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ANNUAL REVIEW OF NUTRITION

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PREFACE

No biologic subject covers a greater span for study and application than does nutrition. The consummate nutritionist should have at least some appreciation of matters molecular and physiologic in order to understand something of the disposition of nutrients within a functional organism, especially one as complex as the human. It seems appropriate, then, to remind the reader that each year there is not only a compilation of some advancements in our discipline as reflected by chapters contained within the current issue of an *Annual Review of Nutrition*, but also there are topics of interest to nutritionists that are to be found in those volumes of *Annual Reviews* that cover findings in other titled disciplines, e.g. biochemistry, cell biology, genetics, medicine, and public health. We call your attention, therefore, to "Related Articles" listed on page ix.

This particular volume ranges through the usual general categories. The prefatory chapter is written by Mark Hegsted, who has fostered the advancement of nutrition. Updates on so-called energy metabolism can be gained from reading about leptin, as addressed in chapters by R. Harris and by Baile et al, mitochondrial uncoupling proteins by Kozak et al, and the transcriptional control of adipogenesis by Rangwala & Lazar. Among selections on nutrients, a tour de force on oligosaccharides in human milk is presented by Kunz et al. Several chapters address lipids and one by Lee & Lin considers present arguments for the role of dietary fat in breast cancer. With expanding research on apoptosis, there is a timely chapter that is written by Watson et al on the diet relationship. Only now have all the steps with regard to intermediates and enzymes been pieced together in the biosynthesis of vitamin B2 (riboflavin), as addressed by Bacher et al. Several chapters bring us up-to-date on inorganic nutrients, particularly calcium, iron, and copper. As concerns other food components, both the benefits and the metabolism of alcohol are reviewed by German & Walzem and by Lieber, respectively. There are other subjects addressed by experts who continue to advance our discipline.

Reputedly the last message of the dying Pasteur, spoken to his devoted assistants surrounding his bed in a home near Saint Cloud, was "Il faut travailler." Indeed there is much work yet to be done in the advancement of nutritional science and its extension to applications in the pursuance of better health. We cannot cover all worthy subjects within the scope of any one volume of an *Annual Review of Nutrition*. This is inevitable given the limitation of space and constraints of what is timely and who should write on a topic. Only over a span of several volumes can an even or balanced coverage be achieved. The reminder to our readership is that we have an Editorial Committee with mixed interests and expertise, which is attentive to your suggestions. My thanks and the credit for content of each volume go to this Committee. The fact that we are produced in a timely, readable timely fashion really is due to Lisa Dean, the production editor, Roberta Parmer, the copyeditor, and Dr. Sam Gubins as president of Annual Reviews.

Donald B. McCormick
Editor

Related Articles

From the ***Annual Review of Biochemistry***, Volume 69 (2000)

Protein Glucosylation and Its Role in Protein Folding, Armando J. Parodi

Cyclooxygenases: Structural, Cellular, and Molecular Biology, William L. Smith, David L. DeWitt, and R. Michael Garavito

Apoptosis Signaling, Andreas Strasser, Liam O'Connor, and Vishva M. Dixit

Autophagy, Cytoplasm-to-Vacuole Targeting Pathway, and Pexophagy in Yeast and Mammalian Cells, John Kim and Daniel J. Klionsky

Protein Tyrosine Kinase Structure and Function, Stevan R. Hubbard and Jeffrey H. Till

Import of Peroxisomal Matrix and Membrane Proteins, S. Subramani, Antonius Koller, and William B. Snyder

Swinging Arms and Swinging Domains in Multifunctional Enzymes: Catalytic Machines for Multistep Reactions, Richard N. Perham

Aminoacyl-tRNA Synthesis, Michael Ibba and Dieter Söll

From the ***Annual Review of Cell and Developmental Biology***, Volume 15 (1999)

Vacuolar Import of Proteins and Organelles from the Cytoplasm, Daniel J. Klionsky and Yoshinori Ohsumi

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Regulation of Nuclear Localization: A Key To A Door, Arie Kaffman and Erin K. O'Shea

