PROCEEDINGS OF THE NUTRITION SOCIETY

The second Boyd Orr Memorial Lecture, organized by the Boyd Orr Memorial Trust, was held in the Edward Lewis Lecture Theatre, The Middlesex Hospital Medical School, Cleveland Street, London W1P 7PN, on 21 May 1976

SECOND BOYD ORR MEMORIAL LECTURE

Regulation of body protein metabolism in relation to diet

By HAMISH N. MUNRO, *Physiological Chemistry Laboratories, Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, Mass. 02139, USA*

Introduction

My links with the late Lord Boyd Orr run deep. Edward Provan Cathcart taught Physiology at Glasgow University and dominated the field of protein metabolism for so many years that it was possible for him to inspire Boyd Orrr in 1913 to study the action of dietary carbohydrate and fat on the protein metabolism of starving subjects (Cathcart & Orr, 1914) and also to be my senior Professor and mentor at a time in 1935 when I too was carrying out studies of the impact of carbohydrate and fat intake on protein metabolism (Cuthbertson & Munro, 1937). Through Cathcart, Boyd Orr and I can trace our scientific ancestry back through Carl Voit to Lavoisier and Joseph Black, the Scottish physician who discovered the first atmospheric gas (CO_2) and thus initiated modern chemistry (Munro, 1964*a*).

I want to relate to you how our knowledge of protein metabolism has evolved since these early days, and what we now understand about the responses of protein metabolism to dietary intake of protein and other nutrients. Since proteins are intimately related to life processes, the regulation of protein metabolism with its twenty amino acids would be expected to have many facets which must be integrated in order to maintain bodily function. My discourse will therefore attempt to demonstrate why an integrated approach to protein metabolism has as much merit today as it had near the beginning of this century when Orr and Cathcart performed their studies in this area. I shall also try to relate the implications of such studies to human disease.

Digestion, absorption and the function of the liver

Digestion of the protein of the diet depends on an attack by pepsin in the stomach and by the proteolytic activity of enzymes secreted by the pancreas and by the mucosa of the small intestine (Fig. 1). It has only recently been appreciated that the quantities of proteolytic enzymes secreted by the pancreas are regulated

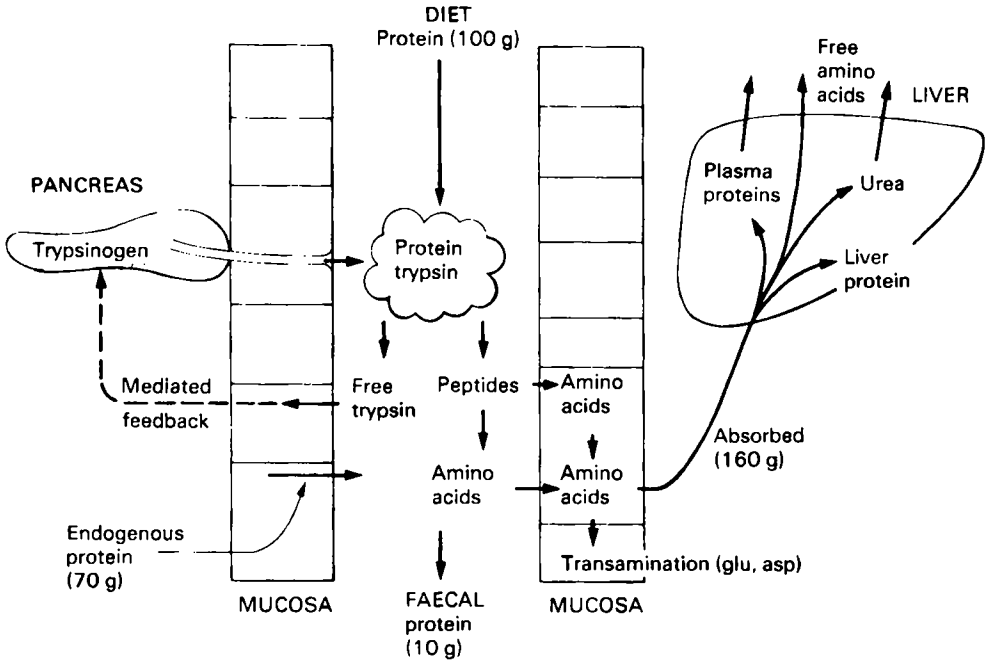

Fig. 1. Diagram showing the fate of dietary protein, the secretion of endogenous protein, and feedback control of pancreatic enzyme secretion (from Crim & Munro, 1976); glu, glutamate; asp, aspartate.

by the presence of dietary protein in the gut contents. For example, Green, Olds, Matthews & Lyman (1974) have shown that trypsin secreted from the pancreas binds to protein in the gut lumen until there is excess of enzyme present. This excess of free enzyme then operates a feedback regulation system to the pancreatic acinar cells which inhibits synthesis of the precursor trypsinogen.

