The relationship between nutritive value of dietary protein and activity of liver arginase and kidney transamidinase enzymes

By E. A. KEAN

Department of Biochemistry, University of the West Indies, Kingston, Jamaica

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1. The protein efficiency ratio of three protein sources was determined with rats by a depletion–repletion method. The sources were: a groundnut product, a methionine-supplemented groundnut product and lactalbumin.

2. Livers obtained from the test animals were assayed for arginase activity, and kidneys for transamidinase activity (glycine amidinotransferase).

3. The measurements indicated that there was an inverse relationship between arginase activity and the nutritive value of the dietary protein.

4. Transamidinase activity was also influenced by nutritive value. Only the unsupplemented groundnut product, which had the lowest nutritive value, failed to produce a significant increase of transamidinase activity over basal levels.

5. The findings are discussed from the standpoint of physiological function and needs. It is suggested that observed levels of arginase activity are not necessarily related to amounts of urea excreted; similarly, transamidinase activity may be well in excess of physiological requirements.

There are two alternative pathways for the metabolism of the amidino group of arginine in mammalian metabolism, namely:

(1) $HN{:}C(NH_2).NH.(CH_2)_3.CH(NH_2).COOH + H_2O \rightarrow CO(NH_2)_2$ (urea) + $H_2N.(CH_2)_3.CH(NH_2).COOH$ (L-ornithine)

(L-arginine)

(2) L-arginine + $CH_2.(NH_2).COOH \rightleftarrows HN{:}C(NH_2).NH.CH_2.COOH$ + L-ornithine

(glycine; guanidoacetic acid)

Reaction (1) is catalysed by the enzyme arginase (L-arginine amidinohydrolase, EC 3.5.3.1); its occurrence in liver is an essential step of the urea cycle (Krebs, 1952). Reaction (2) is catalysed by transamidinase (L-arginine: glycine amidinotransferase, EC 2.1.4.1) (Fuld, 1954; Ratner & Rochovansky, 1956) which is found mainly in kidney. A further reaction, *N*-methylation of guanidoacetic acid, results in creatine synthesis by liver tissue (Borsook & Dubnoff, 1940, 1941).

The studies of Schimke (1962*a*, *b*, 1963) suggested that the activity of arginase and of other urea-cycle enzymes is related, in a direct way, to the rate of urea formation; it was suggested (Schimke, 1962*b*) that the rate of urea formation may be controlled through co-ordinated alterations in the amounts of the urea-cycle enzymes.

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Transamidinase activity in kidney tissue is influenced by the level of protein in the diet (Van Pilsum, Berman & Wolin, 1957), also possibly by the nature of the dietary protein (Borchers, 1964). Van Pilsum *et al.* (1957) found that a marked decrease in activity was produced by protein-free diets, and were therefore led to suggest that the maintenance of this enzyme was dependent on an exogenous protein supply. Borchers (1964) found that growth inhibition produced in rats by a factor present in raw soya-bean meal was accompanied by a decrease in kidney transamidinase activity.

It appeared to the author that arginase activity in liver and transamidinase activity in kidney might each in its own way reflect the nutritive value of dietary protein. Thus, protein of low nutritive value giving poor nitrogen retention could induce a high level of arginase activity necessary for high rates of urea excretion. Simultaneously, low transamidinase activity, due to the inadequacy of the diet for synthesis of enzyme protein, should be observed.

The investigation now described was undertaken to test this hypothesis. The work was based on the use of a protein-rich product from groundnuts. The nutritive value of this food was improved by supplementation with a single amino acid. Enzyme activities were observed in livers and kidneys of rats fed on the unsupplemented or supplemented food, a reference protein, and a protein-free diet. These levels of enzyme activity were found to conform to a pattern determined by the nutritive value of the diets.