Regulation of Mammalian O_2 Homeostasis by Hypoxia-Inducible Factor 1, Gregg L. Semenza

Transport Between the Cell Nucleus and the Cytoplasm, Dirk Görlich and Ulrike Kutay

From the ***Annual Review of Genetics***, Volume 33 (1999)

Ribosome Synthesis in Saccharomyces cerevisiae, Jaap Venema and David Tollervey

Mitochondrial Genomes Evolution and the Origin of Eukaryotes, B. Franz Lang, Michael W. Gray, and Gertraud Burger

From the ***Annual Review of Genomics and Human Genetics***, Volume 1 (2000)

Genetic Screening of Newborns, Harvey L. Levy and Simone Albers

Iron Metabolism: Iron Deficiency and Iron Overload, Nancy Andrews

Apolipoprotein E: Far More Than a Lipid Transport Protein, Robert W. Mahley and Stanley C. Rall, Jr.

From the ***Annual Review of Medicine***, Volume 51 (2000)

Coronary Artery Disease in the Transplanted Heart, M. Weiss and W. von Scheidt

Atherogenic Lipids and Endothelial Dysfunction: Mechanisms in the Genesis of Ischemic Syndromes, Mark R. Adams, Scott Kinlay, Gavin J. Blake, James L. Orford, Peter Ganz, and Andrew P. Selwyn

Role of Cytokines in the Pathogenesis of Inflammatory Bowel Disease, Konstantinos A. Papadakis and Stephan R. Targan

Anorexia and Bulimia Nervosa, W. H. Kaye, K. L. Klump, G. K. W. Frank, and M. Strober

The Role of Protein Traffic in the Progression of Renal Diseases, Piero Ruggenenti and Giuseppe Remuzzi

Current Concepts in Cobalamin Deficiency, Ralph Carmel

Genetic Disorders Affecting Proteins of Iron Metabolism: Clinical Implications, Sujit Sheth and Gary M. Brittenham

From the ***Annual Review of Physiology***, Volume 62 (2000)

Sodium-Calcium Exchange: A Molecular Perspective, Kenneth D. Philipson and Debora A. Nicoll

Mechanisms Underlying the Cost of Living in Animals, A. J. Hulbert and Paul Lewis Else

Leptin, Rexford S. Ahima and Jeffrey S. Flier

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The Na-K-Cl Cotransporter of Secretory Epithelia, Mark Haas and Bliss Forbush, III