A second area of recent knowlege in protein metabolism is the nature of the material absorbed. The end-products of digestion of protein are absorbed through the mucosal cells of the small intestine. Although some of the protein in the gut lumen is finally hydrolysed to free amino acids prior to absorption, the absorption of small peptides, notably dipeptides, has recently been shown to play a significant role in assimilation of dietary protein (Adibi & Soleimanpour, 1974). Due to the presence of peptide hydrolases in the brush border and cytosol of the mucosal cells (Adibi & Soleimanpour, 1974), these peptides undergo resolution to free amino acids as soon as they enter the mucosal cells, so that only free amino acids are transferred from the mucosal cells to the portal vein. The transport mechanism for uptake of peptides by mucosal cells is known to differ from that for free amino acid uptake, since there is no competition between the two. Peptide absorption is probably a major route of amino acid uptake, since patients with Hartnup's disease who cannot transport free tryptophan across the mucosa nevertheless grow almost normally (Asatoor, Cheng, Edwards, Lant, Matthews, Milne, Navab & Richards, 1970).

https://doi.org/10.1079/PNS19760050 Published online by Cambridge University Press

An area of protein metabolism which is now receiving increasing attention is the secretion of protein into the gut. Digestive enzymes represent protein added to the gut contents from endogenous sources. In addition, the epithelial cells of the gut mucosa are continuously being replaced by cell division in the crypts at the base of the villi, followed by passage of each cohort of cells up the villus and sloughing from the villar tip. The magnitude of the daily endogenous protein secretion is, however, controversial. When the over-all process of endogenous secretion is considered, it seems that some 70 g protein (17 g from secreted juices and 50 g from sloughed cells) is added to the intestinal contents daily (Munro, 1969*b*). Together with the approximately 100 g protein consumed in the Western-type diet, this provides a total of 170 g in the gut lumen available for absorption. Since faecal N output is commonly equivalent to 10 g protein daily, the efficiency of digestion and absorption of both dietary and endogenous protein must normally be high. This dynamic state of protein turnover in the gut wall is sensitive to dietary change. Protein deficiency (Munro & Goldberg, 1964) and starvation (Ju & Nasset, 1959) both reduce the rate of cell division in the mucosa, without altering cell size or composition (Munro & Goldberg, 1964). This can be correlated with direct evidence on rats that protein deficiency leads to reduced secretion of endogenous protein into the lumen of the gut (Twombley & Meyer, 1961).

The absorbed amino acids pass to the liver via the portal vein. Following a meal of protein, there is a large change in the amount and pattern of amino acids passing into the general circulation. This occurs because the liver is the main or exclusive site of catabolism of seven of the essential amino acids (Miller, 1962), the exceptions being the branched-chain amino acids which are degraded primarily in muscle and kidney (Ichihara & Koyama, 1966). Using dogs fed a large meat meal, Elwyn (1970) has shown that more than half of the incoming amino acids are degraded to urea, a small proportion is retained as liver protein, some are secreted as plasma proteins and only about a quarter of the incoming load passes into the general circulation as free amino acids, with the branched-chain amino acids predominating.

At the subcellular level, the response of the liver to an increase in amino acid supply is far-reaching (Munro, Hubert & Baliga, 1975). As shown in Fig. 2, protein synthesis on polyribosomes is accelerated, while protein breakdown diminishes. This results in accumulation of enzymes with short half-lives involved in the metabolism of the incoming nutrients. In addition, synthesis of RNA also accelerates, while breakdown of ribosomal RNA is restricted. This latter results in changes in purine metabolism, and de novo synthesis of purine bases accelerates (Clifford, Riumallo, Baliga, Munro & Brown, 1972). In consequence, there are diurnal rhythms in the synthesis of liver proteins on polyribosomes, in accumulation and breakdown of liver RNA, and in purine metabolism, all of which are related to the intermittent intake of meals containing protein (Munro, *et al.* 1975).

At the functional level, there appears to be a sensitive and discriminating mechanism in the liver modulating the degradation of many of the essential amino

