

- Grau, C. R. & Kamei, M. (1950). *J. Nutr.* **41**, 89.
- Harper, A. E. & de Muelenaere, H. J. H. (1961). *Proc. int. Congr. Biochem. v. Moscow*, **8**, 82.
- Hegsted, D. M. (1964). In *Mammalian Protein Metabolism*. Vol. 2, Chapter 14. [H. N. Munro and J. B. Allison, editors.] New York & London: Academic Press Inc.
- Jones, A. S., Hepburn, W. R., Cadenhead, A. & Boyne, A. W. (1962). *Anim. Prod.* **4**, 185.
- McOsker, D. R. (1962). *J. Nutr.* **76**, 453.
- Miller, D. S. & Payne, P. R. (1959). *Brit. J. Nutr.* **13**, 501.
- Miller, D. S. & Payne, P. R. (1961). *Brit. J. Nutr.* **15**, 11.
- Miller, D. S. & Payne, P. R. (1963). *J. theor. Biol.* **5**, 1398.
- Milner, C. K. & Carpenter, K. J. (1963). *Proc. Nutr. Soc.* **22**, xxii.
- Mitchell, H. H. & Block, R. J. (1946-7). *Nutr. Abstr. Rev.* **16**, 249.
- Morrison, A. B., Sabry, Z. I., Gridgeman, N. T. & Campbell, J. A. (1963). *Canad. J. Biochem. Physiol.* **41**, 275.
- Munaver, S. M. & Harper, A. E. (1959). *J. Nutr.* **69**, 58.
- National Research Council (1960). *Publ. nat. Res. Coun., Wash.*, no. 827.
- Njaa, L. R. (1962). *Brit. J. Nutr.* **16**, 185.
- Summers, J. D. & Fisher, H. (1961). *J. Nutr.* **75**, 435.
- Woodman, H. E. & Evans, R. E. (1951). *J. agric. Sci.* **41**, 102.
- Zablan, T. A., Griffith, M., Nesheim, M. C., Young, R. J. & Scott, M. L. (1963). *Poult. Sci.* **42**, 619.

Effects of amino acid excess on metabolism

By H. N. MUNRO, *Institute of Biochemistry, University of Glasgow*

Adverse nutritional effects have been reported when the diet of an animal, usually a growing animal, is supplemented with a single amino acid, a mixture of amino acids, or a protein of poor nutritional quality. In order to facilitate investigation of these adverse effects, Harper (1964) has suggested that all the recorded observations can be classified into one of three groups, namely amino acid antagonisms, amino acid imbalances and amino acid toxicities, on the grounds that these groups probably represent different metabolic mechanisms. The justification for separate classification is most obvious with amino acid antagonisms, in which structurally related amino acids compete with one another in metabolic reactions and thus depress amino acid utilization. There may also be a single type of metabolic mechanism underlying amino acid imbalances, in which a change in the proportion of the dietary amino acids results in a depression of growth rate that can be prevented by adding a supplement of the most limiting essential amino acid in the diet. Toxicities due to excessive intakes of single amino acids are classified as a separate group by Harper (1964) and are regarded as having no common underlying mechanism. For example, an excessive intake of tyrosine produces changes in the corneal epithelium and conjunctiva, accompanied by swelling of the feet (Schweizer, 1947). Excessive intakes of other amino acids generally cause loss of weight without inducing signs as specific as those caused by tyrosine. Furthermore, the amounts of individual amino acids needed to cause toxic effects vary greatly, and Harper (1964) has pointed out that those of greatest toxicity (methionine, tyrosine, tryptophan and histidine) are involved in many metabolic pathways. These various observations suggest that within the group of amino acid toxicities there may be several different types of metabolic change due to overloading the system with a single amino acid.

I should like to describe some investigations into certain effects on liver metabolism produced by giving excessive amounts of single amino acids to rats. These studies suggest one type of metabolic disturbance produced by excessive intake of amino acids, namely as a result of changes in endocrine gland function. Furthermore, the changes observed do not require a threshold level of amino acid excess, but can still be demonstrated on giving small amounts of the amino acids involved. It is thus possible that the toxicities observed with these amino acids are exaggerations of normal actions on endocrine function.

