regulation of enzyme activity and synthesis together with tissue synthesis and repair within the body. A deeper understanding of these topics and, in particular of the control mechanisms operating on the primary biochemical pathways, would appear to be necessary for more precise definition of protein and amino acid needs. At present estimates vary by a factor of two, or more. Closer evaluations are clearly desirable, the more so when calculations are made of the quantities of food, and consequent effort, needed to satisfy demand for whole populations. Provision of only 1 g of $N \times 6{\cdot}25$ per day to each of a population of 50 million people for instance would entail production of about 2×10^5 tons of cereal, 1×10^5 tons of legume, 5×10^4 tons of skim-milk solids or 6×10^7 broiler fowls per year—all requiring a considerable investment in land, labour and materials.

This example indicates the magnitude of the supplies that may be needed and hints at the economic and social problems that must be overcome if such quantities of extra food are to be produced and if the populations at risk are to be provided with the funds to pay for it. Sources of protein other than the traditional foods might well significantly contribute to man's needs. For this reason, therefore, we are fortunate to be able to hear at this meeting of the ways in which protein resources could be greatly increased, by the improvement of some and the better use of others, together with development of entirely novel products.

REFERENCE

Advisory Committee on the Application of Science and Technology to Development (1968). In *International Action to Avert the Impending Protein Crisis*, p. 40. New York: United Nations Organization.

The absorption of protein from the intestine

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This paper was prepared at very short notice and no written version will be published.

Adaptation of mammalian protein metabolism to amino acid supply

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Introduction

The metabolism of the animal is equipped to adapt to changes in both the internal and the external environment. Among internal factors are activity versus rest and sleep, and the menstrual cycle in the case of the female. Metabolism must also respond to variations in the external environment, such as heat and cold, and notably

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the availability of food. Metabolic adaptation to nutrient supply is of two kinds. First, there are transient physiological adaptations to the intermittent intake of nutritionally adequate meals. These short-lived adaptations account for a large part of the diurnal variations that have been observed in the protein metabolism of mammals (Wurtman, 1969). Secondly, long-term adaptive reactions occur when there is a decrease in availability of an essential nutrient in the diet. Under such circumstances, tissue constituents are lost to varying degrees from different parts of the body. It is proposed to discuss here mainly short-term physiological adaptations to variations in amino acid supply and their relevance to the needs of the body for dietary protein. Adaptive changes resulting from long-term protein deficiency are considered in the paper by Waterlow & Stephen (1969).

In order to classify regulatory responses affecting protein metabolism, it can be considered that the free amino acids within the body follow three types of metabolic pathway: first, they can be used for protein synthesis; secondly, they can be employed to synthesize various compounds of low molecular weight, such as creatine and non-essential amino acids; finally, the free amino acids can undergo degradation through the pathways of amino acid catabolism. These competing uses of amino acids are in equilibrium, so that an alteration in one route of disposal is frequently compensated by reciprocal changes in the other pathways. For example, the administration of hydrazine to rats impedes amino acid degradation by inhibiting transamination (McCormick & Snell, 1961). This produces an increase in the free amino acid levels in the tissues, and in the case of the liver it has been demonstrated that protein synthesis is augmented (Amenta & Johnston, 1963), a clear case of cause and effect.

In relation to assessment of protein needs, an obvious question to ask is whether, with increasing intakes of protein, these metabolic pathways adapt linearly to the load of amino acids consumed, or whether there is a sudden increase in catabolism when intakes of protein in excess of requirements are reached. A secondary question is whether adaptive reactions to amino acid supply occur mainly during the period of absorption of the meal or develop more slowly in relation to a change in general level of protein intake. In considering such questions, it should be remembered that protein synthesis occurs actively in many parts of the body, whereas certain pathways in the catabolism of essential amino acids are restricted to the liver, a notable exception being the branched-chain amino acids which are mainly degraded in the carcass (Miller, 1962). Consequently, variations in amino acid supply are likely to evoke different responses in different tissues. Much of our knowledge of adaptive processes has been obtained from studies on liver metabolism. This should not be allowed to obscure the participation of other tissues in adaptive reactions.

