THE ROLE OF MAIZE IN THE PATHOGENESIS OF PELLAGRA

EFFECT OF LEUCINE ON N'-METHYLNICOTINAMIDE EXCRETION IN HUMAN ADULTS

A. S. TRUSWELL, M.D., M.R.C.P., *Department of Medicine, University of Cape Town**

Introduction

Our understanding of the pathogenesis of the 'avitaminoses' has not become complete with the discovery of the vitamins. This generalization is particularly relevant to pellagra. The outstanding epidemiological feature of pellagra is its close relationship to diets based on maize. But it would not be possible to predict this by looking through the niacin content of different foods in a food table. Rice, for instance, contains no more niacin than maize, yet pellagra is not seen in rice-eating communities.[1]

There are 3 explanations which are currently available to fit these facts. They are the modern equivalents of much older theories on the pathogenesis of pellagra.

Niacin deficiency. Over the last 20 years, Kodicek has produced a large amount of evidence indicating that nearly all the niacin in maize is biologically unavailable.[2-5] However, the same probably applies to rice.[6]

Protein intake of poor quantity or quality. The amino acid, tryptophan, is converted to nicotinamide in man,[7-9] as in other organisms, and must provide an important proportion of the nicotinamide requirement of people on a good, mixed diet. Maize differs from most other cereals in containing a low proportion of tryptophan, which is thus one of its first 2 limiting amino acids.[10-12] There is some evidence that protein synthesis has a prior claim on the available tryptophan and can divert it away from the kynurenine pathway to nicotinamide when tryptophan is the most limiting amino acid in the diet.[13]

Toxin in maize. From early in the nineteenth century it has been suggested that maize contains a toxin or antivitamin, but none of the suggestions have been substantiated (e.g. Borrow *et al.*,[14] Pearson *et al.*[15]). The latest suspect on the list is the amino acid, leucine.

POSSIBLE ROLE OF LEUCINE IN THE PATHOGENESIS OF PELLAGRA

Pellagra is less common in Central America than in other maize-eating areas. For some years it has been suspected that this may be because the Central American Indians cook their maize in dilute lime water.[16] Bressani and Scrimshaw,[17] at the Institute of Nutrition of Central America and Panama, produced evidence suggesting that an important result of this lime treatment was an improvement in the proportions of soluble proteins and amino acids, in particular a loss of available leucine in the maize.

In their latest publication on the subject, Bressani *et al.*[18] still adhere to the mechanism they proposed, but other workers in the field have had experimental results which wholly (Harper *et al.*[19]) or partly (Pearson *et al.*[20]) support Kodicek's explanation[3,4] — that lime treatment converts the bound form of niacin to the biologically available free nicotinic acid.

* Present address: MRC Atheroma Unit, Western Infirmary, Glasgow, W2, Scotland.

Meanwhile, in India, Gopalan and Srikantia[21,22] had noticed that pellagra is common in Hyderabad but rare in Coonor. The people eat rice in Coonor, but in Hyderabad a millet, *Sorghum vulgare*, tends to take its place. Maize is not eaten there to any extent. Gopalan and Srikantia compared the composition of millet and maize with that of rice. Millet contains more niacin and tryptophan than maize. It seemed that the only relevant difference from rice that was shared by maize and millet was their high proportion of leucine.

Gopalan and Srikantia tested the effect of leucine on the N'-methylnicotinamide (NMN) excretion of 4 normal subjects and some pellagrins. The graphs they have published from 3 of their normal subjects show a steady excretion of around 3 mg. NMN per day in the preliminary period on a 45 G. protein diet. When 5 G. of L-leucine was given in a single dose with one meal each day, NMN excretion rose 50%. It stayed at this higher level until the leucine was stopped after 7 days. They reported a similar increase of urinary NMN in pellagrins, but have not published the quantitative data.

Five G. of L-leucine had no *clinical* effects on patients with pellagra. A higher dose (20 or 30 G. per day) was given to 2 patients, and Gopalan and Srikantia concluded that this was associated with a temporary deterioration of their mental condition, which was reversed when leucine was discontinued and nicotinic acid was administered. Their detailed protocols of these 2 patients could, however, be interpreted otherwise, for in one patient the acute mental signs appeared in less than a day, whereas the other patient's mental state became suddenly worse 3 days after stopping a 16-day course of leucine.