Effect of administration of single amino acids on liver RNA metabolism

Our investigations arose in the course of studying the action of single meals of protein on the metabolism of RNA in the liver. It was established by these studies that administration of casein to fasting rats causes an increase in incorporation of ^{32}P and $[^{14}\text{C}]$ glycine into liver RNA (Clark, Naismith & Munro, 1957). Subsequently, it was found that the mechanism of response is sensitive to deletion of one essential amino acid from a complete mixture of amino acids given by stomach tube; at time intervals varying from 1.5 to 6 h after giving the amino acid mixtures, the liver RNA showed considerably less uptake of precursor after giving an incomplete mixture than after giving a complete mixture (Munro & Clark, 1959). This suggests that the effect of dietary protein on liver RNA metabolism depends on the nutritive value of the protein. However, the giving of zein, which is deficient in tryptophan and lysine, led to the same stimulus on liver RNA metabolism as that observed after giving casein (Munro & Mukerji, 1958). Zein is not only deficient in tryptophan and lysine; it also contains an excessive amount of leucine. Accordingly, an exploration was made of the effect of excessive intakes of leucine and other individual amino acids on liver RNA metabolism and these researches will now be discussed in detail.

Young adult rats, trained to consume food promptly, were given a protein-free meal to which individual amino acids had been added; ^{32}P was injected 1 h later and they were killed 18 h thereafter. Among the non-essential amino acids, only glycine caused a significant increase in ^{32}P incorporation (+80%). Among the essential amino acids, methionine and leucine produced highly significant increments in ^{32}P uptake (+95% and +68% respectively); tryptophan and cystine had a smaller though still significant action (+56% and +47% respectively). Other amino acids caused increases in ^{32}P uptake that fell short of significance. The action of zein on liver RNA metabolism can be satisfactorily accounted for by its high leucine content. Mixtures of amino acids were prepared, one resembling zein, the other zein with leucine replaced by alanine. The leucine-rich mixture stimulated liver RNA metabolism to a considerably greater extent.

The actions of methionine, leucine and glycine on liver RNA metabolism were then explored more extensively. It was shown that each of these amino acids induces a significant increase in total RNA per liver as well as in incorporation of labelled precursors. Dose-response curves for the three amino acids (Fig. 1) demonstrated that glycine did not increase liver RNA metabolism when the intake was below

0.4 g, whereas the actions of methionine and leucine on ^{32}P uptake were shown to be linear from doses of 0.05 g upwards. We thus have a distinction between the action of glycine and that of the other two amino acids: the effect of glycine requires a threshold dose, whereas leucine and methionine are effective stimulants at all levels of intake.

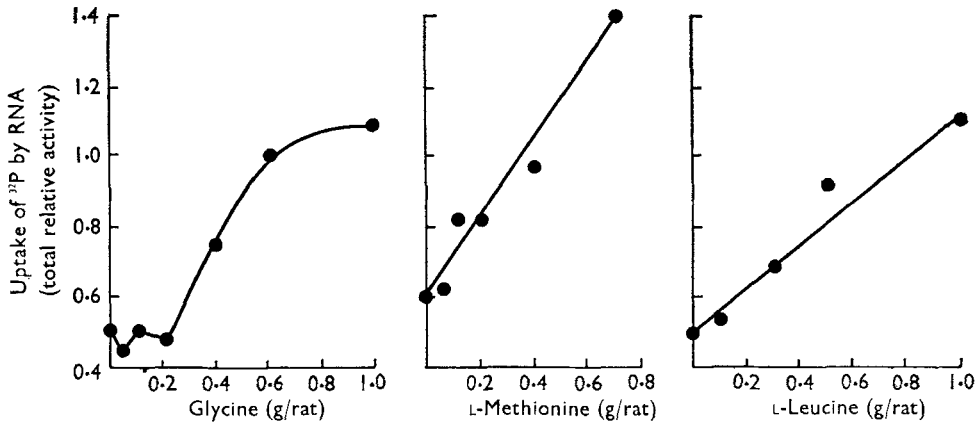


Fig. 1. The effect of giving by mouth different amounts of glycine, methionine or leucine on the uptake of ^{32}P by rat liver RNA 18 h after dosing and 17 h after injection of ^{32}P (from Munro & Mukerji, 1958).