Response of protein metabolism in the liver to a meal of protein

During the absorptive period after each meal containing protein, the liver is subjected to an extensive increase in amino acid supply. The free amino acids in the portal blood rise several fold over those in the systemic circulation (Denton & Elvehjem, 1954). We can therefore ask whether the liver exercises a significant

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selective action on the amounts of amino acids that pass through it and enter the general circulation. Elwyn (1969) has examined this question, using dogs with cannulas implanted in the portal vein, the splenic artery (representing arterial blood going to the liver) and the hepatic vein as it leaves the liver. With these animals he has been able to monitor continuously the exchange of amino acids across the liver during 24 h periods. After feeding a large meal of meat to his dogs, he finds that 57% of the absorbed amino N is converted into urea as it passes through the liver, some 6% leaves the liver as plasma proteins, and only 23% enters the general circulation as free amino acids; the remaining 14% unaccounted for is presumably retained in the liver as hepatic protein. Individual amino acids deviate somewhat from this pattern. In particular, a large proportion of the intake of branched-chain amino acids passes into the systemic circulation, since they are mainly degraded in the peripheral tissues. In general, however, Elwyn's results indicate that, when large amounts of protein are fed, the systemic circulation is protected against excessive changes in free amino acid concentrations by immediate adaptive responses within the liver. Since there are as yet no similar studies on animals receiving smaller meals of protein, we do not know whether these provoke less dramatic adaptive responses from the liver, such that a large proportion of the amino acid load passes into the general circulation.

The evidence obtained by Elwyn allows us to conclude that adaptive reactions in the liver play an important role in the utilization of dietary protein. We have recently examined the response of protein synthesis in the liver during the influx of amino acids. Fasting rats were fed by stomach-tube with nutritionally complete or tryptophan-deficient mixtures of amino acids an hour before killing (Fleck, Shepherd & Munro, 1965). Microsomes were isolated from the livers of such animals and were incubated with [^{14}C]leucine and the various co-factors necessary for cell-free protein synthesis. The microsomes prepared from rats fed with the amino acid mixture lacking tryptophan were some 20% less efficient in incorporating leucine into pep-

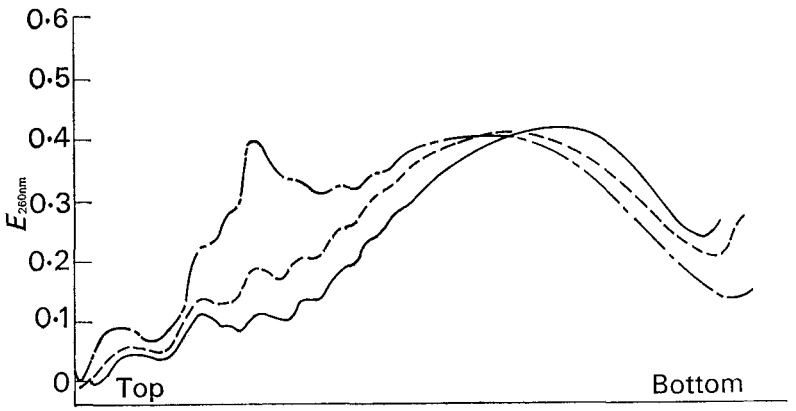

Fig. 1. Changes in liver polysome profile caused by feeding fasting rats with either a complete amino acid mixture (———), or a mixture lacking tryptophan (–.–.–), and killing 1 h later. The broken line (– – –) represents the profile in fasting animals. (From Wunner, Bell & Munro, 1966.)

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tides than were microsomes prepared from rats given the nutritionally complete amino acid mixture. Since the microsome fraction owes its amino acid incorporating capacity to its contained polysomes, it was logical to examine next the profiles of polysomes obtained from the livers of rats fed these two amino acid mixtures. The membranes of the endoplasmic reticulum were removed with deoxycholate and the total population of free and membrane-attached liver ribosomes was resolved into polysome aggregates of various sizes on a sucrose density gradient. Fig. 1 shows that animals fed with the amino acid mixture lacking tryptophan an hour before killing had fewer heavy polysomes and more monosomes and disomes than had rats given the nutritionally complete amino acid mixture. In later experiments (Wunner, Bell & Munro, 1966), the disaggregation caused by feeding the amino acid mixture lacking tryptophan was shown to be rapidly reversible by administering the missing tryptophan. These studies thus demonstrate that the polysome system responsible for protein biosynthesis in the liver is sensitive not only to the supply of amino acids, as confirmed by several authors (e.g. Staehelin, Verney & Sidransky, 1967), but also to the quality of the amino acid mixture.