Gopalan and Srikantia reasoned that the increased NMN excretion which accompanied leucine administration represented wastage and not increased availability of nicotinic acid. But it is difficult to fit this suggestion with the consistently low excretions of NMN that are found in maize eaters who develop pellagra — whether spontaneous[23,24] or experimental.[25]

Gopalan and Srikantia imply that an amino acid imbalance was responsible for their results. But this is unlikely for the following reasons:

(*i*) The percentage of leucine in their diets is not as high as is necessary to obtain growth depression in rats.[26]

(*ii*) They found that the increase of NMN excretion was not prevented by giving isoleucine together with the leucine.

(*iii*) Just as amino acids have a reduced supplementing effect if only given once a day,[27-29] so one would not expect a marked imbalance effect when 2 of the daily meals are eaten without the extra amino acid. In fact, Pearson and Phornphiboul[30] have recently reported that threonine did not induce niacin deficiency in rats when it was fed separately from the diet.

(*iv*) When other workers have produced amino acid

imbalances with low-niacin and -tryptophan diets, by adding leucine in human subjects[5] or threonine in rats,[30] the excretion of NMN was actually decreased.

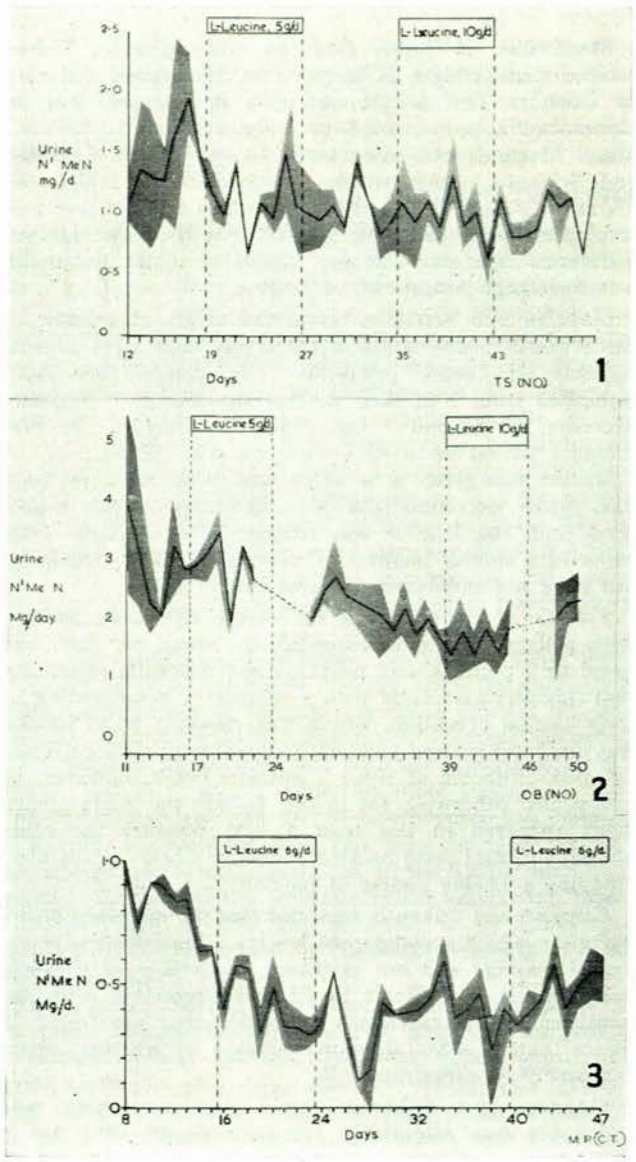

Fig. 1. NMN excretion by subject 1 (T.S. — New Orleans). The days are from the commencement of the low-niacin and -tryptophan diet. There was an 11-day adjustment period before the first period shown here. The continuous line joins the mean values for each day. The shaded area represents the range of replicate determinations. These were in duplicate except for the second leucine period when they were measured in quadruplicate.
Fig. 2. NMN excretion by subject 2 (O.B. — New Orleans). Method of charting as in Fig. 1. On days 23 to 26 and 45 to 47 NMN could not be measured because of menstruation. Duplicate measurements for the first leucine trial, quadruplicate measurements for the second leucine trial.
Fig. 3. NMN excretion by subject 3 (M.P. — Cape Town). Same method of charting as for Figs. 1 and 2. Measurements were in triplicate or quadruplicate except in the first control period (duplicate).