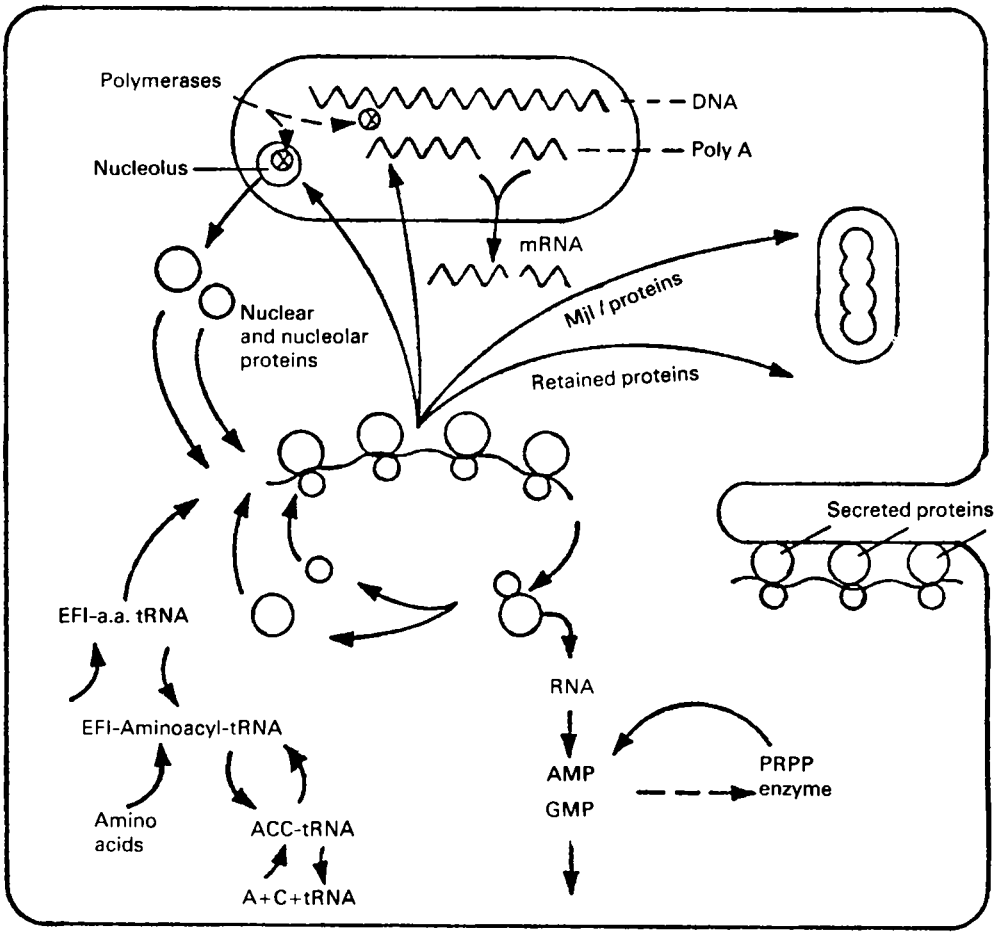

Fig. 2. Mechanisms in the liver cell responding to amino acid intake (from Munro, Hubert & Baliga, 1975).

acids in relation to the needs of the body. When dietary intake of an essential amino acid is increased beyond requirements, induction of the enzymes of the corresponding degradative pathway often occurs. Sometimes, as in the instance of tryptophan oxygenase (*EC* 1.13.1.12) (Young & Munro, 1973), the enzyme is induced by excessive intakes of substrate at the time of absorption of the meal. In other instances, it appears to take a longer time of adaptation to the higher level, as in the instance of lysine (Brookes, Owens & Garrigus, 1972). In this latter study, increasing amounts of [^{14}C]lysine were consumed by young rats. The gain in body-weight reached a plateau of 6·5 g/d at an intake of 100 mg lysine/d. Production of $^{14}CO_2$ showed a clear break point with a rapid increment at intakes above 100 mg lysine/d, indicating that the intake which gave maximal growth corresponded to the point at which the liver began to intervene and to destroy the excess lysine.

The pattern of rise of lysine level in the blood gives a similar curve to that seen with CO_2 production (Pawlak & Pion, 1968) and this has been exploited to study requirements for individual amino acids in animals and man. The point of inflexion

https://doi.org/10.1079/PNS19760050 Published online by Cambridge University Press

when the plasma level starts to rise has been used as an index of requirements. For example, using rats receiving various levels of dietary tryptophan, we found that the point of inflexion varied with age, occurring at lower dietary concentrations for older rats that need less tryptophan (Young & Munro, 1973). This procedure has also been used to determine amino acid needs of humans but some doubts have been expressed about its precision.

The plasma levels of amino acids are also affected by dietary carbohydrate through an insulin-dependent mechanism. Within 1 h of consuming carbohydrate, most plasma amino acids decrease, due to deposition in muscle through insulin-mediated transport (Munro, Black & Thomson, 1959). The effect is maximal on branched-chain amino acids. This mechanism is also the basis of a metabolic interaction between dietary protein or amino acids and carbohydrate consumed in the same meal (Munro, 1964*b*).

The alterations in plasma free amino acid patterns caused by the protein and carbohydrate components of a meal have significance for the availability of amino acids to the peripheral tissues. In the instance of the brain, it has been shown that entry of tryptophan into the cells of this organ is determined by the plasma levels of other competing neutral amino acids, notably the branched-chain amino acids (Fernstrom & Wurtman, 1972). Following a meal of carbohydrate, the extensive fall in plasma levels of branched-chain amino acids results in greater passage of tryptophan into the brain and more serotonin is synthesized (Fernstrom, Madras, Munro & Wurtman, 1974). This mechanism is not only significant in regulating serotonin metabolism, it is also critical to brain function under pathological conditions. As shown in Fig. 3, the loss of normal liver function associated with hepatic cirrhosis results in a series of metabolic alterations relating to protein metabolism (Munro, Fernstrom & Wurtman, 1975). First, ammonia and amines formed by bacterial action in the intestines are no longer trapped by the liver, but pass freely to the peripheral circulation and penetrate the brain cells. In the instance of phenylalanine, for example, bacterial action produces phenylethylamine which is transformed to octopamine in the brain and acts there as a false neuro-transmitter competing with the catecholamines. Secondly, amino acids whose metabolism is normally regulated in the liver, such as tryptophan and phenylalanine, are no longer subject to hepatic control and their levels rise and thus affect availability to the brain. Finally, the liver no longer inactivates part of the insulin as it passes through. In consequence plasma insulin levels rise and this drives branched-chain amino acids into muscle, thus accounting for the low plasma levels of branched-chain amino acids observed in cases of cirrhosis. Because of the low plasma levels of the branched-chain amino acids, a larger proportion of the already elevated plasma tryptophan content passes into the brain and generates excessive levels of serotonin, which contribute to hepatic coma. This theory of the role of excess serotonin formation in hepatic coma receives some confirmation from studies showing that administration of branched-chain amino acids to animals with hepatic coma results in reversal of the comatose state (Fischer, Funovics & Aguirre, 1975).