It will be noted that these actions of single amino acids were obtained by adding each amino acid to a protein-free meal. The influence of dietary protein level on the response to methionine, leucine and glycine was then examined (Munro & Mukerji, 1962a). It was shown that the giving of a protein-free diet for several days before administration of the amino acid had no effect on the response. Inclusion of protein in the same meal as glycine or methionine resulted in no modification in the response at low levels of protein intake, but with larger amounts of protein the actions of glycine and methionine on liver RNA metabolism were inhibited. This agrees with other studies involving excessive intakes of amino acids which generally show less effect at the higher levels of protein intake (Harper, 1964).

Mechanism of action of methionine, leucine and glycine on liver metabolism

In addition to an increase in liver RNA content, a single large dose of leucine produces in rats an increment in liver protein (Munro & Mukerji, 1958), and we have since shown in unpublished experiments that glycine and methionine have a similar effect on liver protein content. Increments in the amounts of protein and RNA in the liver can be caused by other mechanisms in addition to the ingestion of protein. After giving cortisone the amount of RNA and of protein in the rat liver increases considerably, an effect which we (Goodlad & Munro, 1959) have attributed to a flow of amino acids to the liver from breakdown of muscle protein (Fig. 2). Another characteristic feature of increased adrenocortical activity is deposition of glycogen in the liver. Accordingly, rats were fed on leucine, methionine or glycine

and the liver was examined 18 h later for glycogen content. After giving each of the three amino acids, there was an increase in the amount of glycogen in the liver (Munro & Mukerji, 1962*b*). When adrenalectomized rats were subjected to this test, no increase in glycogen content was observed.

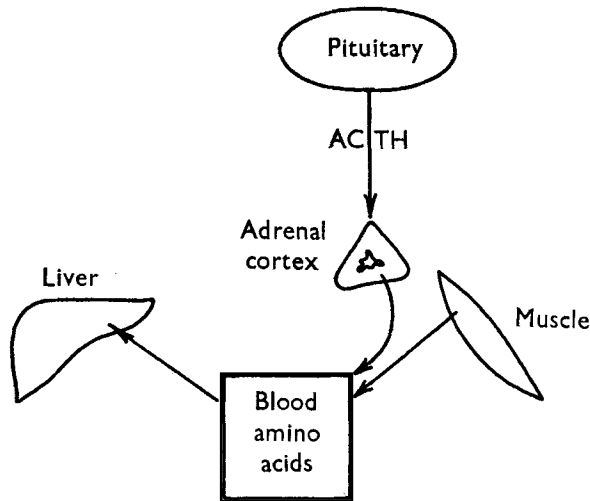


Fig. 2. Scheme showing the relationship of the pituitary-adrenal axis to amino acid redistribution from muscle to liver.

In view of this finding, measurements were made of the effect of adrenalectomy on the response of liver RNA metabolism to these amino acids. Removal of the adrenal glands prevented the action of methionine and leucine, but only moderately diminished that of glycine. This suggests that, after administration of large amounts of leucine and glycine, there is an increase in secretion of adrenocortical hormones. This was tested by measuring the corticosterone content of the plasma and adrenal glands of rats 24 h after they had been given several individual amino acids. After methionine and leucine had been given, there was a significant elevation of the plasma level and a tendency for the corticosterone content in the adrenal gland to be raised (Munro, Steele & Hutchison, 1963).

These findings receive some confirmation from findings of Góth and his colleagues in Hungary, using the well-known depression in blood eosinophil count as an index of adrenocortical activity. Rats were given single amino acids by stomach tube and the eosinophil population was enumerated 3 h later. Highly significant depressions of the eosinophil count followed administration of leucine and methionine, and some reduction was caused by valine, phenylalanine and tryptophan, but no significant change was obtained after giving glycine, histidine or isoleucine (Góth, Lengyel, Bencze, Sávely & Majsay, 1955). When given to adrenalectomized rats, leucine caused no reduction in eosinophil count. It was also shown that leucine is eosinopenic when given by mouth to normal human subjects but not when given to patients lacking adrenal glands (Góth *et al.* 1955). These findings are compatible with an effect of leucine administration on adrenocortical secretion. Other authors

(Harris & Lang, 1952; Vartiainen & Apajalahti, 1953) have also noted reductions in the eosinophil count after oral administration of certain amino acids. However, Harris & Lang (1952) noted that two amino acids found by them to cause significant depressions in eosinophil count, namely aspartic acid and glutamic acid, still did so when given to adrenalectomized animals. This throws some doubt on the eosinophil count as an unequivocal index of changes in adrenocortical secretory rate.