EXPERIMENTS IN NEW ORLEANS

For these reasons it seemed desirable to repeat the experiments.* The first 2 experiments were carried out while the author was working with Dr. G. A. Goldsmith in New Orleans in 1961. The subjects were 2 normal women, studied in a metabolic ward. They received constant diets of mixed foods similar to the diets Dr. Goldsmith had used[31] to induce experimental pellagra in man. These diets contained per day 67 G. of unenriched maize meal, 10 G. of gelatin, and unenriched bread, rice, fruits, vegetables, and a little bacon. Coffee and tea were excluded because they are now known to be quite rich sources of available niacin.[32,33] The diets provided: niacin 6 mg. per day, protein 35 G. per day (7·5% of calories) and tryptophan around 250 mg. per day. A supplement of 2·5 mg. each of thiamine, riboflavine and pyridoxine was given daily. After 10 days of adjustment and a control period, 5 G. of L-leucine (Nutritional Biochemicals Corp.) was added to the lunch for one week. As discussed above, a marked amino acid imbalance effect could not be expected when the excess amino acid was all given with one meal, so in a subsequent period 10 G. L-leucine was given per day, and it was divided equally between breakfast and supper.

Urinary NMN was measured daily by the method of Carpenter and Kodicek[34] modified for the Coleman 12 C fluorometer. In preliminary experiments it was found that there was a mean variation of 10% (range 2-38%) between the NMN values of repeat determinations on the same batch of urines. To eliminate this effect, 8 consecutive urines before and 8 after each change of diet were all measured together. With one unknown, one internal standard and one (urine) blank for each urine plus duplicate external standards and a water blank for the batch, this necessitated reading 51 tubes at a time. Then, to obtain replicate determinations, each such batch was repeated on at least one other day.

The detailed results for the 2 subjects are shown in Figs. 1 and 2. In each figure the continuous line joins the mean NMN values for each day, while the shaded area shows the range of replicate determinations. In subject 2 (OB) measurements had to be broken off twice for 3 or 4 days during menstruation, which contaminates the urine with the pyridine nucleotides of blood. The mean values for each period are shown in Table I.

There was an appreciable day-to-day variation in NMN excretion, but the results show quite clearly that despite allowing 10 days for adjustment there was a gradual decline in NMN excretion as the subjects continued on the low-niacin and -tryptophan diet. Against this declining baseline, it can be seen that there was no rise of NMN during the leucine periods.

The first subject developed mild glossitis and proctitis after 45 days on the low-niacin and -tryptophan diet. It is not possible to say whether this was related to leucine administration. Signs of pellagra usually first appear from around 50 days on diets similar to those that were given.[25] The second subject remained clinically normal up to the 50th day on the low-niacin and -tryptophan diet, when the end of the experiment was reached.

In both subjects, nitrogen balances were slightly more positive in all 4 leucine periods (Table II). However, the

* Of Gopalan and Srikantia.[22]

TABLE I. MEAN URINARY NMN FOR LEUCINE AND CONTROL PERIODS

Subject	Amount of extra L-leucine given per day	Urinary N'methylnicotinamide, mg. per day (mean per period)		
		(I) On leucine	(II) Control*	Difference [(I) − (II)]
T.S.	5 G.	1·11	1·44, 1·03 → 1·24	−0·13
	10 G.	0·98	1·03, 0·98 → 1·01	−0·03 → −0·08
O.B.	5 G.	2·93	2·97, 2·42 → 2·70	+0·23
	10 G.	1·67	1·95, 2·29 → 2·12	−0·45 → −0·11
M.P.	6 G.	0·41	0·81, 0·34 → 0·58	−0·17
	6 G.	0·47	0·42	+0·05 → −0·06
J.M.	6 G.	0·71	0·80, 0·75 → 0·78	−0·07

* In every trial except one there was a control period before and another after the leucine period. Control periods before appear above the control periods after leucine, and the means for the 2 are shown immediately to the right.

TABLE II. NITROGEN BALANCES IN THE NEW ORLEANS SUBJECTS (NITROGEN BALANCE EXPRESSED AS MEAN G. N/DAY FOR EACH PERIOD)

Diet	Basal	Basal + 5 G. L-leucine per day	Basal	Basal + 10 G. L-leucine per day	Basal
Subject 1 (T.S.)	−0·81	−0·29	+0·07, +0·21	+0·44	−0·26
Subject 2 (O.B.)	+0·29	+0·67	+0·26	+0·28	−0·08

nitrogen intake had been increased by the leucine during these periods.