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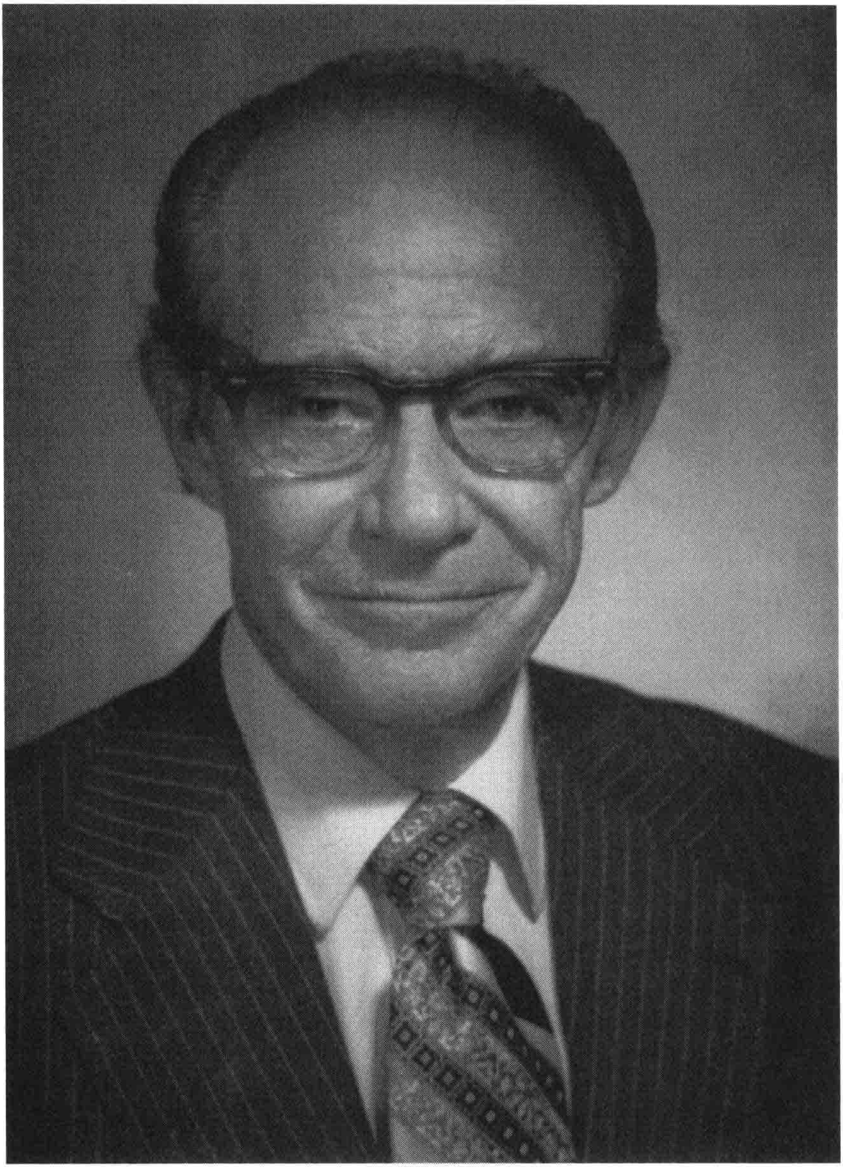

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From Chick Nutrition to Nutrition Policy

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Key Words protein, calcium, fats, cholesterol, dietary guidelines

■ **Abstract** It is difficult to abstract a summary of a lifetime of work. I have chosen to discuss research on protein, calcium, and the effects of dietary fat and cholesterol on serum cholesterol and on my activities that led to the publication of the *Dietary Guidelines for Americans*. Among the conclusions from studies on protein and calcium is that reasonably healthy people are adapted to their current diets. People all over the world, for example, are raised on relatively low calcium intakes yet have less osteoporosis than those who consume western-style diets. They also appear to do reasonably well on low-lysine intakes. Attempts to define requirements need to allow for adaptation, and we need to determine whether such adaptations are beneficial or detrimental to health. The studies on serum cholesterol defined the role of the various classes of fatty acids. The publication of the *Dietary Guidelines for Americans* introduced a new era of nutrition and has radically altered nutrition policy, nutrition standards, and education.

CONTENTS

UNIVERSITY OF IDAHO

The general direction of my career was probably determined during my first few days at the University of Idaho. I had been awarded a Union Pacific Scholarship that paid a small amount of money—I do not recall how much—and a round-trip ticket to the University. This seemed too good to pass up, so I arrived at the University with $75 in my pocket. I had little idea of the fields available, but I was sure I did not want to be a farmer. The County Agent, Don Bolingbroke, and Lou Williams, who taught Agriculture in the high school, were largely responsible for

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my being there, so I thought I would probably do something similar. I had an appointment with Dean Iddings on the third day. He pointed out that all potential students had received a letter saying that a year at the University would cost about $300 and "that there wasn't much point in providing such advice if it was simply ignored." But he must have thought I needed and deserved some support, so a couple of days later, I was told to report to the Experiment Station Laboratory to wash dishes. Don Bolin was the Experiment Station Chemist and he took an interest in me. After I had washed dished for a few months, he began to give me other jobs and opportunities. A major activity in the laboratory was the proximate and calcium and phosphorus analysis of forages collected from around the state. I worked in the lab year round, and by the time I graduated I thought I was a pretty good analytical chemist.

