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BACK TO BASICS: An Evolutionary Odyssey With Reflections on the Nutrition Research of Tomorrow[1]

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PRELUDE

The *Annual Review of Nutrition* provides the rare opportunity and challenge of reflecting on the status of nutrition research within the family of Life Sciences. I shall argue that, like other applied biological sciences, nutrition research needs to be continuously enriched by concepts and techniques developed in the course of basic biological research. This implies that some in the community of nutrition scientists must maintain lively contact with the rapidly moving front in basic biological sciences. Hence the phrase "back to basics" in my title. Not only will this have a beneficial effect on nutrition research, but it will also improve credibility for nutritionists among their basic science colleagues.

The mature scientist always appears to be a more or less autonomous individualist. Yet there are encounters along the way that determine our career

patterns. Looking back on my own progress, I can identify several such factors that, without my seeking them out, have conspired to steer my own progress into basic directions. In its own way, each displays an aspect of evolution, whether it be the evolution of a species or of a scientific discipline.

First, I have always had an interest in history, an interest I share with other prefatory essayists in this *Annual Review* series. In editing the first volume in a series on protein metabolism in the 1960s (21, 22), I decided to explore the history of this area for an introductory chapter, and found that I and the people I had worked with early in my career came from a scientific lineage going back two hundred years to the beginning of modern chemistry, like a family tree that progressively evolves in sophistication.

Second, in continuation of the same series on protein metabolism, I concluded that it needed a section on the role of nutrition as a central factor in the evolution of *animals,* which, I was able to show, share a distinctive common pattern of nutrient needs not found in other life forms, a pattern that can only be satisfied by a supply of food. This need distinguishes unicellular animals from unicellular plants or molds, while multicellular animals can be shown to have made evolutionary adaptations to accommodate their needs for nutrients. In particular, mammals, the class we most often deal with experimentally, show an inverse relationship between body weight and daily intake of nutrients per unit of body weight.

Third, during the past decade it has become possible to explore these evolutionary changes at the level of the genome and this has provided me and others with the exciting possibility of observing both conservation of functional components of proteins along with new adaptations as evolution progresses.

The final molding effect on my career has been my good fortune in being associated with the development of the USDA Human Nutrition Research Center on Aging at Tufts University. It offers the possibility of determining how nutritional and other environmental factors cooperate to allow the human to optimize his or her genomic potential throughout the life span and to ameliorate the gradual aging process.

THE HISTORICAL BACKGROUND OF NUTRITIONAL SCIENCE

Scientific research is an evolutionary process in which the present builds on the past, often without recognition of the latter. In the words of Carl Voit in 1865 [cited in translation by Lusk (17)]: "The man of science ought to realise the factors which have given him the vantage which he holds. But there are textbooks on the animal mechanism which do not even mention the name of Liebig. This anomaly is possible only for those who do not understand history and hold only the new to be worthy of consideration." Here, I want to illustrate

how my early association with the nutritional sciences was predestined from the succession of previous investigators whose work pointed in that direction.

I have written elsewhere (23) about the development of protein metabolism and nutrition. Modern chemical science began with the discovery of carbon dioxide by the Edinburgh physician Joseph Black in 1756 (4). That discovery was rapidly followed by the identification of oxygen and nitrogen in the atmosphere. The biological importance of these gases was appreciated from the beginning. Nitrogen was first recognized by its inability to sustain the life of a mouse (35), a property commemorated in the French word for nitrogen, *azote* (Greek "without life"). In France, Black's contemporary Lavoisier developed the first systematic modern chemistry that included gasometric volume determinations for many compounds. In 1790, he wrote to Black to tell him that his gasometric measurements showed that respiration is accompanied by the disappearance of some oxygen and its replacement by carbon dioxide in the expired air, and that this exchange is accelerated some 50% after meals and several-fold by exercise (23). Thus modern chemistry was born in an atmosphere of physiological function, including measurement of the energy needs of different states. It may be noted that energy needs is as good a place as any to start nutritional research. It remains a major field of nutritional research.