The influence of amino acid supply on liver polysome aggregation appears also to be linked to RNA turnover in the liver cell. When rats are fed with the tryptophan-deficient mixture, not only do monosomes accumulate, but there is also an increase in free ribosome subunits (Wunner *et al.* 1966). Since dissociation of ribosomes into subunits causes activation of their latent ribonuclease, it has been concluded that

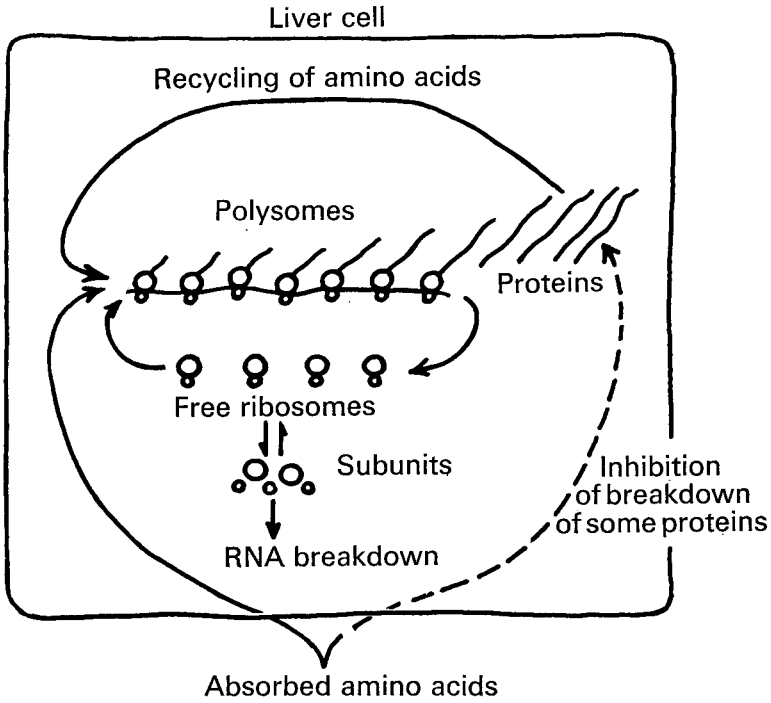

Fig. 2. Scheme showing interrelationship of amino acid supply to protein synthesis and turnover and to RNA turnover in the liver cell. (From Munro, 1968.)

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such an increase in the subunit population would lead to a more rapid loss of RNA from the cell. In fact, indirect evidence of a relationship between rate of RNA degradation and amino acid supply was obtained many years ago (Clark, Naismith & Munro, 1957; Munro & Clark, 1959) and has recently been confirmed directly by prelabelling the RNA of the liver and observing the effect of protein deprivation on the rate of loss of the label (Enwonwu & Munro, 1969, unpublished result). We can thus construct a picture of the events occurring simultaneously in the protein and RNA metabolism of the liver cell after each meal containing protein (Fig. 2). The influx of amino acids causes an acceleration in the rate of protein synthesis; liver protein accumulates because of this and also because turnover of some of the enzymes involved in amino acid catabolism is retarded through substrate stabilization. The increased rate of protein synthesis promotes formation of polysomes from free ribosomes and messenger RNA, and the consequent reduction in the pool of free ribosomes and of ribosome subunits restrains RNA breakdown. Consequently, the RNA and protein content of the liver cell increase in parallel after each meal providing a nutritionally adequate mixture of amino acids.

This description of the response of protein synthesis in the liver cell to an influx of amino acids does not reveal the primary site of their action within the liver cell. If the polysome response involves secretion of additional messenger RNA from the nucleus, it can be blocked by prior treatment with effective doses of actinomycin D. In fact, animals treated in this way and then fed with nutritionally complete or tryptophan-deficient mixtures of amino acids still show a difference in polysome profiles, thus indicating that the response to amino acid influx is dependent on cytoplasmic mechanisms only. Consequently, it should be possible to construct a cell-free protein-synthesizing system from cytoplasmic constituents that will show disaggregation and re-aggregation of polysomes when the supply of amino acids is altered. This was prepared using liver polysomes, an energy source, cell sap dialysed to remove free amino acids, tRNA stripped of amino acids, and [^{14}C]leucine (Baliga, Pronczuk & Munro, 1968). Since this system was low in amino acids available for protein synthesis, it ceased to incorporate the [^{14}C]leucine after a few minutes of incubation at 37°. Fig. 3 shows the polysome profile after 20 min of incubation under these conditions. There are few remaining large aggregates but in their place a large peak of monosomes is present on the left of the profile. When a complete mixture of amino acids was now added to the incubation medium and the polysome profile examined two minutes later, Fig. 3 shows that the monosome peak has diminished considerably and that large polysome aggregates have now appeared. Thus polysomes have been synthesized from ribosomes and surviving messenger RNA in response to an increased amino acid supply. By preparing a series of amino acid mixtures each lacking one amino acid, it has been possible to show that omission of any amino acid (except for isoleucine) results in failure of the polysomes to aggregate.