The next summer I was also asked to help Dr. Ella Woods, the Experiment Station Home Economist, who was estimating the vitamin C content of strawberries. My job was to walk out to the farm every morning, pick a few strawberries, weigh out the appropriate samples, and feed them to the guinea pigs. After a few weeks they and the control animals, who received different levels of lemon juice, were killed and Dr. Woods scored them for signs of scurvy. After that summer I usually had something to do for Dr. Woods, and she also took me under her wing. During those years, Don Bolin and Dr. Woods advised me on course work. I took more math, chemistry, and biology than the usual agricultural student. The Dean said I was trying to get a pre-med degree in the College of Agriculture, but he didn't really object.

By the time my senior year rolled around, it was somehow clear that I should go to graduate school, even though I had no money. I had a rough idea of what was going on in various schools and knew the names of some of the prominent people in nutrition and biochemistry or agricultural chemistry, but Don Bolin and Dr. Woods advised me on where to apply. In those days, nutrition studies were the major effort in many agricultural chemistry and biochemistry departments. In February, I was offered a teaching assistantship by Henry Sherman at Columbia University. I was thrilled and immediately accepted. In a few weeks, however, I received offers from all but one of the other schools I had applied to. After a week of turmoil—Don was from Wisconsin and Dr. Woods was a Columbia graduate—I finally apologized to Dr. Sherman and accepted the offer from Wisconsin. New York seemed far away and a little intimidating for an Idaho farm boy.

UNIVERSITY OF WISCONSIN

At Wisconsin, I was assigned to WH Peterson, a fermentation chemist, to work on AIV silage, which was named after AI Virtanen, the Finnish Nobel laureate. He had shown that alfalfa and other forages that contained little sugar could be made into silage if they were treated with hydrochloric acid or other strong acids. They were higher in protein and carotene than corn silage and might eliminate the need for protein supplements and improve the quality of the ration and the milk. There were many questions about performance of the animals, costs, etc, being studied in animal husbandry. My job was to look at the preservation of carotene and the vitamin A and carotene content of milk from cows fed various rations. It was already known that the standard method for measuring carotene did not work in silages: The silage appeared to have more carotene than the original forage. Over the year, I made all kinds of silage—using different kinds and amounts of acids, from all kinds of green materials, in everything from quart bottles to crocks—and sampled the silos on the farm. I missed an opportunity. Although I could easily set up chromatographic columns that demonstrated various carotenoids and separated them to varying degrees, I never succeeded in getting columns that were easily reproducible. We did develop an improved method for carotene determination (29).

It was becoming clear that AIV silage would not revolutionize the Wisconsin dairy industry. Although I was not privy to the discussions, Professors Elvehjem and Peterson thought I should change problems and considered either putting me in Peterson's fermentation lab, where Frank Strong, Wayne Woolley, Ez Snell, and others were working, or putting me with Professors Elvehjem and Hart to work on new growth factors for chicks. Somehow or other I ended up in the animal work, replacing Herb Bird, who had just graduated.

I cannot imagine a better graduate experience than we had at Wisconsin in those days. The faculty of Professors Hart, Elvehjem, Steenbock, and Link—with Frank Strong, Paul Phillips, Carl Bauman, and others coming on strong—read like a *Who's Who*. There were probably 30–40 students working on almost every current nutritional-biochemical topic. We learned more from bull sessions than from classes. Because practically no one had money, we were, and were expected to be, at the lab from about 7:30 AM to 10–11 PM most of the time. We expected every FASEB meeting to announce something exciting. That was where discoveries were announced—not in the public press. I recall hearing Du Vigneau report on transmethylation, Roger Williams on pantothenic acid, Sam Leprovsky on B6-xanthurenic acid, and of course the Wisconsin group on nicotinic acid in 1938. We searched the literature for every significant advance to report in Professor Hart's Saturday morning seminar. During those years, which have been called the "heyday of nutrition," the vitamin story was nearly completed. I managed some 15 publications, which included studies on silage, vitamin B_6, pantothenic acid, choline, biotin, inositol, arginine, glycine, and the *Lactobacillus casei* factor that became folic acid.