Although Lavoisier did not survive the French Revolution, his school of chemistry flourished and provided fertile ground in which the biological sciences could develop. In 1817, the French physiologist Magendie issued his *Elementary Compendium of Physiology* (18), the first modern text on this discipline. In the preceding century, Haller had published an eight-volume text on physiology written in turgid Latin, and without benefit of the new chemistry. With Magendie, we step from the primeval forests of mystery and speculation created by Haller's *Elementa Physiologiae* (12) into the bright sunshine of scientific observation and deductive reasoning displayed in Magendie's book. Because of his Parisian contemporaries, who had been trained in chemistry in the school of Lavoisier, Magendie was able to state that "the proximate principles of animals are divided into nitrogenous and non-nitrogenous. The nitrogenous principles of animals are: albumen, fibrin, gelatin, mucus, casein, urea, uric acid, red-colouring matter of blood. The non-nitrogenous principles are: olein, stearin, fatty matter of the brain, acetic, benzoic, lactic, formic, oxalic acids, as well as sugar of milk, sugar of diabetic urine, colouring matter of bile." Magendie went on to demonstrate that essential dietary constituents included nitrogenous organic compounds, later to receive the unifying name of "protein" by Mulder (20) in 1839.

Another scientist who benefited from the Lavoisier school of chemistry was Liebig, a German scientist who spent the years 1823–1824 in Paris. He carried the new chemistry to Giessen in Germany, where he applied organic chemistry to the study of animal metabolism. From this emerged his book *Animal*

Chemistry or Organic Chemistry in its Applications to Physiology and Pathology (15) in 1840, which laid the foundations of metabolic principles. In 1852, Liebig transferred from Giessen to Munich, where he established a vigorous school of metabolic studies from which emerged Carl Voit, later to be Liebig's successor in Munich. Although balance studies of carbon, hydrogen, oxygen, and nitrogen had been carried out on cattle by Boussingault (5) in 1844, it was Voit who raised the technique of nitrogen balance to fine precision (3). He taught these skills to various foreign workers who went back to their own countries to perpetuate the science of nutrition: Atwater and Lusk to the US, Rubner to Germany, and Cathcart to Britain, the last-named to be the teacher of the present author.

Voit may be regarded for other reasons as the father of modern nutritional experimentation. In 1853 and again in 1865, Playfair (33), professor of chemistry at Edinburgh University, had surveyed the diets of different classes in the British population and had concluded that the daily diet of the average man contained 119 g protein, 51 g fat, and 530 g carbohydrate. In 1881, Voit (38) summarized these surveys and others, including his own, and assessed the needs of the average working man to be 118 g protein, 56 g fat, and 500 g of carbohydrate. At the time, these intakes were also regarded as desirable quantities of each nutrient class needed to maintain health, but were challenged at the turn of the century by Sivén (37), Chittenden (8), and Hindhede (14). The low intakes of protein advocated by these investigators were later rejected by many who had observed impaired resistance to disease on diets low in protein. Thus Voit and his immediate successors had begun to ask the questions with which we still struggle in the field of nutrition, and which are reflected in another chapter in the present volume (39). It may be noted that, through his long-term role as editor of the *Zeitschrift für Biologie,* Carl Voit identified himself as a generalist in biology.

From this brief history, it will not have escaped the reader that science is made up of a series of successive small steps in which skills in laboratory techniques, and practice in both deductive and inductive reasoning that lead to new concepts, are passed on from the older to the younger investigator. In my own case, I first studied protein metabolism in 1934 with D. P. Cuthbertson (later Sir David Cuthbertson, Director of the Rowett Institute, Aberdeen), who at that time was in the Department of Physiology at Glasgow University headed by Cathcart. Cathcart had also acted as a preceptor for Boyd Orr, the founder of the Rowett Institute and later of the Food and Agricultural Organization in post–World War II Rome. Thus, through Cuthbertson and Cathcart, I learned the concepts developed by Voit, who in turn was influenced by the teachings of Liebig, based on the organic chemistry he had learned from the French school of Lavoisier in Paris. Thus the mature investigator is not an island unto himself, but is part of an ongoing process in the evolution of scientific knowledge!

INTERMISSION

The period of my life from the mid-1930s until the mid-1950s was punctuated by a teaching position in clinical medicine, a war, and other changing events. However, the basic allure of studying nutritional and other factors in protein metabolism persisted. Under the guidance of Dr. Cuthbertson, the relationship of energy intake to protein utilization was extensively explored between the years 1936 and 1952 and it still provides basic data. Indeed, my most recent publication (11) on this topic was in 1979 with John Kinney's surgical metabolism group in Columbia College of Medicine and deals with the influence of energy intake on nitrogen balance in surgical cases. But slowly the desire to delve deeper became irresistible. There was a perceived need to integrate the metabolic events that make protein metabolism the wild ballet that it is, with whirling metabolites answering to the orchestration of the endocrine system as they shuttle among the tissues. To reduce this to order, there followed a period of examining the principles on which protein metabolism is accomplished in different mammals. This was consolidated by an in-depth survey of the evolution of animals, seen from the viewpoint of their nutritional needs and their metabolic responses.