These experiments with a reconstructed cytoplasmic system for protein synthesis confirm that changes in polysome profile can be brought about by alterations in amino acid supply and that the participation of the nucleus is not essential. The cell-free system requires all amino acids to be present simultaneously in order to

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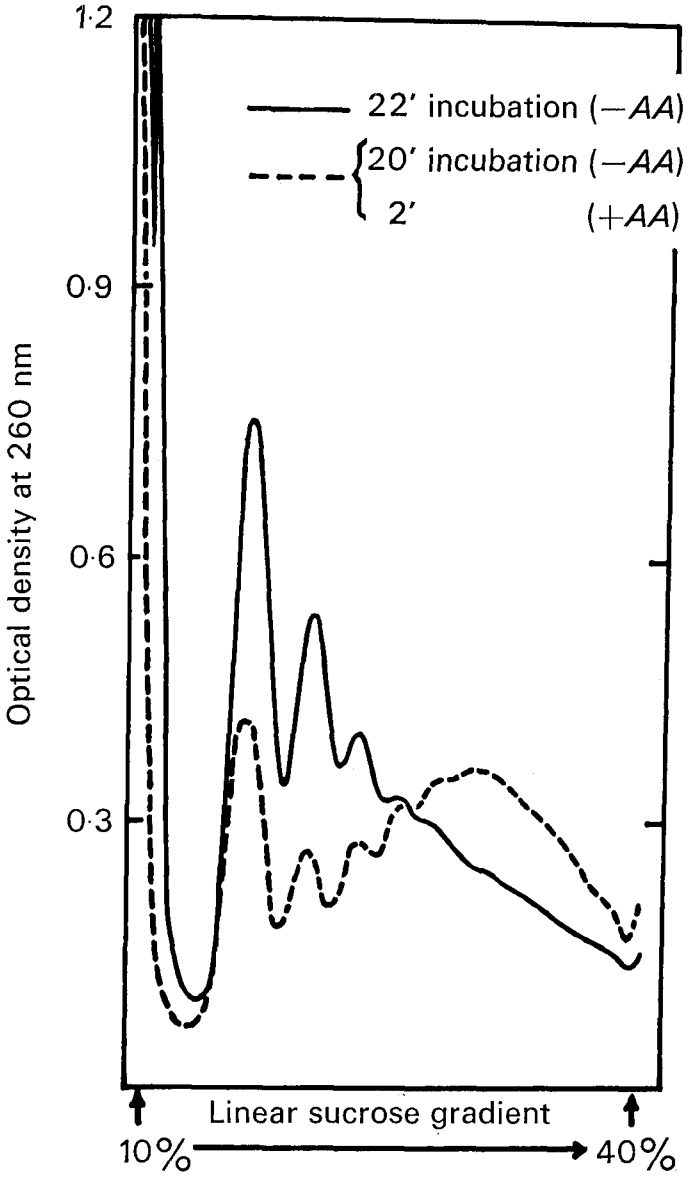

Fig. 3. Effect of delayed supplementation with twenty amino acids on the polysome profile incubated in vitro in a system for amino acid incorporation. The system was incubated for 20 min without amino acids, then all amino acids were added and the profile was taken 2 min later. (From Baliga, Pronczuk & Munro, 1968.)

achieve polysome formation. However, in the case of the intact animal it has been found (Pronczuk, Baliga, Triant & Munro, 1968) that feeding amino acid mixtures deficient in essential amino acids other than tryptophan does not cause disaggregation of polysomes in the way that deficiency of tryptophan does. It is believed (Munro, 1968) that the special sensitivity to tryptophan of the polysome population in the

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liver of the intact animal occurs because tryptophan is normally the least abundant amino acid in the free amino acid pool of the liver, and consequently becomes the rate-limiting factor in protein synthesis. When imbalanced amino acid mixtures are fed under circumstances that are likely to reduce the levels of other free amino acids to very low concentrations, it has been possible to cause changes in liver polysome profiles in relation to intake of threonine and isoleucine (Pronczuk, Rogers & Munro, 1969, unpublished results). The considerable recycling of amino acids within the liver cell (Gan & Jeffay, 1967) probably accounts for the difficulty in demonstrating dependence of liver protein synthesis on amino acids other than tryptophan.

Diurnal rhythms in liver protein metabolism

Protein metabolism is subject to fluctuations in intensity throughout the day (Wurtman, 1969). In view of the preceding discussion, it is hardly surprising to find that, within the liver, diurnal changes in protein metabolism appear to be determined by the intermittent intake of protein in meals; in contrast, plasma amino acids undergo an independent diurnal cycle which may be partly due to amino acid deposition in muscle resulting from absorption of the carbohydrate component of each meal. We have recently correlated the diurnal cycles in liver

