EFFECT OF ADDITIONAL LEUCINE ON NITROGEN BALANCES OF MEN EATING MAIZE DIETS*

PRELIMINARY COMMUNICATION

A. S. TRUSWELL, M.D., M.R.C.P.†, *Department of Medicine, University of Cape Town and Groote Schuur Hospital, Observatory, Cape*

It is well established that the nutritive value of a food protein will be impaired if it contains too low a proportion of any of the essential amino acids. This *amino acid deficiency* is the main reason why the proteins of most vegetable foodstuffs are not as well utilized as animal foods, e.g. egg, for protein synthesis inside the body.

In the case of maize, our Group has previously shown by nitrogen balances in adults and children that its relatively low nutritive value[1] can be improved by giving small supplements of lysine plus tryptophan.[2,3]

A subsidiary concept of protein nutrition is the possibility that *excess* of an amino acid may impair nutritive value. The phenomenon is called *amino acid imbalance*. It has not really been confirmed in man yet, though isolated possible examples have been published.[2,4,5] Almost all the amino acid imbalances reported in rats have been produced by adding excess of a synthetic amino acid to natural proteins, usually ones already deficient in another essential amino acid.

Few natural foods contain an excess of any of the essential amino acids. Of the major foodstuffs, the one with the largest excess is maize; it contains 2·7 times the optimal proportion of leucine (the FAO provisional amino acid pattern). Put another way, leucine makes up 30% of the total of 8 essential amino acids in maize. The Protein Committee of FAO[6] have summarized the position as follows: 'The view has been generally held that imbalance among amino acids is unlikely to occur when only natural foods are ingested. Some evidence has been obtained indicating that the amount of leucine in maize may be sufficiently large to increase the requirement for isoleucine. The addition of an excess of leucine to a diet not deficient in isoleucine has been shown to depress growth in rats, and the depression in growth was eliminated only when the amount of isoleucine in the diet was increased.'

The relevant experiments were reported by Harper *et al.*[7] in 1955. Young rats fed on a low-protein (casein) diet *ad libitum* stopped growing when leucine was added to their ration. The effect was more marked with 3% than with 1·5% of additional L-leucine. It was partly counteracted by giving the isomeric amino acid, isoleucine, at the same time. Even without it, growth was resumed after about a week of continued leucine administration.

*Abstract of one of two papers presented at Research Forum, University of Cape Town, 13 September 1962.

†Present address: Medical Research Council Atheroma Unit, Western Infirmary, Glasgow W.1. Scotland.

The FAO statement was based on these experiments, but it now seems possible that leucine may exert its effect by depressing rats' appetite.[8,9] In recent work the food given to control animals was reduced to the same low level and their growth rate and nitrogen balances were not significantly higher than in the leucine-treated rats. Spolter and Harper were able to increase food consumption with insulin in some of their rats given leucine, and under these conditions they grew quite well. However, most of them died of hypoglycaemia.[8]

Thus it does not seem possible to compare nitrogen balances of leucine-treated rats with controls under natural conditions. To the human taste, leucine is slightly bitter, but not difficult to eat. It was expected that leucine could be given to men without affecting their appetites. This expectation was borne out in the experiments reported here. Maize was used as the basal diet. If maize contains too much leucine, addition of more should increase urinary nitrogen excretion and so reduce the nitrogen balance.

Prolonged nitrogen balances were measured in 5 men. Four were metabolically normal (2 were feeble-minded, one had a mild, old hemiparesis without hypertension, and the fourth had a hysterical abnormality of gait). The fifth subject had hypertension, an old hemiparesis and mild diabetes mellitus. He did not require insulin, never showed ketosis, and his weight was stationary. Two of the subjects had participated in earlier studies of the amino acid deficiencies of maize.[2]

Nitrogen balance methods were as previously described,[2] except that in control periods isonitrogenous amounts of glycine (4 subjects) or L-glutamic acid (1 subject) were given in place of leucine. These amino acids were eaten with the 3 daily meals. The same batch of whole maize meal (97% extraction) was used throughout. With the amino acid supplement it provided 100% of the nitrogen intake in 4 of the subjects (94% in the other subject) and the bulk of the calories. The only other foods given were sugar, coffee, extra vitamins and minerals (and low protein fruits in one subject).