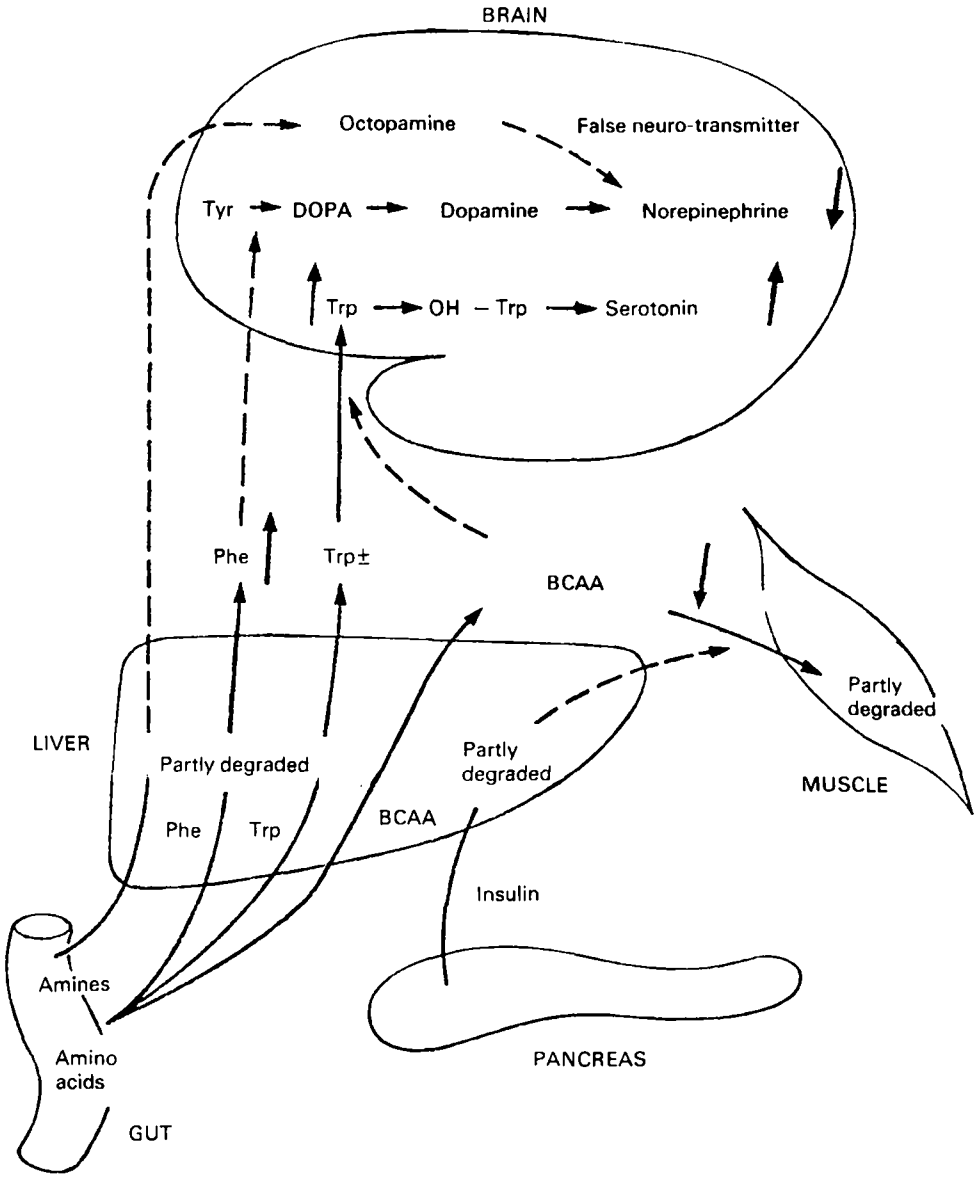

Fig. 3. Role of branched-chain amino acids (BCAA) in hepatic coma. Owing to unrestricted passage of insulin into the general circulation in hepatic coma, BCAA are removed excessively by muscle. In consequence of this lowering of plasma BCAA, there is less competition with tryptophan (Trp) for entry into the brain and thus more serotonin is made (from Crim & Munro, 1975). Tyr, tyrosine; DOPA, 3:4-dihydroxyphenylalanine; OH-Trp, 5-hydroxytryptophan; Phe, phenylalanine; ↓, metabolite decreased; ↑, metabolite increased; ±, metabolite unaffected.

Role of skeletal muscle in regulation of protein metabolism

Two aspects of protein metabolism in muscle, the alanine–glucose cycle and the metabolism of methylhistidine, are relevant to a study of the role of muscle in the protein metabolism of the body. Muscle represents the major depot for retention of

https://doi.org/10.1079/PNS19760050 Published online by Cambridge University Press

free amino acids in the body (Munro, 1970) and is also the major component of whole body protein. In consequence, metabolism of amino acids in this tissue are of considerable significance for general protein metabolism, especially in larger species in which metabolism in the viscera is less intense (Munro, 1969*a*). The daily turnover of muscle protein, estimated by studying the rate of release of alanine, glutamine and other amino acids into plasma, has been put at 75 g/d for a subject fasting up to 24 h (Pozefsky, Felig, Tobin, Soeldner & Cahill, 1969). This estimate is probably too low because it does not allow for reutilization of the liberated amino acids within the muscle cells for synthesis of muscle protein. An amino acid released from the protein but not reutilized would be advantageous. Urinary output of 3-methylhistidine appears to provide an index of muscle protein turnover not subject to reutilization. The data so far gathered on 3-methylhistidine excretion suggest a larger turnover than that obtained from measuring rate of amino acid release from muscle (Pozefsky *et al.* 1969). The evidence is discussed below.