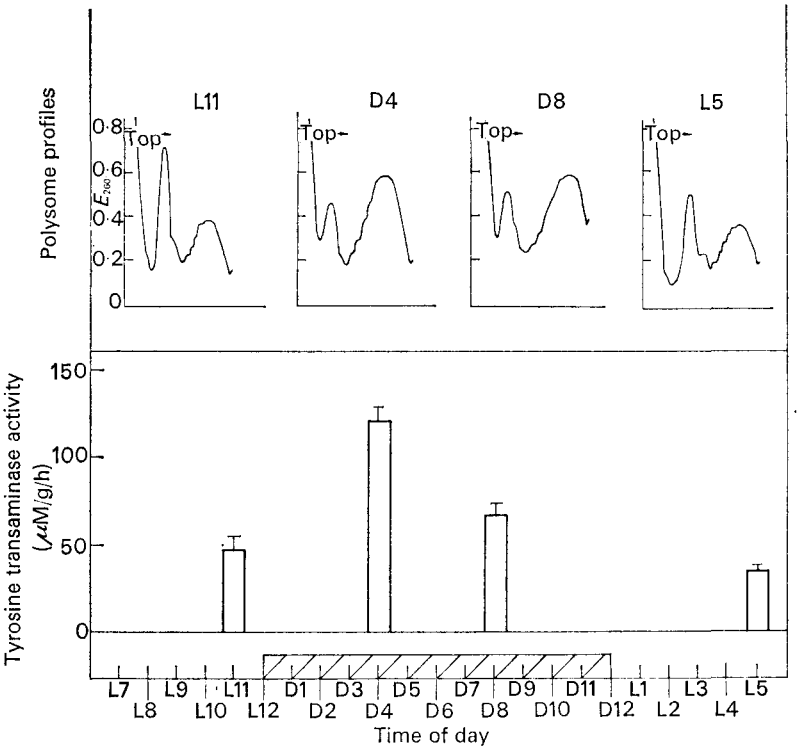

Fig. 4. Diurnal rhythms in polysome profile and tyrosine aminotransferase activity in rats subjected to 12 h of darkness and 12 h of light. Note that spontaneous eating occurs towards the end of the light period and terminates in the middle of the dark period. (Fishman, Wurtman & Munro, 1969 unpublished.)

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polysome aggregation and in the activity of the liver enzyme tyrosine aminotransferase in the case of rats subjected to a 12 h alternating cycle of light and dark (Fishman, Wurtman & Munro, 1969, unpublished results). Under these conditions, the rats adopt a feeding pattern that begins towards the end of the light period, reaches a maximum during the early hours of darkness, and terminates before the light comes on again. Polysome aggregation is least in the middle of the light period, starts to increase shortly after feeding commences, and is maximal in the middle of the dark period; by the start of the next light period, the polysomes have begun to disaggregate again (Fig. 4). The activity of tyrosine aminotransferase, an enzyme initiating the degradation of tyrosine, follows a nearly parallel diurnal pattern. A detailed discussion of these diurnal changes in liver protein metabolism has been provided by Wurtman (1969). From his data, it would appear that the cyclical changes in the activity of tyrosine aminotransferase are likely to be due to the intermittent intake of tryptophan in each meal.

The concentrations of some other liver enzymes involved in amino acid catabolism also appear to be regulated by tryptophan intake. For example, Bojanowska & Williamson (1968) observed that serine administration raises the level of free serine in the liver without increasing the activity of the enzyme serine dehydratase, whereas breakdown of body protein following phlorhizin administration results in an increase in liver serine concentration which is followed by an increment in the activity of the enzyme. This paradox is resolved by the observation that the supply of tryptophan to the liver is the determinant factor in raising serine dehydratase activity (Kaplan & Pitot, 1969). This is, of course, not the only factor involved in regulation of liver enzyme content. Some enzymes, such as tryptophan oxygenase, are stabilized by their substrates and thus undergo a temporary reduction in rate of enzyme protein degradation. Since synthesis of the enzyme continues, the amount of enzyme in the liver increases. This mechanism must be a significant factor in regulating the liver content of many proteins, since Gan & Jeffay (1967) have demonstrated that the proportion of the free amino acid pool of the liver derived from intracellular protein breakdown increases markedly as soon as an animal starts to fast.

From these and other studies, it can be concluded that adaptation of protein metabolism in the liver to variations in amino acid supply occurs after each meal and is of considerable magnitude. This adaptation takes the form of (a) an increase in protein synthesis, which presumably includes increased formation of enzymes involved in protein metabolism and also probably accelerated synthesis of plasma proteins (Kirsch, Frith, Black & Hoffenberg, 1968), and (b) an increase in degradative pathways of amino acid disposal, promoted by larger amounts of degradative enzymes accumulated from increased protein synthesis and also by reduced breakdown of enzyme proteins due to substrate stabilization. The consequent changes in liver enzyme activity will be particularly large for enzymes with short half-lives, such as tyrosine aminotransferase. In consequence of the rapid and extensive nature of these adaptive reactions, the fate of an appreciable part of the incoming flow of amino acids is decided by the hepatic response, which must consequently be an important factor in utilization of dietary protein. However, it should be recognized

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that the experiments of Elwyn (1969) on amino acid metabolism in the liver of the dog and our own studies on diurnal rhythms in polysome aggregation and tyrosine aminotransferase activity have been conducted at only one level of protein intake. In order to assess the role of the liver in utilization of dietary protein with greater understanding, it will be necessary to extend both types of study to animals receiving different levels of protein in the diet, including inadequate levels, and to look for differential effects on protein synthesis and on amino acid degradation.

