Epilogue

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It was inevitable that the phenomenal advances in enzymology should soon provide means for clarifying the heretofore obscure mechanisms of life and disease. The list of these advances grows daily. Established beyond a doubt is the role of enzymes in the biosynthesis of proteins, glycolysis, energy conversions for cellular activities, inborn errors of metabolism, growth and physiology of cancer cells, and a host of as yet unexplored nutritional and biochemical phenomena (1).

Although the human body is deceptively constant in composition, it is now well known that it is in a perpetual state of flux. The dynamic equilibrium existing between anabolism and catabolism of essential constituents within the cells is controlled by enzyme systems and is reflected in the relative level of certain specific factors and metabolic products, e.g., amino acids and other metabolites in the circulating body fluids (2). Consequently, alterations of activity levels of certain enzymes have come to be recognized as an important factor in clinical syndromes of certain human diseases (3) and the existence of biochemical individuality ranging from subclinical to overt aberrations in metabolism (4).

In addition to the classical examples of *biochemical biopsy* via the blood stream, it appears now that measurements of enzymes in body fluids may provide powerful and penetrating tools for the study of the minutiae of body processes. With the advancement of microchemical procedures, the assay of serum enzymes has become increasingly useful in diagnostic evaluations (5). However, the quantitative determination of blood enzyme activity for research purposes may be even more promising, inasmuch as the lack of well-standardized norms for age, sex, or anthropometric categories is not a limiting consideration. Although

interpretation of biochemical and nutritional changes in terms of enzymatic processes is still rudimentary, future development of simple, rapid, and sensitive methods for the determination of specific enzymatic activity in body fluids will permit further investigation of the basic rate of enzyme processes in health and disease.

From extensions of the hypothesis of biochemical individuality of amino acid needs developed by Williams (6), it may be presumed that in the organs and tissues of every individual there will be found more or less distinctive enzyme patterns. This consideration does not suggest that the assortment of enzymes present would actually be different and qualitatively distinctive for different individuals, but that for genetic reasons the efficiencies of the different enzymes and enzyme systems would vary from individual to individual. The existence of these variants has been termed "molecular heterogeneity," and the variants have been termed "isozymes" (7).

In order to obtain comprehensive evidence regarding these variants, and the degree to which they are significant and important, it is necessary to have extensive data on the enzymatic make-up of different individuals derived from serial sampling of the blood elements. From the metabolic diagram in Fig. 1 (8) it is at once apparent that the biochemistry of amino acids is closely integrated with the over-all homeostasis of the body (9). In general, the numerous enzyme systems which constitute the catalysts of the metabolic machinery of cells are now known to affect and to be affected by the utilization of essential nutrients, e.g., vitamins, amino acids (10). Specific evidences of the interrelationship of body enzyme levels and nutritional state have been reviewed by Ashida (11). Additionally, Allison and associates (12) have shown in experimental animals that the levels of ribonuclease, ribonucleic acid, and tissue proteins are closely related to the biological value of dietary proteins. Similar studies of Klain and co-workers (13) lend support to the existence of this relationship with respect to various transaminase and aminoacidase systems. These and other reports indicate that under conditions of nitrogen-limiting diets (specific or total) it is conceivable that loss of body protein involved mainly in structural functions of the organism would not be so detrimental were it not for the fact that tissue enzymes, which are also proteins, are built from the same essential amino acids that are utilized in building body proteins.

The foregoing suggests the feasibility of using blood enzyme patterns to determine the effects of various factors on nutritional requirements and *vice versa*. It has long been known that changes in the diet of animals provoke important variations in carbohydrate metabolism. For instance, glycosuria occurs when carbohydrates are given to a dog that

