Diet and plasma protein composition

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From time to time it has been claimed that the composition of tissue proteins can be altered by changing the proportions of the amino acids made available to the organism. Most of these results, however, are capable of an alternative explanation. For example, Csonka, Denton & Ringel (1947) and Csonka (1950) supplemented the diet of hens with casein and obtained heavier eggs with a higher than average content of methionine and cystine; supplements of methionine and cystine did not have this effect. The authors pointed out that this result could be due either to an alteration in the composition of 'egg protein' or to an alteration in the relative proportions of the different proteins of the egg. They subsequently demonstrated electrophoretically (Csonka & Jones, 1952) that the higher-protein diet of the hens caused a rise in the content of egg-white globulins and a fall in that of ovomucoid.

Similar findings have been reported and similar possible explanations put forward in the microbiological field. Cohen (1958) compared the performance of a valine-sensitive strain of *Escherichia coli* with that of a valine-resistant strain and showed that valine prevented exponential growth in the former, and that its proteins contained more valine and less methionine than did those of the latter strain. The author suggested that valine had replaced other amino acids in the protein, but an alteration in the proportions of the proteins could equally well explain the results. Similarly, the difference in amino acid composition of total cellular proteins of *Mycobacterium tuberculosis* observed by Rao & Wadhwani (1956) on different media may have been due to a change in the proportions of the different proteins.

Freeland & Gale (1947), Camien, Salle & Dunn (1945), Stokes & Gunness (1946) and Work (1949) were unable to alter the amino acid composition of bacterial protein by changing the media on which the organisms were grown.

Against the background of these results the suggestion of Albanese (1953) that there was a change in the blood protein of babies when they were fed on a low-lysine diet, the lysine being replaced by arginine, stands out as unique. There were further reasons for re-examining this finding. Albanese did not analyse the blood proteins for lysine but determined only arginine and total nitrogen. Moreover, there were reasons to doubt the accuracy of the arginine determinations. Therefore rats were given diets low in lysine (diets similar to those used by Albanese) and diets free from lysine, and the arginine and lysine contents of their blood proteins were determined.

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When we failed to reproduce in rats the results reported by Albanese on babies we repeated the experiment on piglets, as these animals are more likely than are rats to show the same reaction as human babies. The piglets also showed no change in plasma content of arginine or lysine.

EXPERIMENTAL

Experiment 1

Animals

Six litters each of four male albino rats aged 47 days and weighing 68–94 g were used. One pair from each litter was given the deficient diet and the other pair from the same litter was given the corresponding supplemented diet, i.e. diets 1 and 2, 3 and 4, 5 and 6.

Diets

Six diets were used, three low or completely deficient in lysine and the same diets supplemented with lysine.

Diet 1, *wheat gluten diet.* This diet was patterned after the diet used by Albanese (1953) with dried malt extract in place of the dextri-maltose no. 2, and with the salt mixture of Hawk, Oser & Summerson (1947) as being more suitable to the requirements of the rat. The diet consisted of wheat gluten 350, dried brewer's yeast 100, olive oil 400, dried malt extract 960, salt mixture 160 and arrowroot starch 230 g. The crude protein content was 19·0 %, that of lysine 0·302 % and of arginine 0·562 %.

Diet 2, *wheat gluten plus lysine diet.* Diet 1 was supplemented with 1·0 g L-lysine monohydrochloride (equivalent to 0·8 % free lysine) per 100 g. The total lysine content was 1·102 % and the arginine content 0·562 %.

Diet 3, *lysine-free amino acid diet.* This was diet 4 in which the lysine was replaced by a mixture of equal weights of arginine monohydrochloride and monosodium glutamate. The protein equivalent was 10·1 %, the arginine content was 0·8 %; there was no lysine.

Diet 4, *amino acid diet.* This diet was patterned on the amino acid composition of whole-egg protein, and prepared by replacing part of the rice starch of the protein-free diet (diet 5) with the mixture of amino acids. The protein equivalent was 10·1 %, the lysine content was 0·56 % and that of arginine 0·66 %.

Diet 5, *protein-free diet.* This diet was composed of starch, glucose and fat, fortified with vitamins and minerals, as described by Miller & Bender (1955).

Diet 6, *stock diet.* Diet 41 B of Bruce & Parkes (Bruce & Parkes, 1949; Bruce, 1950) was used (17 % protein).

The lysine contents of these diets may be compared with the 'target' values set up by Bender (1958, 1960). On this basis diet 1 supplied 33 % of the lysine requirement of the rat, diets 2 and 4 supplied 100 %, diets 3 and 5 contained no lysine.

