Further observations on ribonucleic acid metabolism in the liver after administration of individual amino acids

By H. N. MUNRO and D. MUKERJI
Department of Biochemistry, University of Glasgow

(*Received* 12 *October* 1961—*Revised* 2 *December* 1961)

Toxic effects due to the inclusion of large amounts of individual amino acids in the diet have frequently been noted. In the course of studying the influence of diet on ribonucleic acid (RNA) metabolism in the liver, Munro & Mukerji (1958) gave eighteen amino acids individually in 1 g quantities to rats and observed considerable increases in the uptake of ^{32}P by liver RNA after administration of glycine, methionine and leucine.

The effects of excessive intake of individual amino acids can be modified by changing the protein content of the diet. Harper (1958) has shown that growth retardation caused by addition of single amino acids to the diet is reduced by raising the general level of protein intake. His data demonstrate that an increase in the protein content of the diet from 9 to 18% casein was sufficient to eliminate the toxic effect of adding 3% L-leucine to the diet and reduced the depression in weight gain caused by addition of 2·5% DL-methionine. In view of these observations, we have examined the influence of the protein content of the diet on the response of liver RNA metabolism to large amounts of glycine, methionine and leucine.

EXPERIMENTAL

The influence of the protein content of the diet on the metabolic response to amino acid administration has been examined in two groups of experiments. In the first, the effect of protein depletion was studied. Rats received a normal diet or a protein-free diet for several days. At the end of the period, a final meal was administered containing carbohydrate and fat along with either glycine, methionine or leucine, and the effect of this meal on ^{32}P uptake by liver RNA was measured. In these experiments, the final meal to which the amino acids were added did not include any protein, even when the preceding diet had contained protein. In the second group of experiments, the effect of including protein in the final meal was examined.

The experimental conditions were based on those used previously (Munro & Mukerji, 1958).

Animals and diets. Male albino rats weighing about 180–200 g were housed in separate cages and fed on a synthetic diet (Munro & Naismith, 1953) in two portions: the morning meal provided 4·3 g carbohydrate, 0·2 g fat and 1 g of a vitamin–mineral–roughage mixture (Munro, 1949), and the evening meal contained 1·3 g carbohydrate, 0·5 g fat and all the protein of the diet, namely 2·2 g casein. In the experiments on

the effects of protein deficiency on the response to amino acid administration, the animals were depleted of protein by including 2·2 g carbohydrate in place of casein in the evening meal.

The rats quickly learned to consume these meals promptly. After they had been several days on the diet, the test protein or amino acid was substituted for the protein of the evening meal; control animals received only the carbohydrate and fat of this meal. After 1 h, 40 μc of [^{32}P]orthophosphate were injected intramuscularly. The rats were killed the next day, 18 h after injection of the isotope. The period of labelling thus coincided with absorption of the final meal.

Removal and analysis of liver. Under ether anaesthesia, the liver was perfused with 0·9 % (w/v) NaCl, then excised and analysed for ribonucleic acid phosphorus (RNA P) and deoxyribonucleic acid phosphorus (DNA P) by procedures described previously (Clark, Naismith & Munro, 1957). The amount of RNA P was then expressed as mg/mg DNA P, which takes advantage of the observation of Thomson, Heagy, Hutchison & Davidson (1953) that the DNA content of the livers of mature rats is a measure of cell number which is unaffected by diet and in consequence can be used as a standard of reference for other constituents of the liver cell.

The specific activities of the inorganic phosphate of the liver and of the individual ribonucleotides of liver RNA were measured by procedures described previously (Clark *et al.* 1957). These specific activity values were used to compute the relative specific activities of RNA P (specific activity of RNA P as a percentage of the specific activity of the inorganic phosphate fraction of the same liver). In order to estimate the total incorporation into RNA P per liver, the total relative activity (Campbell, Olley & Blewett, 1949) was obtained by multiplying the percentage figure obtained for relative specific activity by the total amount of liver RNA P in mg/mg DNA P.

Test protein and amino acids. The casein was of unextracted grade (Glaxo Laboratories Ltd, Greenford, Middlesex). The amino acids were commercial preparations (British Drug Houses Ltd, Poole, Dorset). The glycine was AR quality; the L-leucine was stated to contain traces of L-isoleucine.

RESULTS

In the first series of experiments, the effect of protein depletion on the metabolic response to amino acid administration was examined. In confirmation of previous evidence (Clark *et al.* 1957), Table 1 shows that protein deprivation of more than 1 day's duration caused an increase in ^{32}P uptake by liver RNA; this effect was evident both for the animals given no amino acids and for those fed with individual amino acids. Irrespective of the period of protein depletion, more ^{32}P was incorporated by animals receiving an amino acid in the final meal. Analysis of variance of the results confirmed that amino acid administration had a significant effect on RNA metabolism ($P < 0{\cdot}01$), and that the magnitude of the change was not significantly altered by protein deficiency ($P > 0{\cdot}05$ for interaction between treatment and duration of depletion).