NUTRITION AND THE EVOLUTION OF ANIMALS

A defensible case can be made for the view that the main driving force in the evolution of animals from the most primitive forms onward has been the supply of nutrients in the environment (24). It can be presumed that primitive unicellular organisms evolved from non-nucleated (prokaryotic) forms into nucleated (eukaryotic) unicellular forms, such as molds, which still retain the general prokaryotic property of synthesizing all organic compounds required for metabolism. However, it seems likely that, in the early history of the Earth as the fluid surrounding the colonies of such organisms became enriched with their metabolites, it was possible for some mutant cells that had lost the capacity to make certain metabolic pathways to find enough of the missing metabolites to survive. In this way, cells that had eliminated metabolic pathways could multiply even faster than their better-endowed neighbors who had to divert energy to the synthesis of these metabolites. Zamenhof & Eichhorn (40) showed with a histidine-deficient mutant of *B. subtilis* that, in the presence of histidine, this mutant grows faster than the wild type, which continues to make this amino acid and thus has to divert energy to its synthesis.

Eventually, cell populations must have emerged with a stable inheritance of DNA lacking the synthetic pathways for eight to ten amino acids, the B vitamins, and some other essential cell components. These were the first animal cells. Thus the animal kingdom became distinguishable because such cells

lacked the capacity to make a full spectrum of organic compounds. Ever after, evolution of animals was to recapitulate this pattern. For example, a fundamental feature of all animals, from unicellular protozoa up to man, is firmly established to be the dietary requirement for essentially the same series of amino acids whose synthetic pathways were deleted from the DNA of the first animal cells (25).

Nutrition then is a central environmental requirement for the survival and evolution of animal species, in which individual nutrients have to be conserved.

In the case of iron, this element circulates in the blood attached to transferrin. In the mammal, this carrier protein is about 80,000 daltons in size and consists of two nearly identical sequences. However, there was a period in the early evolution of animals when transferrin consisted of only one of these sequences of molecular weight 41,000. It appears that, when the kidney first evolved in *Urochordates,* duplication of the molecule was favored because it then became large enough not to filter through the semipermeable glomerular membrane, whereas the 41,000-dalton transferrin would do so (19). This illustrates how evolution has to adapt nutrient availability to increasing physiological complexity.

In the case of vitamin A, the retinol-binding protein (RBP) is small enough to be filtered through the mammalian kidney and destroyed, but is protected by binding to the much larger pre-albumin of plasma. In fish and tadpoles, RBP lacks the pre-albumin-binding site, since proteins filtered through the kidney are reabsorbed intact into the blood (36). Higher vertebrates obviously adapted by developing the pre-albumin-binding site. The results strongly suggest that the piscine retinol-binding protein is a prototype of the specific vitamin A–transporting protein in plasma of the vertebrates, but was modified later in evolution during phylogenetic development of the vertebrates to acquire a binding site for pre-albumin on the molecule.

Perhaps the evolutionary progression most relevant to us occurred in mammals, where an inverse relationship between mature body size and the intensity of metabolism can be demonstrated. This is expressed by the allometric equation

$$\log M = \log a + b \log W, \qquad \text{or } M = aW^b,$$

where M is the metabolic measurement (e.g. energy needs), W is the weight of the adult animal, a is a constant and b is an exponent of weight expressing the effect of body size on the intensity of metabolism. The exponent b thus tells how body size of mammals affects the metabolic or nutritional measurement. This has been well documented for a range of metabolic processes (25), many of which are related through the 0.75 power of body weight, implying that a 200-g rat has five times the intensity of metabolism of a 70-kg man. For

example, the basal energy metabolism of different mammals is related to the 0.75 power of their body weights. This relationship includes the requirements for essential amino acids, which on the basis of daily needs per kg body weight decline by five-fold between the rat and man. Not surprisingly, protein turnover decreases in parallel, so that, for example, the half-life of plasma albumin becomes longer and longer, being 1.2 days for the mouse, 2.5 days for the rat, 5.7 for the rabbit, 8.2 for a large dog, 18 days for man, and 20.7 days for the cow, a progression related to the 0.66 power of body weight.