EXPERIMENTAL

Animals and diets. Male albino rats were used. They were initially 4–5 weeks old and weighed from 80 to 90 g. After being used for determination of protein efficiency ratio as described below, some of the animals were killed for determinations of arginase activity in liver and of transamidinase activity in kidney. Animals had been fed on a stock diet (Purina Laboratory Chow; Ralston Purina Company, St Louis, Mo.) for 1–2 weeks after weaning. Thereafter and for the entire 14 days of the feeding trials they were supplied *ad lib.* with a diet (protein-free diet) of the percentage composition: sucrose 8·8, maize starch 7·5, butter 10, salt mixture (USP XIV) 4, vitamin mixture in dextrose 2·2. The vitamin mixture (Vitamin Diet Fortification Mixture) was purchased from Nutritional Biochemicals Corporation, Cleveland, Ohio. It contained, per g: vitamin A 9000 i.u., vitamin D 18000 i.u., α-tocopherol 5 mg, ascorbic acid 45 mg, inositol 5 mg, choline chloride 75 mg, menaphthone 2·25 mg, *p*-aminobenzoic acid 5 mg, nicotinic acid 4·5 mg, riboflavine 1 mg, pyridoxine hydrochloride 1 mg, thiamine hydrochloride 1 mg, calcium pantothenate 3 mg, biotin 20 μg, folic acid 90 μg, vitamin B_{12} 1·35 μg.

Rats were removed to individual metabolism cages after 12 days on the protein-free diet; they were then given one of the following protein sources in measured amounts in addition to the protein-free diet. (1) A groundnut product designated GP (Lypro; International Protein Products Ltd, London); according to the supplier, this was a spray-dried lipoprotein powder, free of carbohydrate and fibre, containing 65% protein and 32% lipid (fat). Information about the amino acid composition was also obtained from the manufacturers. (2) GP supplemented with 2·6% L-methionine.

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(3) Lactalbumin (Nutritional Biochemicals Corporation, Cleveland, Ohio) + 32% hydrogenated coconut oil (Puritan cooking oil; Seprod Ltd, Kingston, Jamaica).

These protein-rich foods were prepared in bulk as suspensions blended into 4% (w/v) sucrose solution to improve palatability. Since the aim was to supply similar quantities of nutrients, particularly of N, to all animals (see Rippon, 1959), each mixture was analysed for N to determine the measured volume that should be fed to the animals in each of the different dietary groups. This volume was within the range 9–11 ml/rat per day, and supplied about 170 mg N. Rats soon learned to consume this amount within 3–4 h. Average daily consumption of the protein-free diet was about 11 g/rat per day, so that the daily intake of N represented a level of about 9% protein.

Determination of protein efficiency ratio (PER) (*Frost & Sandy*, 1949; *Rippon*, 1959). Records of weight changes were kept until the rats were killed 2 weeks after being given the protein-rich foods. PER was calculated as (gain in weight (g))/(N intake (g)) from the values for the first 7 days of protein feeding, the very small amount of N in the protein-free diet being neglected. The results for 14 days were found to be somewhat more variable.

N content of protein-rich foods. Total N in the liquid suspensions was determined by a standard semi-micro-Kjeldahl method. After digestion with H_2SO_4 and treatment with alkali, ammonia was steam-distilled into 4% (w/v) boric acid solution and titrated with standard acid (Hawk, Oser & Summerson, 1951).

Urinary N, urea and creatinine. Individual 24 h urine samples were collected from four animals in each group given the protein-rich foods; the period chosen was that immediately before the rats were killed. Toluene was used as preservative. Samples were made up to volume with water, filtered, frozen and stored at $-20°$ until analysed. Total N was determined by the standard semi-micro-Kjeldahl method.

Urea was determined by the method of Archibald (1945) using the colour reaction with an acid mixture containing α-isonitrosopropiophenone. Creatinine was determined by the Folin micro-method based on the Jaffe reaction (Hawk *et al.* 1951).

Enzyme assays. Fed rats were killed by cervical fracture and bled by decapitation. The entire liver and both kidneys were removed, chilled on cracked ice, quickly blotted dry, and weighed.

For determination of arginase activity, a weighed portion of liver was homogenized in ice-cold 0·25 M-sucrose solution, in a Potter–Elvehjem homogenizer with a Teflon pestle. The homogenates containing 20% (wet weight) tissue were frozen in acetone-dry ice mixture and stored for up to 1 week without appreciable loss of arginase activity. Immediately before assay, the frozen samples were diluted with 0·154 M-NaCl to give a final concentration of 5% (wet weight). The procedure then followed was based on that of Van Slyke & Archibald (1946) as modified by Schimke (1962*a*). The incubation medium contained: L-arginine hydrochloride, adjusted to pH 9·7, 500 μmoles; $MnCl_2$ 2 μmoles in 2·0 ml total volume; and 20–50 μl of 5% liver homogenate. Incubation was for 15 min at 37°. Enzymic reaction was halted by adding 1 ml of 30% $HClO_4$. After centrifugation, 0·15 ml supernatant liquid was removed to 4·0 ml acid mixture for the colour reaction with α-isonitrosopropiophenone.