More direct evidence of an action of leucine on adrenal cortical function comes from further studies by the Hungarian workers (Góth, Nadásdi & Stadler, 1958). They showed that the giving of leucine to rats by stomach tube causes depletion of the ascorbic acid content of the adrenal cortex. This action does not occur in hypophysectomized rats, suggesting that the primary site of action is the pituitary gland. The experiments of these authors with parenterally injected amino acids are not relevant to the action of orally administered amino acids, for reasons given later (see below).

The action of methionine on adrenocortical secretory activity receives further confirmation from a rather unusual source. In hepatolenticular degeneration (Wilson's disease) there is an abnormally high excretion in the urine of copper and of amino acids, which is further augmented by administration of cortisone (Bearn & Kunkel, 1954). It has been shown that this effect can also be produced by giving large amounts of methionine (Steger & Steger, 1954; Iber, Chalmers & Uzman, 1957); other amino acids tested have not caused more than transient effects on copper excretion (Matthews, Milne & Bell, 1952), but leucine was not included among those used. The action of methionine in this syndrome is thus compatible with an effect on adrenocortical activity.

The nature of the action of glycine on liver RNA metabolism remains obscure. As noted earlier, adrenalectomy did not abolish the stimulant action of glycine on liver RNA metabolism; it did, however, eliminate the deposition of liver glycogen caused by glycine administration. This has been confirmed by other authors (Todd, Barnes & Cunningham, 1947), who also noted that this action of glycine was restored by giving fixed doses of cortisol to adrenalectomized animals (Todd & Allen, 1958). This suggests a permissive role of adrenocortical steroids in the action of glycine on liver metabolism. It should be pointed out that parenteral administration of glycine and other amino acids leads to accumulation of ammonia in the blood (Gullino, Winitz, Birnbaum, Cornfield, Otey & Greenstein, 1956; Nathans, Fahey & Ship, 1958; Barak, Humoller, Mahler & Holthaus, 1962) and this is the probable cause of the hyperglycaemia due to adrenaline release (Lundsgaard, 1930) and the increase in purine ring synthesis (Feigelson & Feigelson, 1963) which have been observed after injection of amino acids. There is no reason to believe that these effects have any bearing on the mechanism of action of amino acids given orally.

Effects of prolonged administration of single amino acids

In view of the evidence that a single large dose of certain amino acids causes increased secretion of corticosterone, presumably through release of ACTH, it might be expected that repeated administration of these amino acids would lead

to adrenal hypertrophy. This question was explored by Munro, Steele & Hutchison (1965) by feeding rats for 11 days on a basic protein-free diet to which were added large amounts of methionine, leucine or glycine. For comparison, groups receiving the basal diet with casein, gelatin, zein or zein supplemented with tryptophan and lysine were also studied. The weight of the adrenal gland at the end of the 11-day period on each diet was largely dependent on the biological value of the diet given. In particular, repeated dosage with methionine and leucine did not cause any significant hypertrophy of the gland. Nevertheless, all three amino acids increased the corticosterone content of the adrenal gland above the level observed in control animals receiving the basal protein-free diet. The concentration of corticosterone tended to increase in the plasma of these three groups, but the livers from the same animals did not show any change in protein and RNA content attributable to adrenocortical hormone stimulation. It can be concluded that prolonged administration of single amino acids fails to cause hypertrophy of the adrenal glands of the type attributable to overactivity of the pituitary gland, but the accumulation of corticosterone in the adrenal gland nevertheless indicates some continuing effect of amino acid administration on adrenal function. An increase in steroid content without a corresponding increase in adrenal weight does not resemble the action of ACTH, and we have suggested that the effect of the amino acids on gland function may occur through another mechanism.