Analysis of the livers of these mammals (28) showed a similar decline in RNA content per cell. Thus the machinery for intracellular protein synthesis is geared to metabolic intensity. Others have consequently used the RNA content of tissues to predict the intensity of protein synthesis in organs and tissues, and have confirmed the relationship of concentration of RNA to intensity of protein synthesis. Along with the larger body size of most mammals goes a longer life span. Cutler (9) recently demonstrated that the brain and the kidneys of animals of increasing body size show progressively reduced rates of in vitro peroxidation of lipids. Peroxidation is one putative cause of aging, and these findings thus provide an attractive explanation for the different life spans of mammals.

Since the liver is intimately related to metabolism of incoming nutrients, it is not surprising to find that the relative slowing of metabolism in large animals is correlated with reduction in liver size in relation to body weight, from 6% of body weight in the shrew to 1% in the elephant. However, not all tissues share this proportionate decrease. In the slim young adult animal of any size, muscle remains a little below half of body weight. This means that, in larger animals such as man, muscle assumes a more important role relative to the liver and other viscera in the total metabolism of the body. At the time in the late 1960s when we were examining these effects of mammalian evolution on metabolism, new findings were emerging on exchange of metabolites between tissues, notably between muscle and liver. It is thus becoming possible to provide a complex and quantitative picture of metabolism built into the evolution of mammals. This is not restricted to the well-established meal-related surge of branched-chain amino acids from liver to muscle, and the return of amino groups from muscle to the viscera in the form of alanine and glutamine (26, 27). Evidence from our laboratory and elsewhere is emerging to show that incoming dietary carbohydrates follow an equally elaborate path in which they are reduced to three-carbon compounds in the peripheral tissues for return to the liver in order to provide hepatic glycogen deposition through gluconeogenesis.

The unifying features of structure and metabolism in mammals of different size do not extend to the interface between mother and fetus, namely the placenta (30). In evolutionary terms, the placenta is of recent origin and evolution is probably still experimenting with its structure. Fortunately, despite the wide divergence in the architectural features of placentas, they can be

functionally divided for nutrient transport into two types, depending on whether the placental villi are in direct contact with the maternal blood supply (the hemochorial placentas of higher primates and of rodents) or whether nutrients must first pass from maternal blood through a layer of uterine cells before being transferred to the placental villi (the epitheliochorial and syndesmochorial placentas of the sheep, pig, cow, and horse). In the latter case, the surrounding uterine wall imposes a barrier that has to be crossed. For example, in the pig, iron is taken up into the uterine cells from transferrin in the maternal plasma; these cells then synthesize a second iron carrier protein, uteroferrin, which transports the iron into the placenta in order to reach the fetus (34). Uteroferrin belongs to the class of purple acid phosphatases, an example of evolutionary adaptation to provide a new function related to nutrition.

The special reproductive system of the mammal includes the secretion of milk by the mammary gland, which is believed to have developed from the sweat glands of the skin (16). The milk protein α-lactalbumin bears a structural similarity to lysozyme, an antibacterial glycosidic enzyme present in sweat (6). Lactalbumin is responsible for ensuring synthesis of lactose by modifying the action of galactosyl transferase. This enzyme normally adds galactose to the growing carbohydrate chains of glycoproteins. In the presence of α-lactalbumin, glucose becomes the preferred acceptor for galactose and, as a consequence, milk contains lactose in proportion to its lactalbumin content (7).

This brief survey of the role of nutrition in the evolution of animals emphasizes that molecular changes are constantly at work, adapting the species to the environment. To understand the nature of the nutritional factors in this process, one must answer questions demanding analysis of genomic structure and control of its expression. Some years ago, I became involved in studies of the iron-storage protein ferritin, which is unusual in being an enzyme that stores its product, namely oxidized iron (29). In mammals, the ferritin protein shell is made up of subunits of two sizes. Comparison of the amino acid sequences of the two subunits of human ferritin (10) showed that they were only fifty percent similar in sequences. However, the three-dimensional structure showed preservation of the five helical regions in each subunit protein, resulting in similarity of their gross structures. When amino acid sequences of ferritin were compared in different mammals, it was found that they had diverged by 0.16% for each million years of evolution, implying that the two ferritin subunits probably evolved from a single ancestral protein about 350×10^6 years ago (31), which is about the time of divergence of the α- and β-globins from a single globin chain such as occurs in the hemoglobin of the lamprey.