Adaptation of other tissues to amino acid supply

The immediate effect of quality and quantity of amino acid supply on protein metabolism in tissues other than the liver has been much less vigorously explored. Although the liver actively removes amino acids from the portal circulation, nevertheless peripheral blood sampled during the absorptive period after a meal reflects to some extent the amino acid pattern of the dietary protein. Thus, Longenecker & Hause (1959) showed that the feeding of wheat gluten to dogs resulted a few hours later in a rise in the levels of many amino acids in the peripheral blood, but a fall in the concentration of lysine, the least abundant essential amino acid in the administered protein. This must have consequences for tissues served by the peripheral blood supply, as evidenced by studies of the amino acid requirements of these tissues in vitro. Thus, the amount of milk proteins secreted by mammary tissue in vitro is determined by the amino acid content of the medium (Schingoethe, Hageman & Larson, 1967). Similarly, protein synthesis in many mammalian cell lines maintained in tissue culture is dependent on an optimum quantity and pattern of essential amino acids in the medium (Paul, 1958; Eagle, Piez & Levy, 1961; Riggs & Walker, 1963). Consequently, the minor fluctuations in systemic blood amino acid levels occurring after each meal may have immediate effects on the rate of protein synthesis in some of the tissues served by the peripheral blood supply. Not all tissues, however, are likely to be susceptible to such fluctuations. Transportation of amino acids into skeletal muscle is much slower than into liver (Henriques, Henriques & Neuberger, 1955). This may explain why muscle does not appear to lose significant amounts of protein for the first few days of protein deficiency (Munro, 1964; Waterlow & Stephen, 1966). In addition, it is known that many of the tissues contain enzymes capable of degrading amino acids, a notable example being the transaminase for the branched-chain amino acids found most abundantly in kidney and skeletal muscle. While long-term feeding studies reviewed later indicate that dietary protein level sometimes affects the levels of these catabolic enzymes in the peripheral tissues, there appears to be no published evidence regarding diurnal variations in their activities similar to the studies made on liver enzymes.

The impact of amino acid supply on the peripheral tissues does more than change the rate of protein fabrication or even amino acid catabolism in these tissues. There is evidence that other aspects of cell function such as rate of cell division can be influenced, though it is not known whether these changes occur as diurnal reponses to variations in amino acid supply or are a slower response to the general level of available amino acids. There is evidence that amino acid supply can affect the rate of

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cell division in tissues that remain active in mitosis. When rats are briefly given a protein-free diet, there is no alteration in the protein or RNA content of the epithelial cells of the small intestine, but the rate of incorporation of precursors into the DNA of the mucosal cells is retarded, implying a reduction in the normally rapid rate of cell replication (Munro & Goldberg, 1964). This observation is supported by more recent autoradiographic studies (Hopper, Wannemacher & McGovern, 1968), in which a protein-free diet has been shown to retard the cycle of cell division and migration in the intestinal mucosa. Secondly, multiplication of antibody-producing cells in the spleen is less efficient in rodents receiving a protein-free diet for a few days than in control animals adequately fed with protein (Kenney, Roderuck, Arnrich & Piedad, 1968; Cooper & Munro, 1969, unpublished results). Finally, Winick & Noble (1966) have studied rats malnourished for a short period at different phases of their growth. In tissues in which growth by cell division is still proceeding, malnutrition causes a permanent reduction in the cell population of that tissue in the adult animal. Thus, the cell population of the brain is permanently reduced if the food restriction is applied during the first 3 weeks of life but not at a later stage, when the adult cell complement of the brain has already been attained. Miller (1969) considers that the principal nutrient involved in such effects is protein. Consequently, the studies of Winick & Noble suggest that nuclear programming for growth and development is influenced by amino acid supply.

