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PHYSIOLOGICAL ADAPTATION: THE TEST OF NUTRITION

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Physiological adaptation is one of the most important qualities that an individual possesses. It needs to be discussed in contrast to evolutionary adaptation and cultural adaptation, which are distinctly different modes of change. Physiological adaptation should be considered in terms of individual limits that are genetically set, and in terms of physiological cost, which is a subject that seems to be very poorly documented at the present time. If it is true that there is a physiological cost to every physiological adaptation, it should be asked whether the cost can be reduced by providing dietary responses that make the individual more able to meet the particular adaptive problem that he faces. Cultural factors may be involved.

The present work has not begun to explore the role of diet in meeting the onslaught of physiological challenge. Rather, it has attempted to develop a protocol for examining the activity of the liver enzyme tyrosine aminotransferase (TAT), which is extremely susceptible to modulating influences, and to examine an equally responsive parameter simultaneously. This additional parameter is the amino acid transport system. It has been studied by means of two nonmetabolizable radioactive-labeled amino acids, AIB (alphaaminoisobutyric acid) and ACPC (1-amino-cyclopentane-1-carboxylic acid).

Controlled feeding schedules have been applied to rats on diets containing various levels of casein (12, 30, and 60 per cent isocalorically replaced with glucose). Animals were fed in the dark for the first eight hours of a 12-hour dark period in each 24-hour day. Oscillations in TAT and amino acid transport system (TS) were noted and correlated.

Instead of using adaptational challenges, various hormones that represent responses to challenge were administered, along with saline controls. The effect of theophylline alone or with glucagon, when given to animals in a steady

state, was also noted. Marked correlations between the transport system and tyrosine amino transferase activity were observed over periods greatly exceeding the normal diurnal oscillation. The available data suggest important considerations for studying the role of nutrition in physiological adaptations.

ADAPTABILITY AND AMINO ACID REQUIREMENTS

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Homeostasis--the capacity of the body to respond to perturbations in the external environment by adjustments that tend to prevent drastic changes in the internal environment--is particularly well developed in higher organisms.

When protein intake is reduced, urinary nitrogen excretion falls, and, if protein intake is not too low, nitrogen equilibrium is restored at the lower intake with an apparent improvement in the efficiency of nitrogen utilization. In animals, many of the enzymes of amino acid catabolism are adaptive; that is, they increase in activity when protein intake is high and decrease when protein intake is low. These are homeostatic responses that might be expected to have survival value. Elevated activities of amino-acid-degrading enzymes in animals with a high protein intake should facilitate the removal of molecules that cannot be stored and that can cause adverse effects when they accumulate in large quantities in the body. Depressed activities of amino-acid-degrading enzymes in animals with a low protein intake should contribute to the conservation of indispensable amino acids that the body cannot synthesize and that must be redistributed within the body when the protein intake is low so as to maintain those proteins and tissues that are essential for survival.

Waterlow and associates have presented evidence that reutilization of amino acids increases in both man and animals when protein intake is low; that protein synthesis in muscle is suppressed while that in liver is maintained; and that subsequently the rate of catabolism of serum albumin falls.

Whether it is accurate to conclude that these mechanisms tend to reduce amino acid requirements during times of protein deficit is not clear;

nevertheless, they should contribute to improved efficiency in the utilization of available amino acids and delay the adverse effects of protein or amino acid deficiencies. Thus, values for amino acid and protein requirements determined under the usual standard conditions serve only as a guide and do not take into account the adaptability of the body and the capacity of mechanisms that promote survival when protein intake is low. If protein intake is too low, however, the capacity of the homeostatic mechanisms to prevent adverse effects will be exceeded. The concentration of serum albumin and of lipoproteins required for the transportation of fat from the liver will fall, and various other consequences of malnutrition will ensue. More information about the limits of adaptive mechanisms should increase our understanding of nutritional requirements.

ENZYME SYNTHESIS AND DEGRADATION: EFFECT OF NUTRITIONAL STATUS

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There is an extensive and rapid intracellular turnover of enzymes, proteins, and organelles in rat liver. The rates at which specific enzymes are replaced vary markedly; half-lives vary from two hours for tyrosine aminotransferase to 16 days for lactate dehydrogenase isozyme 5. Thus, the amount or level of any given enzyme is a balance between its rate of synthesis and its rate of degradation. For any given enzyme, nutritional variables can lead to altered enzyme levels by affecting the rate of enzyme synthesis or enzyme degradation, or both.