The alanine–glucose cycle in muscle (Fig. 4) has been fully documented (Pozefsky *et al.* 1969). Alanine is formed by transamination from pyruvate and in consequence alanine becomes a carrier of nitrogen to the liver. There, its C skeleton enters the gluconeogenic pathway, while its amino group is transformed into urea. The other carrier is glutamine, which accepts N as its amide group; there is recent evidence that glutamine passes to the intestine where the NH_2 becomes part of alanine, and thence to the liver. Through these reactions muscle has a special mechanism which allows transport of N to the liver equivalent to 75 g muscle protein daily in the fasting subject. Studies of the arterio-venous differences across the forearm have demonstrated that, if insulin or carbohydrate is administered, this leakage diminishes, and after a meal, it is completely reversed so that muscle actually gains protein. Thus, fluctuations in the arterio-venous loss occur throughout the day. This implies that, from the body mass of muscle (45% of body-weight on the average), a considerable amount of C is available in fasting or other emergency.

Interpretation of arterio-venous difference data is difficult, however, because of recycling within the muscle cell. For example, if insulin is given, the arterio-venous difference diminishes and this is interpreted as retardation of muscle protein breakdown. However, insulin may also increase utilization of amino acids released by breakdown, through its known stimulant action on muscle protein synthesis. In order to monitor muscle protein breakdown without reutilization, we have used 3-methylhistidine output in the urine. Methylation of histidine in actin and myosin occurs only after the muscle protein 'backbone' has been synthesized. When the protein of the myofibril is subsequently broken down, methylhistidine is not reused (Young, Alexis, Baliga, Munro & Muecke, 1972). The excretion of this substance in the urine thus offers an index of muscle breakdown.

Several lines of evidence indicate that this 3-methylhistidine may in fact fulfill such a promise. Firstly, we examined the methylhistidine content of various tissues and organs of the rat (Haverberg, Omstedt, Munro & Young, 1975). Although

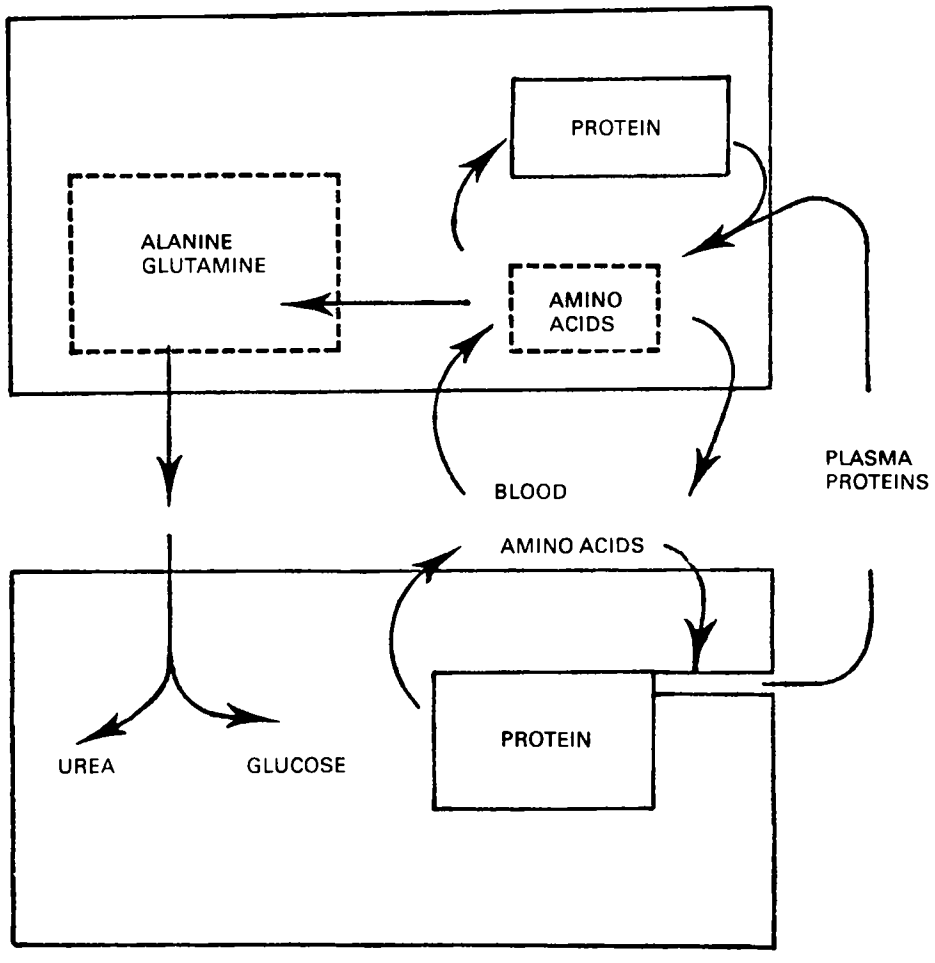

Fig. 4. Release of alanine and glutamine from skeletal muscle.

these data do not represent a complete analysis of the entire body they do include a reasonable number of important tissues and show that skeletal muscle is the overwhelming reservoir of methylhistidine in the body. Secondly, changes in methylhistidine excretion have been examined in young growing rats receiving either a normal diet or diets deficient in either protein alone or protein and energy (Haverberg, Deckelbaum, Bilmazes, Munro & Young, 1975). Fig. 5 shows that normal growth occurred in those rats receiving a synthetic diet with 180 g lactalbumin/kg, whereas animals receiving a diet containing 5 g lactalbumin/kg and adequate in energy lost weight. A third group of rats received a diet low in protein but with an energy intake only half of their normal requirement. Both of the latter groups were repleted on the diet with 180 g lactalbumin/kg after 14 d. Methylhistidine excretion is shown in Fig. 5 (b). The animals on the adequate diet showed an increased output as body-weight increased. The methylhistidine of the protein-depleted group dropped steadily to 20% of their initial output, but upon repletion it again rose. The third group (protein–energy

https://doi.org/10.1079/PNS19760050 Published online by Cambridge University Press