For determination of transamidinase activity, both kidneys of a rat were homogenized

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in a pre-chilled container attached to a Waring Blendor. The homogenizing medium was 0·1 M-tris-(hydroxymethyl)-aminomethane-HCl buffer (tris buffer), pH 7·5 in amounts sufficient to give a concentration of 10% tissue (wet weight). The subsequent procedure was adapted from that of Ratner & Rochovansky (1956) in which arginine production from L-ornithine + guanidoacetic acid is measured; added arginase is present during the incubation and yields urea in amounts equivalent to the arginine formed. The kidney homogenate was diluted with water containing reduced glutathione in an amount sufficient to produce a marked activation without interfering with the α-isonitrosopropiophenone colour reaction with urea. Incubation medium contained tris buffer adjusted to pH 7·5, 200 μmoles, guanidoacetic acid 140 μmoles, ornithine dihydrochloride 10 μmoles, $MnCl_2$ 1 μmole, GSH 2·5 μmoles, arginase 6 mg (120 units) and 0·8 ml of 5% homogenate in a total volume of 4·0 ml. Incubation was for 1 h at 37°. The reaction was stopped by addition of 0·8 ml of 60% (w/v) $HClO_4$. After centrifugation, 3·2 ml supernatant liquid were removed and added to 1·6 ml acid mixture containing α-isonitrosopropiophenone for colour development.

All enzyme assays were done in duplicate or triplicate and included blanks, in which $HClO_4$ was added at zero time. For standards, appropriate amounts of urea were added to reaction mixtures. In assays of both enzymes, yield of urea was found to be proportional to amount of enzyme (i.e. homogenate) over a fourfold range of the latter.

Determination of protein in tissue homogenates. The biuret reaction (Gornall, Bardawill & David, 1949) was used. Standards were prepared from bovine serum albumin.

RESULTS

PER. By this criterion the food GP was inferior to the reference protein lactalbumin in nutritive value. However, the PER of GP was substantially improved by supplementation with methionine and then surpassed that of lactalbumin (Table 1).

Table 1. *Mean values with their standard errors for protein efficiency ratio (PER) of three protein-rich foods by a depletion–repletion method with rats*

Protein-rich food	No. of animals	PER
GP†	16	10·1 ± 0·31
Lactalbumin	12	13·6 ± 0·53***
GP + methionine	12	16·1 ± 0·43***

† Groundnut product.
*** Significantly different ($P < 0·001$) from the preceding value in the table.

Urinary N and urea. The 24 h values for animals given GP, GP + methionine or lactalbumin (four rats each) were expressed as a fraction of the daily N intake from the protein-rich foods. Comparing the values for N output as a fraction of N intake, the means for rats given GP or GP + methionine were the same (0·56, ranges 0·54–0·62 and 0·48–0·61 respectively) whereas that for rats given lactalbumin was much lower (0·17, range 0·13–0·23). The ratios of urea N to N intake were similar for the groups given GP or GP + methionine (ranging from 0·39 to 0·47 and from 0·39 to 0·50

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respectively). The lactalbumin-fed group excreted very little urea N; the mean ratio was 0·06 (range 0·05–0·08).

Urinary creatinine. There were no marked differences among the three dietary groups in the values for 24 h excretion of creatinine. The mean values (mg/24 h, four rats in each group) were: for rats given GP, 3·29; for rats given GP + methionine, 3·33; and for rats given lactalbumin, 3·88.

Table 2. *Mean values for arginase activity in liver samples from rats given a protein-free diet with or without a protein-rich supplement*

Protein-rich supplement	No. of animals	μmoles urea formed/h: Per mg protein	Per g tissue $\times 10^{-2}$	Per 100 g body-weight $\times 10^{-3}$
None	6	133	319	128
GP† + methionine	7	161	394	151
Lactalbumin	7	186**	453**	180**
GP	7	225**	489**	177**
Standard deviation		±37·4	±74·3	±24·4
Smallest difference between means of groups receiving supplements significant at 5% level		43·6	86·7	28·4

† Groundnut product.
** Significantly different ($P < 0·01$) from mean for group receiving no protein-rich supplement.

Table 3. *Mean values for transamidinase activity in kidney samples from rats given a protein-free diet with or without a protein-rich supplement*

Protein-rich supplement	No. of animals	μmoles arginine formed/h per g tissue
None	6	10·3
GP*	7	10·5
GP + methionine	7	13·9
Lactalbumin	7	16·8
Standard deviation		±2·87
Smallest difference between means of groups receiving supplements significant at 5% level		3·35

* Groundnut product.