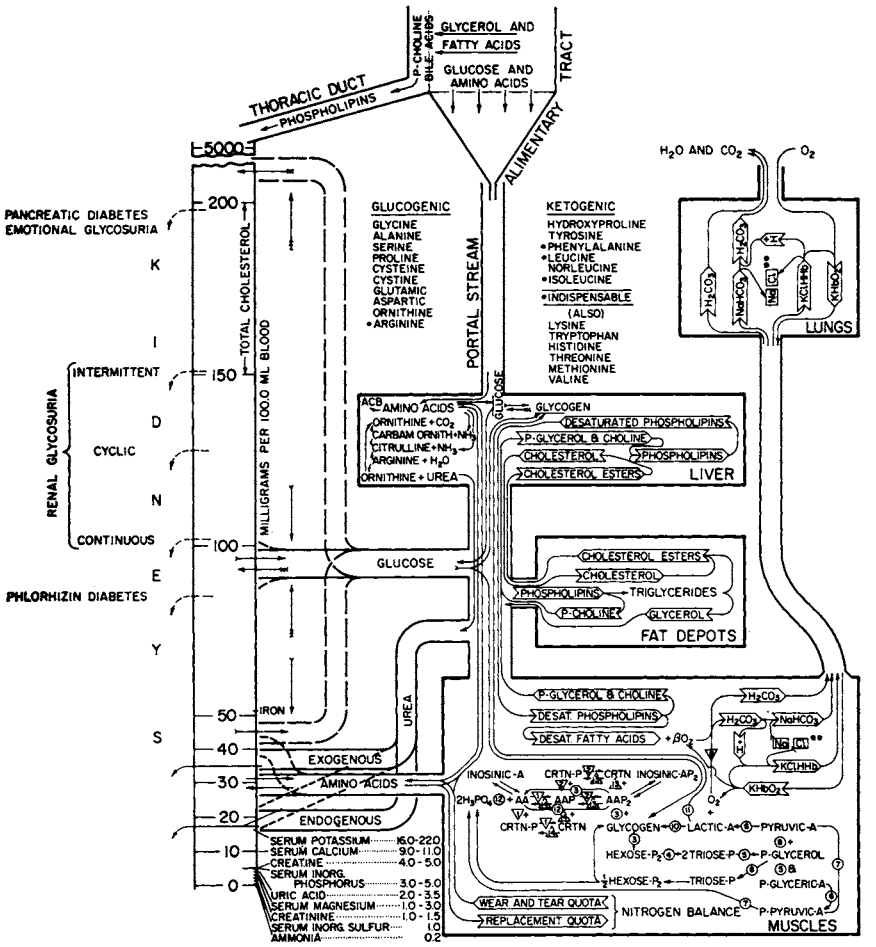

FIG. 1. A graphic representation of intermediary metabolism, showing the utilization of proteins, fats (according to the Verzar and Cahn-Houget theories), and carbohydrates. From Rask (8). KEY: CRTN = creatine; AA = adenylic acid; P = orthophosphate radical; + connects reacting substances; & connects reaction products; ⋙→ indicates epinephrine action; ⟶ indicates insulin action; —▽→ indicates exothermic reaction; —△→ indicates endothermic reaction; AC.B = acetone bodies ** signifies additional anions $^{-}HCO_3$ and $^{--}HPO_4$.

ACTION OF EPINEPHRINE AND INSULIN

Action	Epinephrine	Insulin
Glycogenesis:		
In liver	No action	Facilitates
In muscles	Depresses	Essential
Glycogenolysis:		
In liver	Accelerates	Inhibits
In muscles	Accelerates	No action
Carbohydrate oxidation	Depresses	Nonessential
Release of liver amylase	Facilitates	Inhibits

has been fasted or fed on a low carbohydrate diet for several days. This observation, made by Claude Bernard (14), was called later "hunger diabetes" by Hofmeister. Simultaneously, storage fat is mobilized and used. A decrease in the carbohydrate content in food also diminishes the capacity of the body to utilize carbohydrates and increases glyconeogenesis from protein (15–17).

How can these facts be interpreted at an enzymatic level? Alterations occurring in the metabolism of a cell may well reflect changes in enzymatic activity which lead to variations in the relative rate of certain reactions and metabolic sequences and to discrimination between several alternative pathways (Fig. 1). The question may be asked as to which are the possible mechanisms that bring about changes in the activity of an enzymatic system (18, 19). An excellent review by Knox *et al.* (20) shows that many circumstances, including variations in diet, may provoke changes in the enzymatic composition of the cell.