Addition of L-leucine was tested at 2 levels. In most of the experiments a moderate addition of 2 G. per day was given. This brought the total leucine (from maize plus supplement) to around 1·5% of the calorie intake. The proportion of leucine in the protein was increased from approximately** 2·7 times the FAO pattern to 3·5 times. In all 5 men the mean urine nitrogen was slightly lower in the leucine periods: the mean nitrogen retention** for the group was 0·094 G. per day with a range in individual subjects from 0·008 to 0·250 G. per day. Faecal nitrogens showed the usual variation and no obvious difference between leucine and control periods. These results are based on a total of 153 balance days, not counting adjustment periods.

Three of the same subjects have been given larger additions of 6 G. of L-leucine per day, bringing the proportion of this amino acid up to approximately 5 times the FAO amino acid pattern and the total leucine intake to 2·3% of the calories. In one man the urine nitrogen was reduced by leucine, in the second it was increased and in the third subject there was no change. Mean differences of urine nitrogen between leucine and control periods in the individual subjects were: (1) 0·294 G. per day less on leucine, (2) 0·237 G. per day more on leucine, and (3) 0·008 G. per day more on leucine. These results represent a total of 117 balance days. Subjects 1 and 3 received low nicotinamide intakes[10] during the experiments with a large addition of leucine.

Leucine was deliberately obtained from 3 different manufacturers, i.e. British Drug Houses, Merck, and Nutritional Biochemicals Corporation, and the different preparations were used in different periods. On paper chromatography[11] none of the leucine was contaminated with detectable amounts of isoleucine.

Thus, with moderate increments of leucine not only was there no nitrogen loss, but there appears to have been a very slight retention. One might speculate that this resulted from increased insulin secretion. It has been reported that L-leucine causes a slight fall of blood sugar in normal people.[12]

In conclusion, the present experiments have shown no evidence that the proportion of leucine naturally present in maize exerts any deleterious effect on the nutritive value of its protein for human adults.

I should like to thank Prof. J. F. Brock for the provision of facilities, Mr. F. Baumgartner for laboratory assistance, and Sister N. Daniels for supervision of the metabolic ward. Financial support was received from the South African Council for Scientific and Industrial Research and from the US Public Health Service (grant no. A 3995).

**Based on world average figures.[4] Results of amino acid analysis of the batch of maize meal used are not yet at hand.

**(leucine minus control)

REFERENCES

1. Truswell, A. S. and Brock, J. F. (1962): Amer. J. Clin. Nutr., **10,** 142.
2. *Idem* (1961): *Ibid.*, **9,** 715.
3. Hansen, J. D. L. (1961): in *Progress in Meeting Protein Needs of Infants and Preschool Children*, p. 89. Washington, D.C.: National Academy of Science — National Research Council.
4. Scrimshaw, N. S., Bressani, R., Béhar, M. and Viteri, F. (1958): J. Nutr., **66,** 485.
5. *Idem* (1958): *Ibid.*, **66,** 501.
6. Report of F. A. O. Committee (1957): *Protein Requirements*. Rome: F.A.O.
7. Harper, A. E., Benton, D. A. and Elvehjem, C. A. (1955): Arch. Biochem., **57,** 1.
8. Spolter, P. D. and Harper, A. E. (1961): Amer. J. Physiol., **200,** 513.
9. Benton, D. A. and Krauss, R. (1962): Fed. Proc., **21,** 8.
10. Truswell, A. S. (1962): S.Afr. Med. J., in press.
11. Boissonnas, R. A. (1950): Helv. chim. Acta, **33,** 1966.
12. Di George, A. M. and Auerbach, V. H. (1960): Amer. J. Med. Sci., **240,** 792.