Calculation of the aminogram of the basal diet from Orr and Watt's tables[35] indicated that, after tryptophan, the next most limiting amino acids were the sulphur-containing amino acids, methionine and cystine. The methyl group of N'-methylnicotinamide is provided by methionine.[36] It is thus conceivable that shortage of methyl groups prevented a rise of NMN excretion when leucine was given. Gopalan and Srikantia did not give details of the basal diet they used. It would almost certainly have been different from what was given to the first 2 subjects reported here. Regardless of what Gopalan's diet comprised, it seemed that the most important one to try would be an almost pure maize diet. If leucine still had no effect when added to this, the high content of leucine in maize could not be held responsible for the pathogenesis of pellagra. A basal diet of maize has the further advantage that methionine is not one of its first limiting amino acids.[10]

EXPERIMENTS IN CAPE TOWN

The next 2 subjects were studied in Prof. J. F. Brock's department. In Cape Town it is possible to find Bantu subjects who are accustomed to eating large quantities of maize.

Subject 3 (M.P.) had an old hemiparesis, essential hypertension and mild diabetes mellitus. His body weight did not change during the experiment. He ate 520 G. per day of yellow, whole ground (straight run) maize meal plus a little sugar and fruit juices. This diet was supplemented with 3 vitamins (thiamine, riboflavine and pyridoxine — no niacin) and minerals. No coffee or tea were given. This diet provided (per day) 60 G. of protein* (measured as N), 7·96 mg. of niacin (from food tables — 91% of it from the maize) and 327 mg. of tryptophan (98% from maize).

Subject 4 (J.M.) was normal except for a mild old hemiparesis. He ate 420 G. of the same maize meal. The rest of his diet was as for subject 3, except that he was also given low-niacin fruits and vegetables. His total daily intakes were: protein 51 G.,† niacin 5·88 mg. (83% from maize) and tryptophan 275 mg. (almost all from maize).

In these Cape Town experiments the diets of each subject were kept *isonitrogenous*, and leucine was replaced by glycine in the control periods. Some of the L-leucine used was obtained from British Drug Houses and some from Merck. Six G. were given each day and this was divided into 2 G. given with each of the 3 meals.

Two of the recommendations of Gassman and Scheunert[37] were incorporated in the NMN method. The tubes were left for exactly 20 minutes after adding the NaOH, and the heating step was omitted. The fluorescence developed was read in a fluorometric attachment to a Beckman DU spectrophotometer. As before, each batch of NMNs measured included 8 urines on each side of a change of diet.

Subject 3 (M.P.) was given leucine in 2 separate 8-day periods. His detailed results are shown in Fig. 3. The mean results for this subject and for subject 4, who was only given one trial of leucine, are presented in Table I. Again, there was no rise of NMN excretion when leucine was given. The absolute levels of NMN were lower in the Cape Town experiments. This presumably reflects the lower availability of the niacin in the maize than in the mixed diets used in New Orleans.

Subject 3 first developed mild glossitis on the 48th day, the day after the last result shown in Fig. 3. Two days later he developed an intercurrent infection; the low niacin regime was therefore stopped. Subject 4 was only on the low-niacin diet for 32 days and showed no clinical abnormalities.

Contrary to expectation, nitrogen balances did not decrease in the leucine periods. These results have been published in detail elsewhere.[38]

SUMMARY

Gopalan and Srikantia recently claimed that leucine administration results in increased urinary excretion of N'-methylnicotinamide. They suggested that this phenomenon might explain the association of pellagra with *Sorghum vulgare* and, what is more important from our point of

* This is total N intake × 6·25. 7% of it came from pure amino acids (leucine or glycine) and 90% of it was maize protein.

† 8% of this was from the pure leucine or glycine supplement, 86% was from maize protein.

view, with maize. Both these cereals contain unusually high proportions of leucine.

In 7 trials of leucine in 4 normal or near-normal adult subjects I have been unable to confirm their findings. For 2 of the subjects the basal diet consisted of mixed foods, low in niacin and tryptophan: for the other 2 it was an almost pure maize diet. In some experiments leucine was added to the diet; in others it was substituted for an isonitrogenous amount of glycine that was given in control periods. The dose of L-leucine used was 5, 6 or 10 G. per day, and it was either given all with one meal or distributed between 2 or 3 of the daily meals.