Generally speaking, there is a good correlation between enzymatic activity and cell functions. Enzymatic changes appear to be an adaptation, favorable or necessary for metabolic changes in the tissues or the body as a whole, in response to variations in diet. Several examples can be given to illustrate this statement. Thus, when fructose is substituted for glucose as a source of carbohydrate, metabolic alterations must be expected because the liver must convert an important amount of fructose into glucose and glycogen (gluconeogenesis) in order to satisfy the demand of other tissues which cannot use fructose efficiently, or even are strictly dependent on glucose (21). It has been shown that slices of liver tissue taken from rats on a fructose diet utilize more fructose and less glucose than similar tissue slices from rats on a normal diet (22). An analysis of the enzymes in these tissues shows enzymatic changes that are an adaptation to a diet of fructose. There is an increase in fructokinase (23), fructose diphosphatase (24), phosphohexoisomerase (25), and glucose-6-phosphatase (25, 26).

The decrease in the utilization of glucose found in intact animals, and in tissue slices, as a consequence of fasting or a high fat diet can be correlated to the decrease in glucokinase (27–29) and many other enzymes directly concerned with the metabolism of its immediate product, glucose-6-phosphate (Table I). Some of the enzymes, however, diminish less than others, and glucose-6-phosphatase increases somewhat (26, 35). Levels of this enzyme appear to be related with gluconeogenesis from carbon residues of amino acids. The maintenance of the activity of other enzymes such as glutamic dehydrogenase in fasting animals (42) and the increase in glutamic pyruvic transaminase caused by fasting and high protein diets (43) are also related to gluconeogenesis. It is interest-

TABLE I

CHANGES IN THE ACTIVITY OF SEVERAL ENZYMES CAUSED BY CHANGES IN THE DIET[a]

Enzyme	Dietary conditions[b,c]						
	Fast	Fat	Protein	Carbohydrate	Glucose	Fructose	Galactose
Glucokinase	−(27, 28)	−(27, 29)	−(29)	=(27)			
Fructokinase	−(23)					−(23)	
Phosphoglucomutase	−(31)				=(25)	=(25)	
Phosphorylase	−(32, 33)	−(29, 34)	=(29, 34)	=(34)	=(34)		
Phosphohexoisomerase	−(31)					+(25)	
Glucose-6-phosphatase	+(35); −(31, 36)	+(26)	+(26)	−(37)	=(25)	+(25, 26)	+(26, 38)
Fructose-d-phosphatase	−(39)		+(24)			+(24)	
Glucose-6-phosphate dehydrogenase	−(27, 40, 41)	−(27)			+(25)	+(25)	
6-Phosphoglucomutase dehydrogenase	−(27, 40, 41)	−(27)			+(25)	+(25)	
Glycogen synthetase	−(27)	−(27)		+(27)			

[a] From Niemeyer (30).

[b] Fasting was of variable duration. In the fat and carbohydrate diets there was usually a minimum of protein. In the diets indicated as glucose, fructose, and galactose, these hexoses were the only source of carbohydrates.

[c] Key: minus sign (−) indicates diminution of activity; plus sign (+) indicates an increase; equals sign (=) indicates no change. The numbers in parentheses indicate references.

ing to point out, in order to establish new correlations, that an increase in transaminase has been observed in animals treated with hydrocortisone (43), a hormone which is known to stimulate gluconeogenesis. It must be pointed out, however, that changes caused by feeding a high protein diet occurred in adrenalectomized animals (43); therefore this effect of the diet is not mediated by adrenal hormones.

Recent reports indicate that serum enzyme levels are affected not only by changes of diet, but also by physical activity (44). In the most thorough studies carried out to date on exercise in normal subjects, Fowler and his associates (45) studied trained and untrained males and females undergoing varying degrees of exercise. Serum enzyme activities were measured at 5, 15, 30, and 60 minutes after exercise. These measurements included serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), aldolase (ALD), lactic dehydrogenase (LDH), and malic dehydrogenase (MDH). The subjects were exercised for 15 minutes, and it was found that the degree of increase in serum enzyme activity was related to the previous training of the individual and the severity of exercise over the 15-minute period.

In a more recent publication, Swaiman and Awad (46) reported that eleven volunteers subjected to 10 minutes of strenuous controlled exercise showed no significant increase at 10- and 60-minute intervals in the serum concentration of the aforementioned enzyme systems, as well as

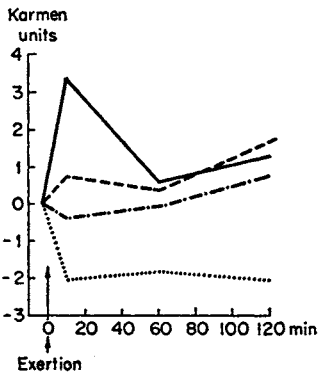

FIG. 2. A comparison of the average changes in SGOT after exercise in normal individuals and cardiovascular patients (47).