In the case of hemoglobin, it is well established that the two globin chains are associated with improved capacity to take up and release oxygen as compared to

the single-chain hemoglobin of the lamprey. The advantage of the two subunits of ferritin has not been precisely identified, but must relate to efficiency of iron storage, since free ions of iron in the cell can cause peroxidative damage to unsaturated fatty acids in membranes, to proteins, and to DNA (13). The structure of the messenger RNAs for the two ferritin subunits reflects the importance of this protective function. In the rat, these were found to be stored in the cell cytoplasm in an inactive form that can be rapidly transferred into polyribosome form for active protein synthesis when iron enters the cell (2). In this way, the balance between the need for iron and the risk of peroxidation is achieved by varying the proportion of ferritin messenger that is in the active form. We (31) now have reason to believe that this regulation is achieved through certain features of the secondary structure of the ferritin messenger that make it sensitive to the free iron content of the cell (J. K. Vass and H. N. Munro, unpublished information).

It appears to me that the application of modern techniques for studying genomic function will provide an increasingly deep understanding of the role of nutrients, singly and in combination, on the long-term function and health of animals and man.

THE CHALLENGE FOR TOMORROW'S NUTRITION SCIENTIST

In most sciences, individual scientists are specialists in narrow areas of their discipline that are advancing so rapidly that it is academically suicidal to be other than expert in their own specialized area. Nutritional research tends to impose the need for integration of much biochemical data to meaningful terms for whole animals, so that the generalist is more common. This finds a market because nutritionists are often called upon to make pronouncements on the overall effect of diet on health and disease.

The challenge of integrating an imperfect background of widely diverse but pertinent knowledge was impressed on me by my experiences in developing a program to explore the role of nutrition in the aging process. Nutrition may interact with aging in three ways—first, as a potential means to ameliorate the continuous loss of organ function throughout adult life; second to influence the onset of age-related degenerative diseases; and third, to provide guidelines for intakes of nutrients at different ages, especially for the elderly (32).

I want to emphasize how difficult it is to make judgments in broad fields in which no one view prevails. For example, osteoporosis is an important degenerative condition in which the only agreement seems to be that the loss of bone strength associated with it can be extensive enough to predispose to

fracture (32). Is calcium intake an important factor in osteoporosis? If so, would a high intake be most important in ensuring extensive deposition of bone calcium during adolescence or during middle life, and can it contribute to restoring bone strength after fracture? What is the importance of dietary protein in causing osteoporotic calcium loss from bone and can this be prevented by raising the phosphorus content of the diet through natural foods? What is the importance of vitamin D and its metabolic derivatives in age-related metabolic bone disease? Is there any role for fluoride or for fiber in the prevention of osteoporosis? How much does exercise contribute to maintaining bone strength? Should estrogens be used to prevent post-menopausal osteoporosis, and does supplementary calcium reduce the effective dose of the steroid? Some of these measures, such as raising calcium intake, could be public health options affecting the general population, whereas others, such as administration of estrogens or even of vitamin D metabolites, might be regarded as involving medical decisions for individuals.

To solve nutritional problems of this kind requires an integrated approach for which research training programs in nutrition have in the past usually not provided the appropriate mixture of disciplines. Indeed, university departments of nutrition have often become extinct or nearly extinct, as if after the departure of their leaders nutrition was an aberration in the college's offerings. Fortunately, the interest in nutrition as a health factor that characterized the 1970s was instrumental in triggering government action, one consequence being the establishment of five nutrition research centers working within the Department of Agriculture. This cluster of research centers and the establishment of clinical nutrition core units under the Department of Health and Human Services are solid evidence of government commitment to answering important questions involving human nutrition. In the case of the five USDA centers, the primary objective is to identify human nutrient requirements that will optimize the genetic potential of the individual at all stages of life, as expressed in official objective Number 5 of the Agricultural Research Service Program Plan (1): "To develop the means for promoting optimum human health and well-being through nutrition and family resource management."

The present is clearly an appropriate time for encouraging a renaissance of nutritional science, provided that it can take full advantage of the exciting advances in the basic sciences, as have many other applied sciences.

We must emulate the spirit of Drs. Goldstein and Brown on hearing of their award of the Nobel Prize on October 15, 1985. Dr. Brown said on interview that, in their basic work on lipoprotein receptors, their goal has been to determine why diet has a determining role in atherosclerosis. No team of research workers could better demonstrate the resounding success of basic science as applied to a nutrition-related problem. Back to basics!

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