 28 days produced a small but constant positive balance of approximately 0.05 gm nitrogen/day/kg. These data, illustrated by the solid circles in Figure 2, may be interpreted to mean that this amount of casein nitrogen is just a bit over that needed to maintain the animal in the depleted state with very little filling of the protein reserves. The circles with vertical bars record data obtained while feeding 0.6 gm of casein nitrogen/day/kg body weight in a diet equivalent to 80 calories/day/kg. The slope of the line drawn through these points may be interpreted to represent the rate of filling of the protein reserves; the area under the curve is an estimate of the grams of nitrogen retained during repletion. The line drawn through the open circles in Figure 2 measures the increased rate and amount of filling of the reserves in dogs fed a higher intake of 1.12 gm casein nitrogen/day/kg and approximately 140 calories/day/kg. The circles with cross bars demonstrate the improvement in nutritive value obtained while feeding 1.12 gm casein nitrogen/day/kg supplemented with an optimum amount of methionine *(18)*. In general, these data demonstrate the increased rates of filling of the protein reserves and amounts of nitrogen retained, both values being correlated with a rise in nitrogen intake and with supplementation to correct for a deficiency in an essential amino acid.

The results of feeding approximately 0.6 gm of wheat gluten nitrogen/day/kg and 80 calories/day/kg to depleted dogs for 28 days are recorded by the circles with vertical bars in Figure 3. The open circles illustrate the increased rate and amount of nitrogen retention obtained while feeding 1.1 gm of wheat gluten nitrogen/day/kg at the higher caloric intake. The circles with cross bars record the still