Arginase activity. Values are given in Table 2 as μmoles product/h on the basis of either protein content, tissue weight or body-weight. On either basis, rats given only the protein-free diet had the lowest values, followed in ascending order by rats given GP + methionine, lactalbumin and GP alone. Analyses of variance of the results showed that there were real differences ($P < 0·05$) between the mean values for the groups, however expressed, but the differences between any two consecutive means were not always significant. In general, no real difference was found between diets with GP + methionine and with lactalbumin, or between diets with lactalbumin and with GP.

Transamidinase activity. The mean values for each dietary group are given in ascending order, as μmoles product/h per g kidney in Table 3. The protein content of kidney did not differ appreciably between the various dietary groups, and total kidney

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weight was, in general, proportional to body-weight. An analysis of variance showed that there were real differences between the dietary treatments ($P < 0.01$). The mean values found with the protein-free diet and with the GP-supplemented diets were not significantly different; each of these values was significantly lower than that for rats given GP+methionine ($P < 0.05$) or lactalbumin ($P < 0.002$). The values for the latter two groups were not significantly different from one another.

DISCUSSION

Supplementation of the groundnut product with methionine had the effect of increasing the PER. The amount of methionine added was estimated as sufficient to raise the content of sulphur-containing amino acids to their level in lactalbumin. It appears that there was a relative deficiency of methionine or of cysteine, or of both, in this food.

An unexpected finding was the relatively low PER for lactalbumin. This protein is known to have an amino acid composition which well satisfies the essential amino acid requirements of the rat (Block & Mitchell, 1946–7; Block & Bolling, 1951). In parallel feeding trials, egg albumin similarly mixed with hydrogenated coconut oil gave a mean PER of 21·2 (Kean, 1966). There are some indications, as discussed below, that the PER value reported here for lactalbumin did not truly reflect the adequacy of its amino acid composition for supporting growth or repletion.

The lowest values for arginase activity were obtained for rats which received no protein-rich supplement (Table 2). This is in accord with earlier reports (Mandelstam & Yudkin, 1952; Ashida & Harper, 1961). Other values in Table 2 together with those in Table 1 show an inverse relationship between nutritive value and arginase activity; the highest value for PER (methionine-supplemented groundnut product) was associated with the lowest value for arginase activity, and the lowest value for PER (unsupplemented groundnut product) with the highest value for arginase activity.

On this basis, and in view of the suggestion of Schimke (1962*b*) quoted earlier, some correlation between urea excretion and arginase activity might have been expected. None was evident, however, among the groups receiving protein-rich supplements. The lactalbumin-fed group excreted much less urea than the other two, but did not differ significantly from either in liver arginase activity. Again, urea excretion was similar for the two groups given the groundnut product or methionine-supplemented groundnut product, although their levels of arginase activity were different. It is evident that these diets resulted in levels of arginase activity which were not related in any simple way to urea excretion, but which were rather a function of N retention as indicated by growth rate.

It is probable that an explanation lies in some previous reports on factors affecting arginase activity. Schimke (1963) found that the effect of adrenalectomy, or of giving arginine-free diets, on arginase activity was different from the effect on other urea-cycle enzymes; he suggested that arginase differed from these other enzymes as regards the controlling factor or agents. Freedland & Sodikoff (1962), applying hormonal and other treatments to rats, found that arginase activity was increased only

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when increased protein catabolism was accompanied by maintenance or increase of body-weight. In this respect arginase differed from arginine synthetase (L-citrulline: L-aspartate ligase (AMP)), the enzyme of lowest activity in the urea cycle (Brown & Cohen, 1959; McLean, 1961). Freedland & Sodikoff (1962) suggested that arginase is influenced by two factors, namely degree of protein catabolism and N balance, using change in weight as an index of the latter. It seems feasible that, since the amount of arginase in liver greatly exceeds physiological needs, its observed activity need not be correlated with urea excretion.