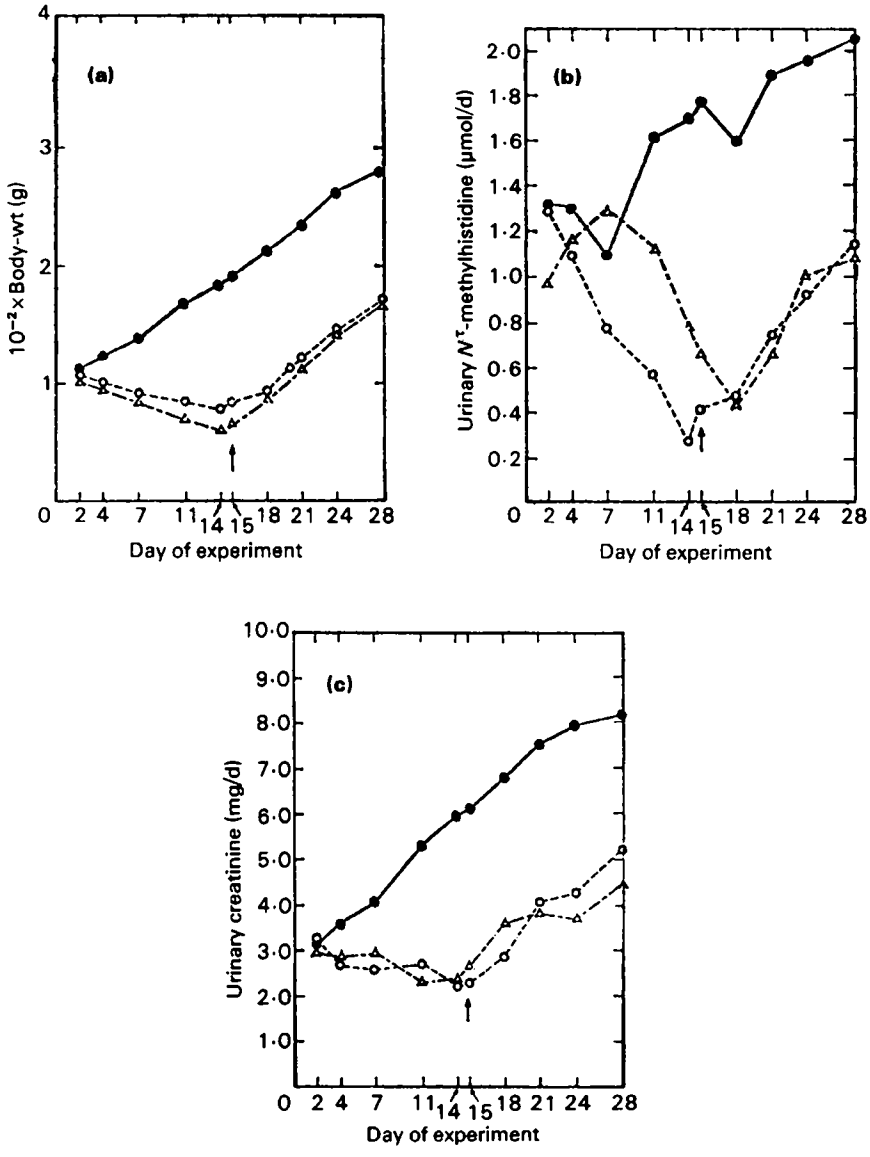

Fig. 5. Growth, and 3-methylhistidine output and creatinine output in the urine of rats given: (*a*) an adequate diet (●——●), (*b*) a diet with 5 g protein/kg (○— —○), or (*c*) a diet with 10 g protein/kg at half the energy intake of diet *b* (△— – —△). After 14 d on the two deficient diets, the rats were repleted on the diet with 180 g protein/kg (from Haverberg, Deckelbaum, Bilmazes, Munro & Young, 1975).

deficient) showed an initial rise, followed by a gradual fall. As in the second group, methylhistidine output rose with repletion. In contrast, creatinine output (Fig. 5 (c)) showed changes paralleling those of body-weight.

Thus muscle responds to protein depletion by shutting off breakdown. However, with semi-starvation, breakdown at first increases and then diminishes so that the responses to the two situations are different. The increase seen during repletion

https://doi.org/10.1079/PNS19760050 Published online by Cambridge University Press

was not fully adequate to restore output to normal level, even when allowance is made for body-weight. Thus, if the methylhistidine output is divided by the weight of the animal during the 14 d of repletion, the value obtained is slightly below that of an adequately nourished rat. Evidently, during repletion, the breakdown of muscle protein is still being restrained. It is pertinent to note that malnourished children in India (Narasinga Rao & Nagabhushan, 1973) show a low output of methylhistidine, which rises when recovery occurs. We have also observed that grossly obese subjects undergoing prolonged fasting show a progressive reduction in 3-methylhistidine output (Young, Haverberg, Bilmazes & Munro, 1973).