In the second group of experiments, the effect of including protein in the final meal

was examined. Animals trained on an adequate diet to consume food rapidly were given a final meal which included different amounts of casein along with glycine or methionine. Casein administration by itself has been shown to stimulate incorporation of ^{32}P into liver RNA (Clark *et al.* 1957). Table 2 shows that this did not occur as a linear response to the amount of casein administered, no effect on RNA metabolism being noted until 1 g casein had been included in the final meal. Combination of

Table 1. *Liver ribonucleic acid metabolism after administration of glycine, methionine or leucine to rats receiving a protein-free diet*

(The rats were given the protein-containing diet for several days and were then fed on the protein-free diet. After 0, 3 or 7 days of depletion, the animals were given a final meal of carbohydrate and fat, to which was added 1 g glycine, L-methionine or L-leucine; 40 μc ^{32}P were injected 1 h after the final meal and the rats were killed 18 h later. Each entry is the mean value, with standard error, for three rats. Values in parentheses are the percentage increases in ^{32}P uptake caused by amino acid administration at the different stages of depletion)

Addition to final meal	Uptake of ^{32}P by RNA (total relative activity)*		
	No depletion	3 days' depletion	7 days' depletion
None	0·47 ± 0·04	1·04 ± 0·20	1·08 ± 0·33
Glycine	0·85 ± 0·22 (+81 %)	1·67 ± 0·16 (+61 %)	2·03 ± 0·36 (+88 %)
Methionine	0·61 ± 0·10 (+30 %)	1·38 ± 0·25 (+33 %)	1·81 ± 0·25 (+68 %)
Leucine	0·85 ± 0·12 (+81 %)	1·36 ± 0·10 (+31 %)	1·63 ± 0·21 (+51 %)

* See p. 258.

Table 2. *Liver ribonucleic acid metabolism after administration of 0·5 g glycine or methionine along with different quantities of casein to rats*

(After 4 days of training on a diet containing protein, rats were given a final meal of carbohydrate and fat to which had been added different quantities of casein with or without 0·5 g glycine or L-methionine. After 1 h, 40 μc ^{32}P were injected and the rats were killed 18 h later. Each entry is the mean value, with standard error, for four rats. In addition, at each level of casein in the final meal, the results have been examined by analysis of variance and the minimum values needed to establish a difference between groups of rats significant at the 5 % level were computed from the error variances)

Casein added to final meal (g)	Uptake of ^{32}P by RNA (total relative activity)*					
	Value			Change		
	No addition	Glycine added	Methionine added	With glycine	With methionine	Minimum significant difference
0·0	0·67 ± 0·09	1·14 ± 0·07	0·99 ± 0·07	+0·47	+0·32	0·25
0·5	0·68 ± 0·07	1·20 ± 0·07	1·08 ± 0·21	+0·52	+0·40	0·44
1·0	0·96 ± 0·07	1·51 ± 0·22	1·02 ± 0·10	+0·55	+0·06	0·47
2·0	1·42 ± 0·11	1·48 ± 0·06	1·13 ± 0·21	+0·06	−0·29	0·45

* See p. 258.

casein with 0·5 g glycine had an additive effect on RNA metabolism up to 1 g casein but not when 2 g casein were given with the glycine. The combined stimulus due to casein and methionine was no longer additive when the meal included 1 g casein and the stimulant action of 2 g casein was in fact reduced by addition of methionine to the meal. This was presumably due to interference by methionine with the digestion and

absorption of casein, since rats receiving 0·5 g methionine in the final meal invariably had a considerable amount of food in their stomachs when killed 18 h later. This effect of methionine on gastric emptying has been observed previously (Munro & Mukerji, 1958).

DISCUSSION

The toxic effects of large doses of amino acids are greater when the acids are added to a diet deficient in protein (Harper, 1958). Table 1 shows that the action of excessive amounts of glycine, methionine or leucine on liver RNA metabolism was not modified by the protein content of the diet when the amino acids were given apart from the dietary protein. This effect can be contrasted with the action of excessive amounts of glycine or methionine when given in a meal along with protein. Table 2 shows that, at low levels of protein intake, the stimulant actions of the dietary protein and of the amino acid on RNA metabolism were additive, but at higher levels of protein intake they ceased to be so. This may have represented a reduction in rate of absorption of the amino acid in the presence of appreciable amounts of protein in the same meal.

Wiseman (1955) has described a powerful inhibitory effect of methionine on the absorption of other amino acids from the intestinal lumen. This phenomenon is unlikely to have played a part in the effects we have observed in the liver, since our results showed an action of methionine on liver RNA metabolism even in animals receiving a protein-free diet. Other evidence (Munro & Mukerji, 1962) demonstrates that stimulation of adrenocortical hormone secretion is the most likely explanation for the action of methionine on liver RNA metabolism.

SUMMARY

1. The uptake of ^{32}P by liver ribonucleic acid was measured in rats receiving large doses of glycine, methionine or leucine.

2. When given along with carbohydrate and fat, each amino acid caused a considerable increase in ^{32}P uptake which was not significantly influenced by the protein content of the preceding diet.

3. Inclusion of protein in the same meal as the amino acid resulted in no modification of the response at low levels of protein intake, but with large amounts of protein the actions of glycine and of methionine were inhibited.

We are grateful to the Medical Research Council for a grant to one of us (H.N.M.) for part-time scientific assistance.

REFERENCES

Campbell, I. G., Olley, J. & Blewett, M. (1949). *Biochem. J.* **45**, 105.
Clark, C. M., Naismith, D. J. & Munro, H. N. (1957). *Biochim. biophys. Acta*, **23**, 587.
Harper, A. E. (1958). *Ann. N.Y. Acad. Sci.* **69**, 1025.
Munro, H. N. (1949). *J. Nutr.* **39**, 375.
Munro, H. N. & Mukerji, D. (1958). *Biochem. J.* **69**, 321.
Munro, H. N. & Mukerji, D. (1962). *Biochem. J.* **82**, 520.
Munro, H. N. & Naismith, D. J. (1953). *Biochem. J.* **54**, 191.
Thomson, R. Y., Heagy, F. C., Hutchison, W. C. & Davidson, J. N. (1953). *Biochem. J.* **53**, 460.
Wiseman, G. (1955). *J. Physiol.* **127**, 414.

Printed in Great Britain