The values in Table 3 together with those in Table 1 show the relationship between nutritive value and kidney transamidinase activity. Table 3 shows that, after a protein-depletion period, the groundnut product produced no significant change in enzyme activity; groundnut product + methionine and lactalbumin each produced a significant increase. Therefore, a certain minimum nutritive value of dietary protein was required for increase in enzyme activity; unsupplemented groundnut product did not achieve this minimum. The difference between enzyme activity levels produced by methionine-supplemented groundnut product and by lactalbumin was significant only at a level of P between 0·05 and 0·1. A more significant difference would have required some explanation as to why lactalbumin, in spite of its lower PER value, was better for renewal and maintenance of this enzyme than was the methionine-supplemented groundnut product.

It is likely that decreased values for transamidinase activity were unimportant in relation to physiological requirements; this is suggested by the fact that urinary excretion of creatinine was similar in all groups receiving supplements. This is in agreement with the observation of Van Pilsum (1957) that even after a decrease of up to 85% in kidney transamidinase activity brought about in rats by protein depletion, total body creatine and creatine phosphate remained at normal levels.

A report by Kiriyama & Ashida (1964) suggested that, with diets containing equal amounts of protein, urea excretion decreases as the nutritive value of the protein increases, and also that a lower proportion of urinary N then appears as urea. If such a relationship applied to the investigation described here, then on the basis of the values for urea excretion lactalbumin should have had the highest nutritive value of the three protein-rich foods. It appeared that with lactalbumin some undetermined factor, such as relatively poor absorption of the protein, influenced the results for PER and N excretion. However, this limitation does not affect the general conclusions of the work reported here as to the relationships between nutritive value of dietary protein and the activity of the two enzymes investigated. Attention is drawn to two points in particular: (1) the absence of any obvious relationship between arginase level and the amount of urea excreted, and (2) the possible usefulness of transamidinase levels as an additional index of the state of N nutrition in animals.

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REFERENCES

Archibald, R. M. (1945). *J. biol. Chem.* **157**, 507.
Ashida, K. & Harper, A. E. (1961). *Proc. Soc. exp. Biol. Med.* **107**, 151.
Block, R. J. & Bolling, D. (1951). *The Amino Acid Composition of Proteins and Foods*, 2nd ed. Springfield, Illinois: Thomas.
Block, R. J. & Mitchell, H. H. (1946–7). *Nutr. Abstr. Rev.* **16**, 249.
Borchers, R. (1964). *Proc. Soc. exp. Biol. Med.* **115**, 893.
Borsook, H. & Dubnoff, J. W. (1940). *Science, N.Y.* **91**, 551.
Borsook, H. & Dubnoff, J. W. (1941). *J. biol. Chem.* **138**, 389.
Brown, G. W. Jr & Cohen, P. P. (1959). *J. biol. Chem.* **234**, 1769.
Freedland, R. A. & Sodikoff, C. H. (1962). *Proc. Soc. exp. Biol. Med.* **109**, 394.
Frost, D. V. & Sandy, H. R. (1949). *J. Nutr.* **39**, 427.
Fuld, M. (1954). *Fedn Proc. Fedn Am. Socs exp. Biol.* **13**, 215.
Gornall, A. G., Bardawill, C. J. & David, M. M. (1949). *J. biol. Chem.* **177**, 751.
Hawk, P. B., Oser, B. L. & Summerson, W. H. (1951). *Practical Physiological Chemistry*, 12th ed. New York: McGraw-Hill.
Kean, E. A. (1966). *J. Nutr.* **90**, 91.
Kiriyama, S. & Ashida, K. (1964). *J. Nutr.* **82**, 127.
Krebs, H. A. (1952). In *The Enzymes*. Vol. 2, part 2, p. 866. [J. B. Sumner and K. Myrback, editors.] New York: Academic Press Inc.
McLean, P. (1961). *Nature, Lond.* **191**, 1302.
Mandelstam, J. & Yudkin, J. (1952). *Biochem. J.* **51**, 681.
Ratner, S. & Rochovansky, O. (1956). *Archs Biochem.* **63**, 277.
Rippon, W. P. (1959). *Br. J. Nutr.* **13**, 243.
Schimke, R. T. (1962*a*). *J. biol. Chem.* **237**, 459.
Schimke, R. T. (1962*b*). *J. biol. Chem.* **237**, 1921.
Schimke, R. T. (1963). *J. biol. Chem.* **238**, 1012.
Van Pilsum, J. F. (1957). *J. biol. Chem.* **228**, 145.
Van Pilsum, J. F., Berman, D. A. & Wolin, E. A. (1957). *Proc. Soc. exp. Biol. Med.* **95**, 96.
Van Slyke, D. D. & Archibald, R. M. (1946). *J. biol. Chem.* **165**, 293.

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