KEY: ——— patients with coronary disease and ischemic changes in the electrocardiogram (ECG) after exercise; ----- patients with coronary disease and ischemic changes in the ECG after exercise, treated with nitroglycerin; -·-·-·-·- patients with cardiovascular disease and no ischemic changes in the ECG after exercise; ..... individuals with healthy hearts and normal ECG after exercise.

creatine phosphokinase (CPK). They concluded that the exercise was not of sufficient duration or severity to produce changes previously reported in the literature.

The findings of Nerdrum and Nordøy (47) are summarized graphically in Fig. 2. It will be noted that the SGOT after exercise varied with clinical state of the subjects. Of the thirteen normal healthy subjects in this study, only one subject, who was in training for competitive sport, showed an increase in SGOT at both the 10- and the 60-minute intervals after exercise. All the others showed SGOT decrements at 10, 60, and 120 minutes after exercise. As an aside, it is of interest to note the normalizing effect of the administration of nitroglycerine on this biochemical parameter in patients with coronary disease.

These prior observations, and the many military and civilian implications of the protein catabolic effects of hypokinesia, encouraged our group to study the protein biosynthetic aspects of the phenomena (44). To this end, ten healthy, normal young adults were studied on 2 consecutive days, 2 hours after a standard breakfast which provided an average of 300 calories and 4 gm of protein. On the first day blood samples were collected before and after 20 minutes of bed rest. On the second day the same procedure was employed before and after 20

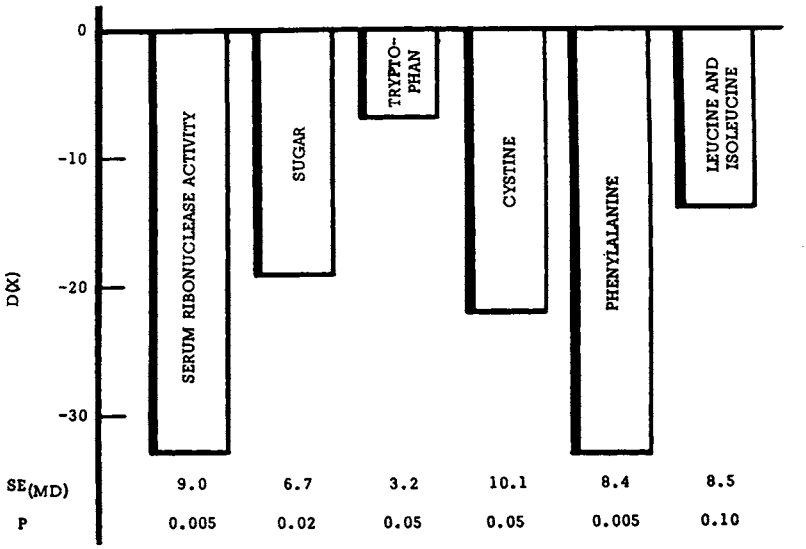

FIG. 3. Representative changes in some blood constituents associated with physical activity of ten young, healthy normal adults. Observed changes in plasma-free lysine, threonine, methionine, glycine, alanine, the prolines, and aspartic and glutamic acid (not shown) were all below the acceptable level of significance.

minutes of operation of a stationary bicycle at fixed tension and at 10 mph. Measurements of serum ribonuclease (RNA) activity and other blood constituents were initiated immediately following collection of the blood specimens. Detailed tests of accuracy of the serum RNA method showed a high degree of reproducibility (±3%). Results of other measurements were well within the necessary degree of confidence. The findings are shown diagrammatically in Fig. 3. These data reveal a remarkable uniformity in the decrease of the blood constituents listed, and particularly so for the decrements of RNA activity, sugar, and plasma-free phenylalanine, all of which show an exceptionally high probability. The changes in plasma-free tryptophan and cystine were also of significant probability. The changes in other amino acids were below the level of significance.