It is likely that repeated administration of large doses of amino acids results in their more rapid destruction, as exemplified by tryptophan pyrrolase, the hepatic concentration of which rises after giving tryptophan (Knox, 1951). Snyderman (1965) has found that a single dose of leucine administered to infants causes a large increase in plasma leucine content, accompanied by changes in the concentrations of some other amino acids; on repeated administrations of leucine, these changes in plasma pattern became minimal. This indicates the capacity of the body to adapt rapidly to an excessive intake of one amino acid.

Finally, we may ask whether the study of amino acid toxicity under such highly artificial conditions as we have used has any bearing on practical nutrition. Although the levels of methionine and leucine used to induce metabolic changes in our experiments were usually very large, it should be remembered that the dose-response curves for changes in liver RNA metabolism are linear over a wide range of intake (Fig. 1). This implies that small variations in intake of these amino acids may produce changes in metabolism, and that, in consequence, adrenocortical function may well be sensitive to the level of intake of these amino acids under normal nutritional conditions.

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REFERENCES

- Barak, A. J., Humoller, F. L., Mahler, D. J. & Holthaus, J. M. (1962). *Gastroenterology*, **43**, 35.
Bearn, A. G. & Kunkel, H. G. (1954). *J. clin. Invest.* **33**, 400.
Clark, C. M., Naismith, D. J. & Munro, H. N. (1957). *Biochim. biophys. Acta*, **23**, 587.
Feigelson, P. & Feigelson, M. (1963). *J. biol. Chem.* **238**, 1073.

- Goodlad, G. A. J. & Munro, H. N. (1959). *Biochem. J.* **73**, 343.
- Góth, A., Lengyel, L., Bencze, E., Sávely, C. & Majsay, A. (1955). *Experientia*, **11**, 27.
- Góth, A., Nadásdi, N. & Stadler, E. (1958). *Z. Vitam.-Horm.- u. Fermentforsch.* **9**, 184.
- Gullino, P., Winitz, M., Birnbaum, S. M., Cornfield, J., Otey, M. C. & Greenstein, J. P. (1956). *Arch. Biochem. Biophys.* **64**, 319.
- Harper, A. E. (1964). In *Mammalian Protein Metabolism*. Vol. 2, p. 87. [H. N. Munro and J. B. Allison, editors.] New York: Academic Press Inc.
- Harris, M. M. & Lang, B. (1952). *Proc. Soc. exp. Biol., N.Y.*, **80**, 664.
- Iber, F. L., Chalmers, T. C. & Uzman, L. L. (1957). *Metabolism*, **6**, 388.
- Knox, W. E. (1951). *Brit. J. exp. Path.* **32**, 462.
- Lundsgaard, E. (1930). *Biochem. Z.* **217**, 147.
- Matthews, W. B., Milne, M. D. & Bell, M. (1952). *Quart. J. Med. N.S.* **21**, 425.
- Munro, H. N. & Clark, C. M. (1959). *Biochim. biophys. Acta*, **33**, 551.
- Munro, H. N. & Mukerji, D. (1958). *Biochem. J.* **69**, 321.
- Munro, H. N. & Mukerji, D. (1962a). *Brit. J. Nutr.* **16**, 257.
- Munro, H. N. & Mukerji, D. (1962b). *Biochem. J.* **82**, 520.
- Munro, H. N., Steele, M. H. & Hutchison, W. C. (1963). *Nature, Lond.*, **199**, 1182.
- Munro, H. N., Steele, M. H. & Hutchison, W. C. (1965). *Brit. J. Nutr.* **19**, 137.
- Nathans, D., Fahey, J. L. & Ship, A. G. (1958). *J. Lab. clin. Med.* **51**, 124.
- Schweizer, W. (1947). *J. Physiol.* **106**, 167.
- Snyderman, S. E. (1965). In *Significance of Changes in Plasma Amino Acid Patterns*. [J. H. Leatham, editor.] New Brunswick, NJ: Rutgers University Press. (In the Press.)
- Steger, J. & Steger, R. (1954). *Dtsch. Z. Nervenheilk.* **172**, 321.
- Todd, W. R. & Allen, M. (1958). *Amer. J. Physiol.* **195**, 643.
- Todd, W. R., Barnes, J. M. & Cunningham, L. (1947). *Arch. Biochem.* **13**, 261.
- Vartiainen, I. & Apajalahti, J. (1953). *J. clin. Endocrin.* **13**, 1502.