Regulation of over-all body protein metabolism

From the preceding information, a composite picture of the daily flux of amino acids in various compartments of the body of an adult man can be assembled. These estimates of the daily amino acid flux in different compartments of the body of a 70 kg man are shown in Fig. 6. Balance experiments show (Munro, 1972*a*) that the average 70 kg man can be maintained in N equilibrium on an intake of 32 g high quality dietary protein/d. However, the customary protein intake in Western

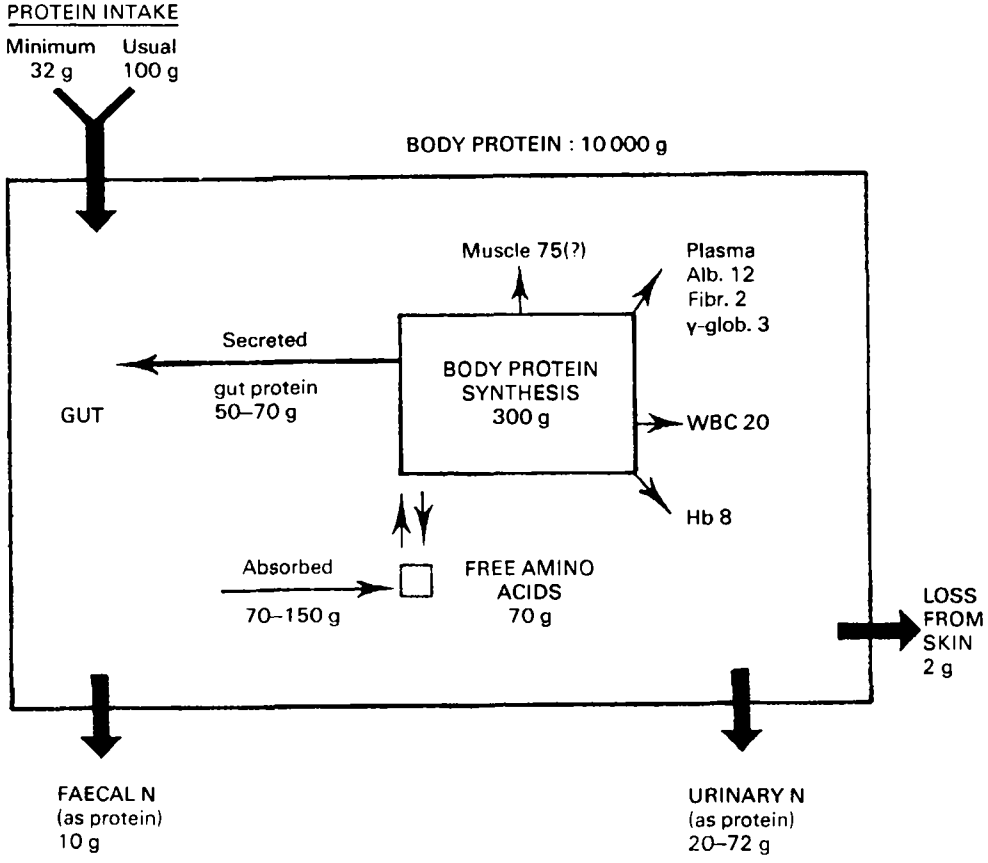

Fig. 6. Over-all protein metabolism in a 70 kg man (from Munro, 1972*b*).

https://doi.org/10.1079/PNS19760050 Published online by Cambridge University Press

countries is about 100 g daily, and this is augmented by addition of at least 70 g of protein secreted into the gastrointestinal tract, so that the total protein load for absorption is at least 170 g. The pools of free amino acids in the tissues are about 70 g, mostly in the form of four non-essential amino acids. These free amino acid pools exchange with body protein and experiments involving ^{15}N suggest that some 300 g protein is synthesized daily in the body of the adult (Munro, 1969*a*). The discrepancy between the intake of 100 g protein and the daily turnover of 300 g emphasizes the amount of reutilization involved in protein metabolism.

We need more accurate estimates of amino acid fluxes in different tissues, not only in adult, healthy subjects but also in patients with injuries or debilitating diseases, and also for children at various stages of development. We also need to know more about the effects of starvation and of hormones on the daily flux of amino acids within the body (see Munro, 1964*b*).

Finally, it is important to recognize that the intensity of protein metabolism varies in proportion to the body size of the mammal. This is well known in energy metabolism, where basal energy output is related to the three-fourths power of body-weight. Most parameters of protein metabolism follow the same general relationship, e.g. requirements for threonine and methionine per kg body-weight of the mature rat that are about four times that of man (Nasset, Anderson & Siciliano, 1951). Table 1 demonstrates that, over a wide range of species, the turnover of serum albumin shows a slowing down in rate with increasing body size. Analysis of the livers of these species demonstrates a parallel reduction in the RNA content per liver cell which is compatible with the reduced intensity of plasma protein synthesis in the larger species. Nevertheless, the free amino acid pools of the tissues do not decrease in the same way (Munro, 1969*a*). We can conclude that the amino acid pools are thus replaced more slowly in the larger species. A complete description of amino acid homeostasis therefore requires acknowledgement of the effects of body size on these parameters.