Relationship of adaptive changes in protein metabolism to protein needs

It would be helpful in the biochemical diagnosis of protein needs if the adaptive reactions discussed above showed a sharp differentiation between intakes of protein that are ample and intakes that are inadequate for the needs of the body. Harper (1968) has indeed suggested that, when rats are fed increasing levels of dietary protein, there is no increase in the activities of enzymes catabolizing essential amino acids until the protein requirements of the animal are attained, after which further increases in protein intake result in a rapid rise in the activities of these enzymes. He instances the case of threonine-serine dehydratase activity in the liver, which remains low until the protein (casein) content of the diet reaches some 20% when activity begins to increase and finally attains high levels in the livers of animals on a diet containing 70% casein. On the other hand, Harper finds that transaminases handling non-essential amino acids, such as glutamic acid, exhibit activities in the liver that are linearly related to the protein content of the diet from the lowest levels upwards. Fig. 5 combines some of Harper's findings.

In an empirical way, such an approach may have predictive value in the study of adequacy of dietary protein intake. There are, however, limitations to the general application of this principle in attempting to relate the magnitude of adaptive changes in protein metabolism to the adequacy of the diet. First, many of the enzymes degrading essential amino acids are confined to the liver (Miller, 1962), but the transaminase for the branched-chain amino acids (Mimura, Yamada & Swendseid, 1968) and the enzyme threonine-serine dehydratase (Fallon, Davis & Goyer, 1968) are

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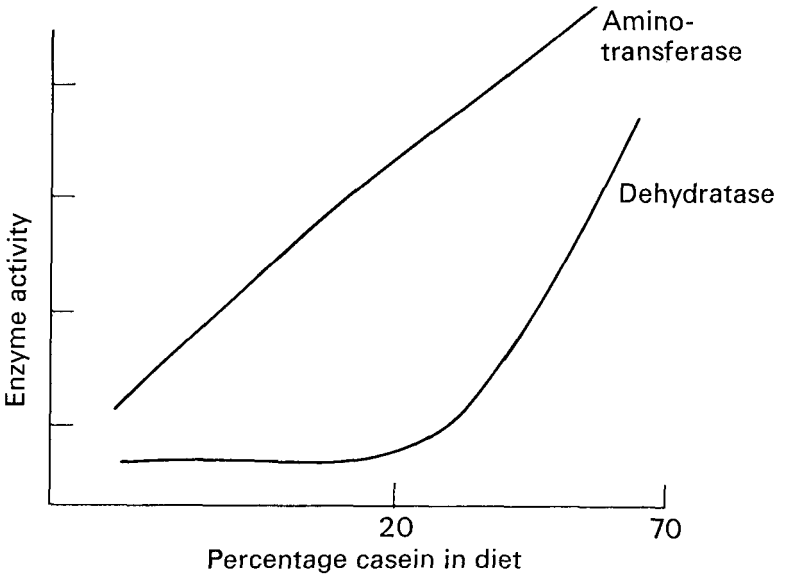

Fig. 5. Activity in the liver of glutamic acid—oxaloacetate aminotransferase and threonine-serine dehydratase at different levels of protein intake. (Drawn from the results of Harper, 1968.)

more widely distributed. Both of these degradative enzymes show different responses in the various tissues when protein intake is varied. For example, rats on a protein-free diet exhibit higher branched-chain amino acid transaminase activity in both liver and muscle than do rats on an 18% casein diet (Mimura *et al.* 1968); similarly, animals maintained at these two levels of protein intake show similar activities for threonine-serine dehydratase in kidney and in brain (Fallon *et al.* 1968). It would consequently be impossible to identify an adequate level of protein intake from studies based on branched-chain transaminase activities in muscle or serine dehydratase activities in brain or kidney. Secondly, the time of sampling a tissue for enzyme activity can be significant. Enzymes with short half-lives, such as tyrosine aminotransferase in liver, can undergo extensive variations in activity at different times of day, whereas the urea-cycle enzymes increase more slowly in activity when dietary protein intake is raised and do not undergo these rhythmic changes. In consequence, a single determination of the latter type of enzyme may well be representative of the general level obtaining throughout the day, whereas the activities of more labile enzymes such as tyrosine aminotransferase can only be adequately studied by making repeated observations throughout the twenty-four hours. Finally, it has already been pointed out that the level of serine-threonine dehydratase and possibly of other enzymes can be determined by tryptophan intake without reference to the effect of the diet on nitrogen balance and other criteria of adequacy (Kaplan & Pitot, 1969). In view of these limitations, it would be premature to claim a general relationship between degradative enzyme activities and level of protein intake until more is known about how the degradative enzymes in different tissues respond at various times of day to different levels of protein intake.

https://doi.org/10.1079/PNS19690042 Published online by Cambridge University Press