In the course of these studies a preliminary attempt was made to relate prior physical activity states with biochemical response. Results of the first study are shown in Fig. 4. It is apparent from these data that

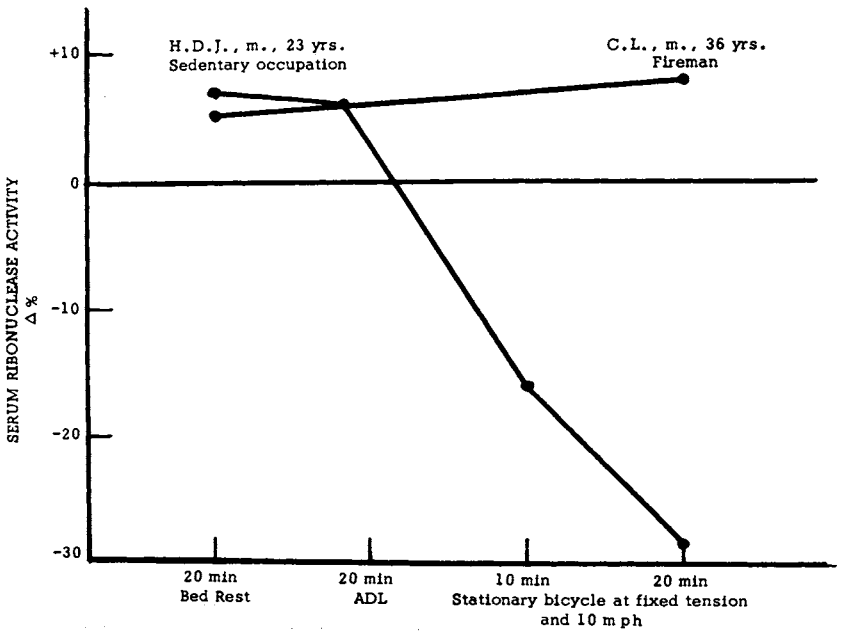

FIG. 4. The effect of bed rest, activities of daily living (ADL), and exercise on serum ribonuclease activity of a sedentary and an active individual.

lack of prior training is reflected in the magnitude of RNA change in the blood following exercise and parallels the aforementioned findings with SGOT.

It may be speculated that these observations have some significance in clarifying the protein losses of immobilization which have been observed by a number of investigators in both healthy normal and convalescent adults (Fig. 5).

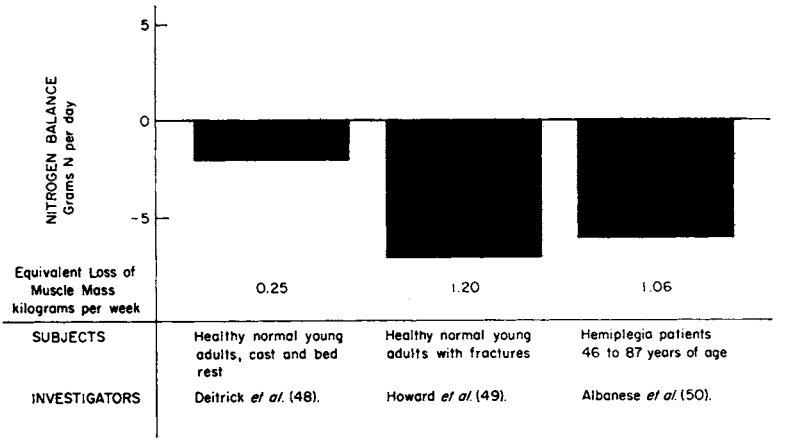

FIG. 5. Protein catabolic effects of immobilization.

It is apparent from the results shown in Fig. 6 that the protein losses of inactivity are substantially reduced by increased physical exercise.

The juxtapositions of these observations suggest that alterations in serum RNA and GOT levels may be involved in the nitrogen retention effects of exercise. The mechanism of this process remains to be explored.

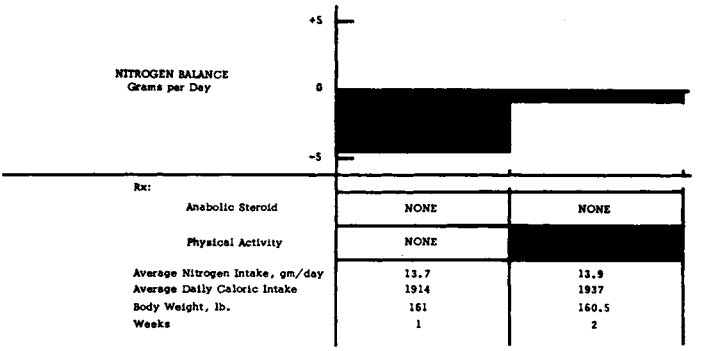

FIG. 6. The effect of physical activity on nitrogen balance of an elderly male patient [J. E., 62-years old, 161 lb (with brace), 103% S (per cent of standard weight, according to the Tables of the Metropolitan Life Insurance Co., revised 1951)] with right hemiplegia after a cerebrovascular accident (CVA) in September, 1962. Study made in December, 1963.