I did not find a suitable job in 1940. After an interview with an industrial company, Professor Hart told me that such companies "prostituted his boys," so I continued to work another year at Wisconsin. The next year I went to the Abbott Laboratories as a research chemist. I did not find it all that stimulating and made no significant contributions. I did try to convince the Director of Research that Abbott should put some effort into the isolation of what we called the *L. casei* factor, that this would be the next important vitamin. The Lederle Laboratories succeeded in isolating folic acid soon after.

Fortunately, Fred Stare recruited me and Jack McKibbin—all Wisconsinites—to be the faculty at the new Division of Nutrition in the Harvard Schools of Medicine and Public Health. Fred described these beginnings in his contribution to this *Review* (51), so I do not discuss them. Fred built one of the better departments of nutrition, provided us with good facilities, and gave us a rather free hand in research. We were fortunate to grow during the time when the National Institutes of Health (NIH) was expanding and NIH funds were usually available. It seems remarkable now that when I was chairman of the Nutrition Study Section, 80%–90% of applications were approved and paid.

Before discussing some of the issues that have interested me over the years, I would note the transformation that occurred in nutrition just after the war. As I indicated, in the 1930s, the major interest was in identifying the essential nutrients and trying to determine their requirements. When World War II was over, however, the nutrition community was surprised to find that deficiency diseases had become rare in the United States, Europe, and in some other areas. Interest in nutrition in medicine and public health fell accordingly. Why teach medical students about diseases they would never see? During the first few years, our group in nutrition had a fair block of teaching time in the biochemistry department at the medical school, but this was gradually whittled down until I had one lecture to teach, "Nutrition," which seemed less than useless. The situation is probably best exemplified by what happened at Oxford. Funds had been obtained to develop a nutrition department just after the war, but the University refused to accept it. Sir Donald Acheson notes that, "[f]or most students in Oxford in those days there were no remaining unsolved problems in nutrition. All of the accessory food factors had been identified. All that was necessary was to eat a good mixed diet, preferably three square meals daily, avoid obesity, and all would be well" (see 50a). Of course the functions of the nutrients had to be elucidated, but this would be done by the biochemists. Enzymology bloomed. Many erstwhile nutritionists, perhaps the more enlightened ones, became enzymologists. I never found sitting in front of a Warburg apparatus very appealing, but my guess is that had the decision been delayed a few years, there would have been no nutrition department at Harvard.

My bibliography reads like a potpourri of nutrition topics, including nutrition of ducks, gerbils, agoutis, cats, dogs, monkeys, and a few other species, with papers on most of the B vitamins, ascorbic acid, vitamin D, vitamin A, inositol, iron, fluoride, and others. I regret that space does not permit a discussion of some of these here.

PROTEIN AND AMINO ACIDS

Protein requirements were of concern during the war years because of the possibility that war-time rationing might lead to deficiencies. Our early studies (30) (which now look meaningless to me), but more important the data in the literature, convinced me that nitrogen balance was possible at relatively low intakes, even with largely vegetarian diets, and that the role of protein had been generally overemphasized. Various issues related to protein have been a continuing interest over 45 years.

It had long been obvious that proteins varied in nutritional value. This was a function of their amino acid content since it could be modified by amino acid supplementation. The traditional method of estimating the nutritional quality of proteins with young rats was the protein efficiency ratio (PER), originally proposed by Osborne et al (43). This was the gain in weight divided by the grams of protein eaten, and there was a rather voluminous literature, some of it indicating obvious problems. We could rather easily show that this was an unsatisfactory method, for several reasons (31), but it took many years of thought and effort to devise a more satisfactory approach (47). We eventually developed a slope-ratio assay similar to those used in microbiologic assays. This was never accepted because it required larger numbers of animals and was, thus, more expensive. Much later we applied a similar technique to estimate available calories and could show that the calories in fats are more efficiently used than those in carbohydrates (9). It is particularly interesting that even when the total energy intake was not sufficient for maximal growth, the animals receiving more calories from fat had more body fat than those receiving comparable levels of carbohydrate. I believe fat helps make you fat.