Experiment 2

Animals

Two pigs, each about 60 lb weight, were fed for 6 weeks on the wheat gluten diet described below; two pigs of the same weight were fed on the same diet with zein replacing wheat gluten, so that the diet was free from lysine; two more pigs were fed on a commercial pig diet.

Diets

The experimental diets were composed of 16% protein (as zein in the lysine-free diet, and as wheat gluten in the low-lysine diet), 5% yeast, 50% starch, 20% glucose, 4% maize oil, with a supplement of vitamins and mineral salts.

Methods

Collection of plasma and preparation for analysis. At the end of the experimental period the rats were killed with ether, the thorax was opened and blood was withdrawn from the heart into a 5 ml syringe moistened with one drop of heparin solution (1 mg/ml). The blood was discharged into a test tube, in which one drop of 30% (w/v) potassium oxalate solution had been dried, and mixed immediately by repeated inversion. Plasma was separated by centrifuging, removed with a Pasteur pipette, and stored in deep freeze until the analyses were performed. Two rats in each group were killed after 14 days and the remaining two after 28 days.

In the second experiment blood was drawn from the ears of the pigs into oxalate tubes.

Precipitation of plasma proteins. Proteins were precipitated with trichloroacetic acid by the procedure of Albanese, Irby & Saur (1946).

Hydrolysis and preparation of samples for amino acid assay. The precipitated proteins were suspended in 1·0 ml of 3 N-HCl in a 15 ml centrifuge tube, the tube was closed with a small beaker and the protein was hydrolysed by autoclaving at 15 lb/in^2 for 8 h. The solution was cooled, the humin was removed by centrifuging and the hydrolysate was extracted twice with 5 ml portions of diethyl ether. Dissolved ether was removed in a warm water bath, the pH was adjusted to 6·8 and the volume made up to 20 ml.

Arginine determination. The method of Albanese & Frankston (1945) is a development of that of Sakaguchi (1925) and depends upon the production of an orange colour when 0·06 N-sodium hypochlorite and urea are added to an alkaline solution of arginine and α-naphthol. Under the conditions given by these authors we obtained very little development of colour and the fault appeared to lie with the method given for standardization of the hypochlorite.

It is usual to standardize sodium hypochlorite by titrating the iodine liberated from potassium iodide in acid solution (Furman, 1939). Albanese & Frankston (1945), Albanese *et al.* (1946) and Albanese (1953), however, titrated the iodine liberated without the addition of acid. When a commercial hypochlorite solution was standardized without the addition of acid variable results were obtained, averaging 0·142 N. When acid was added consistent results were obtained and the solution was found to be

4·42 N. Thus, a solution of apparent normality 0·06 as used by Albanese might have a true normality of $0{\cdot}06 \times (4{\cdot}42/0{\cdot}142) = 1{\cdot}87$.

We found that the maximum colour was developed with 0·5 N-hypochlorite and that both 0·06 and 1·8 N-hypochlorite gave very low readings. However, when the estimation was carried out with 0·5 N reagent the blanks were high (extinction 0·300) and we used the modification of Janus (1956) of Macpherson's (1946) method as our final choice of procedure for arginine determination.

Lysine estimation. Lysine was determined microbiologically with *Leuconostoc mesenteroides*. The method, a modification of that of Henderson & Snell (1948) and Henderson, Brickson & Snell (1948), was essentially that in use in the University of Wisconsin and details were provided by Dr A. E. Harper. The proteins from 0·10 ml of plasma were hydrolysed as described previously and assayed in triplicate at four levels. Recovery of added lysine was 96 %.

Nitrogen estimation. Nitrogen was determined on the precipitated proteins by the method of Jacobs (1959).

RESULTS AND DISCUSSION

Expt 1. The results are summarized in Table 1. No significant differences were observed in the lysine or arginine content of the blood proteins of animals on lysine-low or lysine-supplemented diets.

Expt 2. The results in Table 2 did not indicate any replacement of lysine by arginine even after 8 weeks' feeding, so the experiment was terminated at this stage.

Table 1. *Lysine and arginine content of plasma proteins of rats given different levels of lysine*

(Mean values with their standard errors for four rats)

Diet	Lysine (%)	Arginine (%)
Wheat gluten	10·02 ± 0·12	6·33 ± 0·18
Wheat gluten + lysine	10·14 ± 0·13	6·25 ± 0·18
Lysine-free amino acid	9·36 ± 0·09	6·56 ± 0·25
Amino acid	9·57 ± 0·14	6·59 ± 0·15
Protein-free*	9·44 ± 0·13	6·04 ± 0·18
Stock	9·69 ± 0·16	6·38 ± 0·13

* Mean for three rats.