Table 1. *Plasma albumin turnover, liver cell composition and composition of liver microsomes of different mammals arranged in ascending order of body-weight (from Munro & Downie, 1964)*

Species	Half-life of plasma albumin (d)	Whole liver composition		Liver microsome composition (mg/g dry matter)	
		RNA:DNA	Phospholipid:DNA	RNA	Phospholipid
Mouse	1·2	4·45	14·2	96	242
Rat	2·5	3·05	9·8	73	229
Rabbit	5·7	2·44	11·6	62	292
Dog	8·2	1·69	9·4	—	—
Cow	20·7	1·29	8·7	59	313

REFERENCES

Adibi, S. A. & Soleimanpour, M. (1974). *J. clin. Invest.* **53**, 1368.
Asatoor, A. M., Cheng, B., Edwards, K. D. G., Lant, A. F., Matthews, D. M., Milne, M. D., Navab, F. & Richards, A. J. (1970). *Clin. Sci.* **39**, I.P. (Abstract).
Brookes, I. M., Owens, F. N. & Garrigus, U. S. (1972). *J. Nutr.* **102**, 27.

Cathcart, E. P. & Orr, J. B. (1914). *J. Physiol., Lond.* **48**, 113.
Clifford, A., Riumallo, J. A., Baliga, B. S., Munro, H. N. & Brown, P. R. (1972). *Biochim. biophys. Acta* **277**, 443.
Crim, M. C. & Munro, H. N. (1975). In *Defined Formula Diet* [M. E. Shils, editor]. Chicago: American Medical Association.
Cuthbertson, D. P. & Munro, H. N. (1937). *Biochem. J.* **31**, 694.
Elwyn, D. (1970). In *Mammalian Protein Metabolism*, vol. 4, p. 523 [H. N. Munro, editor]. New York and London: Academic Press.
Fernstrom, J. D. & Wurtman, R. J. (1972). *Science, N.Y.* **178**, 414.
Fernstrom, J. D., Madras, B. K., Munro, H. N. & Wurtman, R. J. (1974). *Ciba Fdn Symp. Aromatic Amino Acids in the Brain*, p. 153.
Fischer, J. E., Funovics, J. M. & Aguirre, A. (1975). *Surgery, St Louis* **78**, 276.
Green, G. M., Olds, B. A., Matthews, G. & Lyman, R. L. (1973). *Proc. Soc. exp. Biol. Med.* **142**, 1162.
Haverberg, L. N., Omstedt, P. T., Munro, H. N. & Young, V. R. (1975). *Biochim. biophys. Acta* **405**, 67.
Haverberg, L. N., Deckelbaum, L., Bilmazes, C., Munro, H. N. & Young, V. R. (1975). *Biochem. J.* **152**, 503.
Ichihara, A. & Koyama, E. (1966). *J. Biochem., Tokyo*, **59**, 160.
Ju, J. S. & Nasset, E. S. (1959). *J. Nutr.* **68**, 633.
Miller, L. L. (1962). In *Amino Acid Pools*, p. 608 [J. T. Holden, editor]. Amsterdam: Elsevier.
Munro, H. N. (1964*a*). In *Mammalian Protein Metabolism*, vol. 1, p. 3 [H. N. Munro and J. B. Allison, editors]. New York and London: Academic Press.
Munro, H. N. (1964*b*). In *Mammalian Protein Metabolism*, vol. 1, p. 381 [H. N. Munro and J. B. Allison, editors]. New York and London: Academic Press.
Munro, H. N. (editor). (1969*a*). In *Mammalian Protein Metabolism*, vol. 3, p. 133. New York and London: Academic Press.
Munro, H. N. (editor). (1969*b*). In *Mammalian Protein Metabolism*, vol. 3, p. 237. New York and London: Academic Press.
Munro, H. N. (editor). (1970). In *Mammalian Protein Metabolism*, vol. 4, p. 299. New York and London: Academic Press.
Munro, H. N. (1972*a*). In *Parenteral Nutrition*, p. 34 [A. W. Wilkinson, editor]. London: Churchill Livingstone.
Munro, H. N. (1972*b*). In *Symposium on Total Parenteral Nutrition*, p. 7 [P. Vanamee and M. E. Shils, editors]. Chicago: American Medical Association.
Munro, H. N. & Downie, E. D. (1964). *Nature*, **203**, 603.
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.
Munro, H. N., Black, J. G. & Thomson, W. S. T. (1959). *Br. J. Nutr.* **13**, 475.
Munro, H. N., Fernstrom, J. D. & Wurtman, R. J. (1975). *Lancet* i, 722.
Munro, H. N., Hubert, C. & Baliga, B. S. (1975). In *Alcohol, Nutrition and Protein Synthesis*, vol. 1, p. 33 [M. Rothschild, M. Oratz and S. S. Schreiber, editors]. New York: Pergamon Press Inc.
Narasinga Rao, B. S. & Nagabhushan, V. S. (1973). *Life Sciences* **12** (II), 205.
Nasset, E. S., Anderson, J. T. & Siciliano, A. M. (1951). *J. Nutr.* **45**, 173.
Pawlak, P. & Pion, R. (1968). *Annls Biol. anim. Biochim. Biophys.* **8**, 517.
Pozefsky, T., Felig, P., Tobin, J. D., Soeldner, J. S. & Cahill, G. F. Jr (1969). *J. clin. Invest.* **48**, 2273.
Twombley, J. & Meyer, J. H. (1961). *J. Nutr.* **74**, 453.
Young, V. R. & Munro, H. N. (1973). *J. Nutr.* **103**, 1756.
Young, V. R., Alexis, S. D., Baliga, B. S., Munro, H. N. & Muecke, W. (1972). *J. biol. Chem.* **247**, 3592.
Young, V. R., Haverberg, L. N., Bilmazes, C. & Munro, H. N. (1973). *Metabolism* **22**, 1429.

Printed in Great Britain