It turned out that a completely satisfactory assay for protein quality is not possible because the dose-response lines do not have a common intercept. The reason for this became apparent when we investigated the amino acid requirements of adult rats. Various levels of each essential amino acid were fed to define the maintenance requirement (46). When a threonine-free diet was fed, they lost weight rapidly, similarly to those fed a protein-free diet. In contrast, a lysine-free diet caused a very slow loss of weight; some of the animals maintained their original weight for weeks. The response to other amino acid deficiencies fell between these extremes. Obviously, the animals had the capacity to adapt to a low-lysine diet and conserve their body lysine but little capacity to conserve body threonine. Sue Chu (6) then demonstrated that liver lysine-ketoglutarate reductase—the first enzyme involved in lysine catabolism—fell markedly in lysine-deficient animals, whereas liver theonine dehydratase fell little during threonine deficiency. Both enzymes increased on a high-protein diet. Thus, animals have varying capacity but can adapt to different levels of protein and amino acids in the diet.

These data also undermined other basic nutritional tenets (17, 18). The Biological Value and Amino Acid Score (4), measures of protein quality, had been based upon the idea that because all essential amino acids are required for protein synthesis, a deficiency of any essential amino acid should be the nutritional equivalent of a protein-free diet. Clearly, that is not true.

Rapidly growing animals have limited capacity to adapt to nutritional deficiencies because new tissue is being formed. Thus, it is easy to demonstrate differences in protein quality with growing rats. However, even these animals demonstrate adaptation when fed diets that only allow maintenance of weight. I believe the conclusion must be that in adult animals or in very slowly growing species, such as man and other primates, protein quality is of much less significance than is generally believed. It is true that differences in protein quality have been repeatedly demonstrated in nitrogen-balance trials with adults, but these have been in short-term studies, which may not allow ability to adapt. How long this may take is unknown.

We do not know how similar man may be to adult rats, but some adaptation to low-protein diets must occur. This would explain why amino acid supplementation has generally failed to improve performance, as well as the ability of populations to perform reasonably well on largely cereal, low-lysine diets. All future studies on either amino acid or protein requirements should be designed with this in mind. Because adaptation is a universal phenomenon, the requirement to maintain the metabolic status quo is simply the content of the current diet of the individual. Measuring the requirement to maintain what is provided by the current diet has little or no nutritional significance.

In 1957, I (15) concluded that growth was a minor determinant of protein or other nutrient needs after the first months of life. The amount of new tissue protein deposited per day in growing children or during the adolescent growth spurt is very small compared with the total maintenance requirement of protein. This distinguishes humans and other primate species from common laboratory and domestic species. The young of most common species require diets higher in protein than adults, but this is not true of humans. Breast milk is a very low-protein diet but obviously nutritionally adequate. During the rapid growth of the first few months of life, 6%–7% of calories as protein is adequate and there appears to be no reason to believe that needs later in life are higher.

Brock & Autret (5) reported the wide-spread occurrence of infant malnutrition in Africa, and it was soon apparent that similar problems were common throughout the developing world. Because edema occurred in some infants and plasma albumin levels were low, it was soon labeled "protein deficiency" (54a). I was skeptical (16, 17). There followed a long period of study and debate, sometimes acrimonious debate. A number of committees, such as the Protein Advisory Committee of the Food and Nutrition Board (FNB), were developed for advice and guidance. To make a long story shorter, these extensive efforts to promote everything from protein to amino acid supplementation reached their peak with the UN conference on Averting the Coming Protein Crisis (45) and almost disappeared after McLarin (38).

During this period, amino acid fortifications was rather widely recommended. Although I was skeptical, the department participated in field trials testing the effects of lysine supplementation of wheat in Tunisia (10) and threonine-lysine supplementation of rice in Thailand (14). The results were essentially negative. As discussed above, it appears that such negative results are explained by adaptation to low-protein diets.

Another tenet of protein nutrition that has confused the issue over the years has been the conclusion that energy restriction increases protein needs. This seemed obvious from the repeated and common finding in nitrogen balance trials that urinary nitrogen excretion increases when energy intake is restricted. Thus, the