Apart from the biochemical aspects of the protein catabolic effect of immobilization, its implications in efforts to determine protein and amino acid needs are clear. Specifically, the protocol of such studies must include a measure and standardization of physical activity of the test subjects during the course of study. The broad range of values of protein needs of man, which are now in the literature, may well be due to a failure to recognize the nutritional significance of physical activity as a decisive parameter in protein nutrition (51, 52).

Indeed, failure to recognize the multitude of endogenous and exogenous factors affecting protein metabolism has led to some serious errors in interpretation of nitrogen balance data. This position has recently been re-emphasized by Mitchell and Edman (53) who note that there are significant but ignored losses of nitrogen through the skin as sweat and as sloughed off skin, hair, and nails. Since all nitrogen balance data obtained in studies of amino acid needs have failed to recognize these losses, Mitchell feels that the requirement values thus obtained are inaccurate. There is some discussion as to the magnitude of these losses, which range from 0.5 to 1.0 gm per day. If it is assumed that the lower

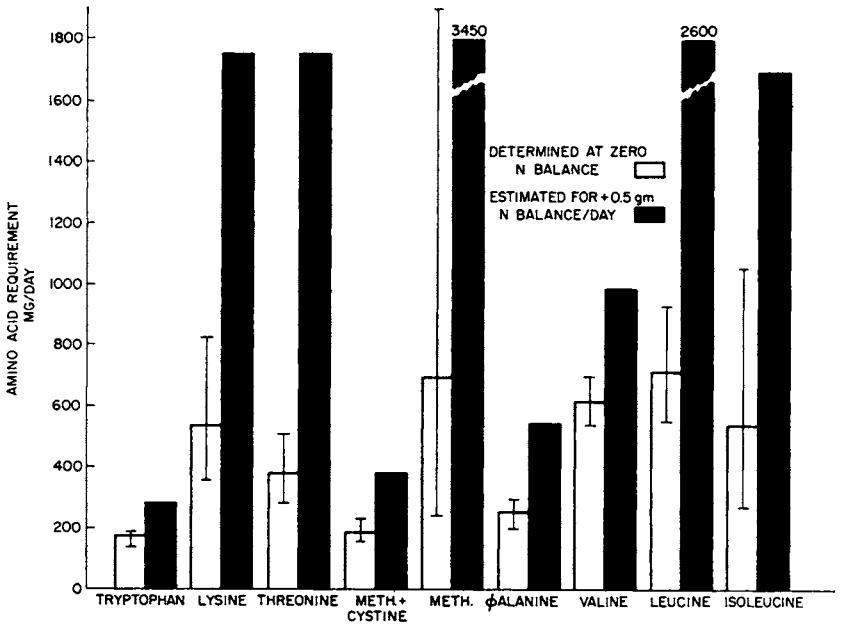

FIG. 7. Estimates of amino acids required to produce zero and +0.5 gm per day nitrogen balances. The capped vertical bars indicate the range of reported requirements.

value, namely 0.5 gm, of nitrogen per day is thus lost, then all amino acid requirement data should be corrected to a balance of +0.5 gm of nitrogen balance from the zero balance end point employed by Rose, Leverton (54), and others.

Since the relationship of individual amino acid requirement to nitrogen balance is a logarithmic one, the percentage increase in requirement is related to the slope of the regression line. These calculations are summarized in Fig. 7. It will be noted that the increases are large and vary considerably for each of the amino acids. This error is further compounded and complicated by the broad range of nutritional individuality of the amino acid needs. These, it will be seen from the figure, are quite extensive.

In conclusion, it is clear from these few examples that the biochemical approach to nutritional problems must be constantly encouraged and considered in order to obtain data of the utmost fundamental and practical usefulness.

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