The steady state level of rat liver arginase is directly proportional to the dietary intake of protein. This difference is due to an altered rate of enzyme synthesis; the rate constant of degradation remains unchanged (half-life of five days). Under starvation conditions, after animals have been maintained on an 8 per cent protein diet, the rate constant of degradation decreases, whereas the rate of synthesis does not change. Thus, during starvation there is actually an increase in total arginase content because it is no longer degraded. Conversely, when changing from a 70 to an 8 per cent protein diet, there is an increase in degradation, and synthesis is diminished (Schimke, J Biol Chem 239:3803, 1964).

Another example demonstrates the same theme: the level of rat liver acetyl CoA Carboxylase of rat liver is increased 10-fold on a fat-free diet. Under steady state conditions, this difference is due to a difference in the rate of enzyme synthesis. The half-life of the enzyme is 48 hours in both nutritional states. When rats are starved, there is a precipitous decrease of enzyme as a result of both a decreased synthesis and an increased degradation (Majerus and Kilburn, J Biol Chem 244:6254, 1969).

Thus, shifting nutritional (and hormonal) conditions can modify the enzymated machinery of the liver by altering both rates of synthesis and degradation, thereby allowing for various mechanisms for controlling enzyme levels in animal tissues (Schimke, Ganschow, Doyle, and Arias, Fed Proc 27:1233, 1968).

DIETARY AND HORMONAL EFFECTS ON LIVER GLUCOKINASE

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The enzyme pattern of the liver is tightly related to the diet. Normal levels of certain enzymes depend on an appropriate supply of either protein or carbohydrate, or both. For instance, normal glucokinase levels can be maintained for several days even when carbohydrate is the only source of calories. Carbohydrate deprivation causes a drastic fall in glucokinase activity, which is quickly reversed by glucose. This increase in enzyme activity will be referred to as induction, since indirect evidence indicates that de novo synthesis is involved.

Changes in the diet lead to perturbations in the endocrine balance. Thus, glucose stimulates insulin release, and, in fact, not only glucose but also insulin is essential to maintain glucokinase activity and to initiate the induction of the enzyme. The relationship between glucose and insulin effects may explain peculiarities in the kinetics of glucokinase induction by glucose in normal animals, which will be discussed.

Glucocorticoids, and perhaps other hormones, modulate the rate of glucokinase induction. Adrenal glands are not required for induction, but the rate of enzyme increase is lower in adrenalectomized than in sham-operated rats, and it can be brought to normal values by cortisol.

Glucagon and catecholamines inhibit glucokinase induction. Inhibition is complete when the hormones are administered at the initiation of induction, but not if they are administered when induction is already in progress. These results may be interpreted as hormonal effects at the transcriptional level of protein synthesis, probably mediated by cyclic AMP. The dibutyryl derivate of the cyclic nucleotide inhibits glucokinase induction.

The inhibition by epinephrine can be counteracted partially either by insulin or by α-adrenergic blocking agents. This may be interpreted as an indication that part of the action of epinephrine involves inhibition of insulin release from the pancreas. On the contrary, the inhibitory actions of glucagon and of dibutyryl cyclic AMP on glucokinase induction cannot be reversed by insulin.

THE PHYSIOLOGICAL SIGNIFICANCE OF CHANGES IN TISSUE ENZYME LEVELS AS AFFECTED BY DIET

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Tissue enzyme levels vary according to the quality and the quantity of food ingested. A review of these factors is beyond the scope of the present paper; however, an effort will be made to bring into focus some of the aspects bearing on the mechanism and significance of the changes that take place.

It is not desirable to directly extrapolate the knowledge derived from microorganisms in order to explain enzyme changes that occur in higher organisms. The increasing complexity brought about by differentiation superimposes other mechanisms--namely, the nervous and endocrine systems--and the variations thus depend on an intricate interplay of nutritional, hormonal, and neural factors.

Changes in the synthesis and catabolism rates of a given enzyme affect its quantity. Modification in the concentration of small interacting molecules, such as substrates, coenzymes, activators, inhibitors, and metal ions, affect its activity. Release of enzymes from subcellular particles should also be considered. The control of catabolism becomes important in higher organisms, which seem, significantly, to regulate protein synthesis at the level of translation.

Higher organisms ingest food periodically. They are thus submitted to repeated loads of metabolites, and biochemical oscillations are introduced. Daily variations have been observed in some of the enzyme activities.

In order to fully understand enzyme changes, it is necessary to evaluate other intervening factors. Is it possible to extrapolate from <u>in vitro</u> assays

many times removed from the conditions prevailing in vivo? Is enough known about the biomolecular organization of the multi- or single enzyme systems to understand their physical connections--their actual Km and Vmax values operating inside the cell? This question leads to the problem of channeling and compartmentalization.