Table 2. *Lysine and arginine content of plasma proteins of pigs after 8 weeks' feeding on different levels of lysine*

(Each value is the mean of two)

Diet	Plasma protein (g/100 ml)	Lysine in plasma protein (g/100 ml)	Arginine in plasma protein (g/100 ml)
Zein	5·31	10·30	9·80
Wheat gluten	4·35	12·45	9·90
Stock	6·18	11·00	10·20

Albanese (1953) drew attention to the finding of Chow (1950) that wheat gluten replenished the total circulating proteins of depleted dogs more efficiently than did egg albumin despite the higher biological value of egg albumin (97) compared with that of wheat gluten (40) (cf. Mitchell, 1950). This anomaly caused Albanese (1953) to investigate the effect of wheat gluten on human infants. After finding a rise in plasma protein arginine without any corresponding rise in total plasma protein levels, Albanese suggested that the explanation could be (*a*) the substitution of arginine for lysine, or (*b*) an increase in a plasma protein fraction rich in arginine. As no change in albumin:globulin ratio was detected he accepted the first explanation.

We were unable to confirm this suggestion in rats and pigs even with a diet completely free from lysine. Hundley, Sandstead, Sampson & Whedon (1957) were also unable to find any change in the lysine content of the blood proteins of human adults on a rice–fruit diet, poor in lysine and rich in arginine. Similarly, Gray, Olsen, Hill & Branion (1960) gave lysine-deficient diets to chicks and found a lower content of plasma lysine, a higher one of threonine and tyrosine, but no significant changes in contents of other amino acids.

Thus, there is no reason to disagree with the generalization of Vaughan & Steinberg (1959) that the specificity of protein synthesis is probably absolute under physiological conditions.

Albanese drew his conclusions from a determination of arginine and total nitrogen; lysine was not determined. Moreover, using his method of determining arginine, we were unable to obtain valid figures.

A considerable amount of evidence has been presented in the literature to show that some part of the nitrogen of diets completely deficient in one amino acid can be retained in the animal body (Nielson & Corley, 1939; Benditt, Wooldridge, Steffee & Frazier, 1950).

Benditt *et al.* (1950), Harris, Neuberger & Sanger (1943), Kligler & Krehl (1950, 1952), Bothwell & Williams (1952) and ourselves (unpublished) have all shown long survival and comparatively slow weight loss on lysine-free diets. Diets lacking tryptophan, threonine, histidine, phenylalanine or leucine plus isoleucine have a net protein utilization (NPU) value of about 20, and diets lacking lysine one of 30–50 (Bender, 1960).

The results reported here do not explain the retention of incomplete amino acid mixtures but merely add to the evidence that the formation of abnormal tissue proteins is not the explanation. A number of other explanations remain, notably that the animal can synthesize submaintenance amounts of some of the essential amino acids (which would still remain within the definition of an essential amino acid) or, more likely, that certain of the amino acids obtained from degradation of tissue proteins can be recycled to a limited extent and used, in conjunction with the incomplete amino acid mixture from the diet, to synthesize new tissues. This last explanation would seem to be the most likely to account for the maintenance of rats for periods as long as 6 months on a diet completely free from lysine (Bender & Doell, unpublished). Other results suggest that it is most unlikely that replacement of lysine by any other amino acid is the explanation. For example, lysine cannot be replaced by α-aminoadipic acid, in which

the ε-amino group is replaced by carboxyl (Geiger & Dunn, 1949), by homoarginine (Stevens & Bush, 1950), by D-lysine (Berg, 1936; Neuberger & Sanger, 1944), or by compounds in which the α-nitrogen of lysine is replaced by an hydroxy group (Gordon, 1939). Only ε-*N*-acetyl and ε-*N*-methyl derivatives of lysine can replace lysine in the rat (Neuberger & Sanger, 1943; Gordon, 1939).

SUMMARY

1. The conclusion of Albanese (1953) that babies fed on low-lysine diets suffered a change in the composition of their plasma proteins, lysine replacing arginine, was examined by giving diets either low in lysine or free from lysine to rats for 2- and 4-week periods.

2. No changes were detected in the lysine or arginine content of their total plasma proteins.

3. The experiment was also carried out on piglets by giving them low-lysine or lysine-free diets for 8 weeks, and no changes were detected in their total plasma protein content of arginine or lysine.

4. These results, together with the fact that Albanese did not estimate the lysine but only the arginine, and the evidence offered here to question the validity of the arginine assays leads to the conclusion that there is no evidence that alterations in the diet cause changes in the amino acid composition of the plasma proteins.

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