REFERENCES

Amenta, J. S. & Johnston, E. H. (1963). *Lab. Invest.* **12**, 921.
Baliga, B. S., Pronczuk, A. W. & Munro, H. N. (1968). *J. molec. Biol.* **34**, 199.
Bojanowska, K. & Williamson, D. H. (1968). *Biochim. biophys. Acta* **159**, 560.
Clark, C. M., Naismith, D. J. & Munro, H. N. (1957). *Biochim. biophys. Acta* **23**, 581.
Denton, A. E. & Elvehjem, C. A. (1954). *J. biol. Chem.* **206**, 449.
Eagle, H., Piez, K. A. & Levy, M. (1961). *J. biol. Chem.* **236**, 2039.
Elwyn, D. (1969). In *Mammalian Protein Metabolism.* Vol. 4, Ch. 37. [H. N. Munro, editor.] New York: Academic Press. (In the Press.)
Fallon, H. J., Davis, J. L. & Goyer, R. A. (1968). *J. Nutr.* **96**, 220.
Fleck, A., Shepherd, J. & Munro, H. N. (1965). *Science, N.Y.* **150**, 628.
Gan, J. C. & Jeffay, H. (1967). *Biochim. biophys. Acta* **148**, 448.
Harper, A. E. (1968). *Am. J. clin. Nutr.* **21**, 358.
Henriques, O. B., Henriques, S. B. & Neuberger, A. (1955). *Biochem. J.* **60**, 409.
Hopper, A. F., Wannemacher, R. W. & McGovern, P. A. (1968). *Proc. Soc. exp. Biol. Med.* **128**, 695.
Kaplan, J. H. & Pitot, H. C. (1969). In *Mammalian Protein Metabolism.* Vol. 4, Ch. 35. [H. N. Munro, editor.] New York: Academic Press. (In the Press.)
Kenney, M. A., Roderuck, C. E., Arnrich, L. & Piedad, F. (1968). *J. Nutr.* **95**, 173.
Kirsch, R., Frith, L., Black, E. & Hoffenberg, R. (1968). *Nature, Lond.* **217**, 579.
Longenecker, J. B. & Hause, N. L. (1959). *Archs Biochem. Biophys.* **84**, 46.
McCormick, D. B. & Snell, E. S. (1961). *J. biol. Chem.* **236**, 2805.
Miller, L. L. (1962). In *Amino Acid Pools.* [J. T. Holden, editor.] Amsterdam: Elsevier.
Miller, S. A. (1969). In *Mammalian Protein Metabolism.* Vol. 3, Ch. 26. [H. N. Munro, editor.] New York: Academic Press.
Mimura, T., Yamada, C. & Swendseid, M. E. (1968). *J. Nutr.* **95**, 493.
Munro, H. N. (1964). In *Mammalian Protein Metabolism.* Vol. 1, Ch. 10. [H. N. Munro, editor.] New York: Academic Press.
Munro, H. N. (1968). *Fedn Proc. Fedn Am. Socs exp. Biol.* **27**, 1231.
Munro, H. N. & Clark, C. M. (1959). *Biochim. biophys. Acta* **33**, 551.
Munro, H. N. & Goldberg, D. M. (1964). In *The Role of the Gastro-intestinal Tract in Protein Metabolism*, p. 189. [H. N. Munro, editor.] Oxford: Blackwell.
Paul, J. (1958). *J. exp. Zool.* **142**, 475.
Pronczuk, A. W., Baliga, B. S., Triant, J. W. & Munro, H. N. (1968). *Biochim. biophys. Acta* **157**, 204.
Riggs, T. R. & Walker, L. M. (1963). *J. biol. Chem.* **238**, 2663.
Schingoethe, D. J., Hageman, E. C. & Larson, B. L. (1967). *Biochim. biophys. Acta* **148**, 469.
Staehelin, T., Verney, E. & Sidransky, H. (1967). *Biochim. biophys. Acta* **145**, 105.
Waterlow, J. C. & Stephen, J. M. L. (1966). *Br. J. Nutr.* **26**, 461.
Waterlow, J. C. & Stephen, J. M. L. (1969). *Proc. Nutr. Soc.* **28**, 234.
Winick, M. & Noble, A. (1966). *J. Nutr.* **89**, 300.
Wunner, W. H., Bell, J. & Munro, H. N. (1966). *Biochem. J.* **101**, 417.
Wurtman, R. J. (1969). In *Mammalian Protein Metabolism.* Vol. 4, Ch. 36. [H. N. Munro, editor.] New York: Academic Press. (In the Press.)

Assessment of protein requirements by nitrogen balance

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The earliest estimates of protein requirements, such as those of Smith (1863) and Pavy (1874) who recommended about 125 g per day for the average working man, were based on studies of the diets of individuals or of groups of subjects who were considered to be healthy and leading normal active lives. Such estimates were simply a reflection of the dietary habits of those under study, but helped to establish minimum standards of feeding in institutions. About the turn of the century, similar

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