In view of the focus of the present symposium, it is relevant to emphasize the enzyme changes caused by faulty nutrition. Some of the tissue protein--part of it presumably enzymes--is removed under a starvation or protein-free diet. The protein biosynthetic machinery is also affected. It may be asked whether the changes in enzyme activity are due to metabolic adaptation or whether they are a consequence of damage caused by malnutrition. Is the capacity to carry out a given function necessarily hampered by a lowering of the activity of the enzymes involved? These questions are discussed.

Illustrative examples of the different situations mentioned, some of them worked out in the author's laboratory, are given.

THE CONCEPT OF "NORMAL" IN NUTRITION

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As medicine concentrates more on the preservation of health, rather than on the cure of disease, the question of normal standards and normal ranges comes increasingly to the fore. The extensive studies done in recent years on blood pressure levels in different populations, stemming from the current interest in cardiovascular disease as a major cause of death in developed countries, is a good example of this new focus.

In nutrition and in hematology, the definition of normal ranges for biochemical measurements is of great importance, because this is the means by which "subclinical" deficiency states are detected in individuals at risk. It seems that at present the basis on which such measurements are interpreted is very unsatisfactory.

Human beings can adapt to different levels and patterns of food, and it is therefore likely that for most of the parameters measured there is a range of adaptation within which normal function is preserved. The problem is to define this range.

It is argued in this paper that normal levels and ranges cannot be defined on a statistical basis. Alternative ways of approaching the problem include (1) comparative or prospective studies permitting an accumulation of experience; (2) functional studies; and (3) increased knowledge of the physiological and biochemical mechanisms that control the elements in question.

PHYSIOLOGICAL AND SOCIAL ADAPTATION IN FAMINE

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No abstract available.

STANDARDS FOR THE DIAGNOSIS OF VITAMIN DEFICIENCY IN MAN

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The spectrum of vitamin deficiency states, like any other nutritional deficiency, extends between two theoretical limits: optimal nutrition, when the metabolic needs for the vitamin are satisfied, and extreme deficiency, when the lack results in such severe metabolic and organic alterations as to interfere with the life process. In between, there is a continuum of stages approaching either extreme to different degrees. The first task in the development of standards is to define the characteristics of subjects at the two extremes. The second would be to design quantitative means of expressing values over the range between. Dietary, biochemical, clinical, and functional criteria have been tried for this purpose. Actually functional tests should be the ultimate criterion, but unfortunately they are also the most difficult to evaluate. In view of this problem, efforts have been concentrated on the development of dietary and biochemical indexes. In general, these are numerical expressions of measurable characteristics which ideally may be related to a state of functional alteration, but which can feasibly be applied to human subjects as well. They express arbitrary estimates of the position occupied by a given subject on the spectrum between the two extremes. Unfortunately, in many cases the relationship of the indices to the function is still poorly defined. The present paper discusses three cases that can be taken as models of what may be done to develop standards when relatively sufficient knowledge is in hand.

Improvement of currently available standards cannot be accomplished through discussion or critisism; it can only be done by research.

ANEMIA AND NORMALITY

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The automation of diagnostic laboratory procedures has made it possible to extend the scope of clinical assessment to the community outside the hospital. Fundamental to the interpretation of each observation is a knowledge of the range of values that should be considered normal for the person concerned. Widespread use of automated techniques should make it possible to reassess normal values for hemoglobin and other body constituents, making use of large numbers of observations and also of improvements and standardization of methods.

The differentiation between anemia and normality based on an arbitrary Hb concentration remains a statistical concept. The results of random surveys of Hb concentrations, although representative of the group under study, may not represent optimal levels. This shortcoming may be largely of academic interest when the main objective is the detection and treatment of overt anemia in the developing countries, where nutritional anemia is a serious public health problem. On the other hand, in clinical medicine the individual patient's optimal Hb concentration is the significant parameter, and the acceptance as normal of an Hb concentration within the statistical "normal" range may result in delayed diagnosis of latent deficiencies of iron, B_{12}, or folate, which in turn may reduce the Hb concentration below its optimal level in apparently healthy subjects. Careful screening of the blood film and specific tests for iron, B_{12}, and folate deficiencies, especially in patients and population groups at risk, will help greatly in the early detection of these deficiencies before the onset of overt anemia or other complications. While it is still essential to define anemia as a parameter of health, the Hb concentration cannot be regarded as a sensitive index of nutritional status. The latter can better

be determined by direct measurements of the nutrients concerned. To make this a feasible proposition for clinical screening and nutritional surveys, it will be necessary to automate the measurement of as many nutritional parameters as possible.

ENDOCRINE MECHANISMS IN NUTRITIONAL ADAPTATION

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The restriction of nutrients during the period of rapid growth produces numerous biochemical alterations, particularly in the endocrine system. In general, responses are related to the intensity of malnutrition, the length of time the child has suffered from malnutrition, and the type of nutritional deficiency.

The response of the endocrine system seems to differ in patients whose malnutrition is due to a low calorie and protein intake as compared to those with a pure protein deficiency. These two etiologies correspond to two different clinical syndromes: marasmus and kwashiorkor. In typical cases, the symptoms are quite characteristic. Pure cases, however, are exceptional; most of them are of mixed etiology and consequently exhibit a mixed symptomatology. To distinguish between the two syndromes, the term "typical kwashiorkor" will be used in referring to conditions in which the etiology corresponds to a protein deficiency alone. In this type of malnutrition, the disease is acute and the full symptomatology appears after a short time on a protein-deficient diet. The experience with typical marasmic patients, on the other hand, has been that malnutrition starts during the first months of life and that they reach one year of age with almost the same weight and size they had at birth. These patients have a chronic disease and in some way get adapted to this condition by decreasing their calorie consumption.

Marasmic infants have diminished functional capacity of the adrenal glands. ACTH administration is able to induce an adrenal response, but two or three days later the gland becomes exhausted, suggesting a limited adrenocortical reserve. In kwashiorkor, plasma cortisol levels have been found to

be consistently about twice the normal values.

The thyroid function in marasmic patients is also decreased, as demonstrated by low oxygen consumption, low iodine uptake, and low plasma butanol extractable iodine. The administration of TSH corrects the iodine uptake and the levels of butanol extractable iodine but only partially corrects the oxygen consumption. In kwashiorkor, the oxygen consumption is normal.

The levels of plasma HGH in marasmic patients is lower than normal, and the administration of arginine does not change the picture. In kwashiorkor, plasma HGH levels are normal or higher than normal, and the response to arginine stimulation is also normal.

All these data point to the conclusion that in marasmic patients there is a decrease of hypophysial function and, as a consequence, a decrease of thyroid and adrenal function. In kwashiorkor, these changes cannot be demonstrated, and the response appears to be similar to what has been described in acute stress.

HOMEOSTATIC MECHANISMS IN THE REGULATION OF SERUM ALBUMIN LEVELS

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In human subjects or experimental animals exposed to low protein diets, diminished hepatic synthesis leads to lowering of the serum albumin level. A decrease in the rate of catabolism of albumin has been shown to occur, and at the same time albumin is probably transferred from extravascular sources to the plasma pool. If adequate dietary protein is introduced at this point, the changes are reversed: albumin synthesis increases; the albumin pools are restored; and catabolism returns to its normal rate.

Adaptation of catabolism can also be shown to occur in cases of hypoalbuminemia resulting from liver disease and experimental plasmapheresis, although if loss takes place through the kidney (nephrotic syndrome) or gut (protein-losing enteropathy) local hypercatabolism might obscure the expected compensatory fall. A similar adaptive response is seen when serum albumin levels are raised by infusion; in this case, maintenance of a normal fractional catabolic rate allows increased breakdown of albumin in absolute terms. Alterations in the rate of catabolism thereby provide the body with a powerful means of regulating the level of albumin in the plasma.

The isolated perfused rat liver may be used to study adaptation to dietary protein deprivation. Organs taken from animals deprived of protein for two or three weeks show diminished rates of albumin synthesis, although these lower levels are normal in relation to the size of the liver. This finding needs to be considered in the light of known alterations in polysome profile--disaggregation to polysomes of lower ribosomal content--that occur after protein restriction. Livers taken from rats refed a normal diet after a period of deprivation show albumin synthesis rates that are greater than normal. This is consonant with <u>in vivo</u> observations.

The isolated rat liver may also be used as a model for studying albumin catabolism. Livers taken from normal animals maintain a fixed fractional rate of breakdown even when the albumin level of the perfusing fluid is drastically increased. Low rates of catabolism are shown by livers taken from protein-deprived animals, but these can be restored to normal levels by addition of albumin to the perfusate pool. Reduced pinocytic activity or lysosomal proteolysis is thought to occur after protein deprivation.

On the basis of these observations, it is possible to formulate a concept of the control of albumin metabolism and its adaptation to changes in dietary protein content or other similar types of disturbance.