In the past, there have been proposals of various pellagra-producing toxins in maize from time to time. None have been substantiated. It appears from the present work that the latest proposal, leucine, cannot be substantiated either.

Dr. G. A. Goldsmith of Tulane University, New Orleans, suggested starting this work. She provided the facilities for the first 2 experiments and gave me much valuable instruction and advice. I am also grateful to Dr. W. N. Pearson of Vanderbilt University for helpful discussions on methods for NMN. Nitrogen estimations on the first 2 subjects were carried out by Mr. C. Novel.

The second 2 experiments were conducted in the Metabolic ward at Groote Schuur Hospital, Cape Town, and in the Department of Medicine, University of Cape Town, under the direction of Prof. J. F. Brock. Financial support for this part of the work was provided by the South African Council for Scientific and Industrial Research and by a research grant (PHS A 3995) from the US Public Health Service.

I should like to thank Miss C. Liadsky for her painstaking assistance with the NMN determinations from subjects 3 and 4.

Dr. A. E. Harper of the Massachusetts Institute of Technology kindly read and commented on the manuscript.

REFERENCES

1. Chick, H. (1951): Nutr. Abstr. Rev., **20,** 523.
2. Kodicek, E. (1940): Biochem. J., **34,** 712 and 724.
3. Kodicek, E., Braude, R., Kon, S. K. and Mitchell, K. G. (1956): Brit. J. Nutr., **10,** 51.
4. *Idem* (1959): *Ibid.*, **13,** 363.
5. Kodicek, E. and Wilson, P. W. (1960): Biochem. J., **76,** 27P.
6. Chaudhuri, D. K. and Kodicek, E. (1960): Brit. J. Nutr., **14,** 35.
7. Perlzweig, W. A., Rosen, F., Levitas, N. and Robinson, J. (1947): J. Biol. Chem., **167,** 511.
8. Horwitt, M. K., Harvey, C. C., Rothwell, W. S., Cutler, J. L. and Haffron, D. (1956): J. Nutr., **60,** suppl. 1.
9. Goldsmith, G. A., Miller, O. N. and Unglaub, W. G. (1961): *Ibid.*, **73,** 172.
10. Truswell, A. S. and Brock, J. F. (1961): Amer. J. Clin. Nutr., **9,** 715.
11. Scrimshaw, N. S., Bressani, R., Béhar, M. and Viteri, F. (1958): J. Nutr., **66,** 485.
12. Rosenberg, H. R., Rohdenburg, E. L. and Eckert, R. E. (1960): *Ibid.*, **72,** 415.
13. Vivian, V. M., Chaloupka, M. M. and Reynolds, M. S. (1958): *Ibid.*, **66,** 587.
14. Borrow, A., Fowden, L., Stedman, M. M., Waterlow, J. C. and Webb, R. A. (1948): Lancet, **1,** 752.
15. Pearson, W. N., Valenzuela, J. S. and van Eys, J. (1958): J. Nutr., **66,** 277.
16. Laguna, J. and Carpenter, K. J. (1951): *Ibid.*, **45,** 21.
17. Bressani, R. and Scrimshaw, N. S. (1958): J. Agric. Food Chem., **6,** 774.
18. Bressani, R., Gomez-Brenes, R. and Scrimshaw, N. S. (1961): Food Technology, **15,** 450.
19. Harper, A. E., Punekar, B. D. and Elvehjem, C. A. (1958): J. Nutr., **66,** 163.
20. Pearson, W. N., Stempfel, S. J., Valenzuela, J. S., Utley, M. H. and Darby, W. J. (1957): *Ibid.*, **62,** 445.
21. Gopalan, C. (1961): In *Meeting Protein Needs of Infants and Preschool Children*, p. 216. Washington, D. C.: National Academy of Sciences — National Research Council.
22. Gopalan, C. and Srikantia, S. G. (1960): Lancet, **1,** 954.
23. Sarrett, H. P. and Goldsmith, G. A. (1950): J. Biol. Chem., **182,** 679.
24. Prinsloo, J. G., Joubert, C. P., du Plessis, J. P. and Höjby, T.: Proc. Nutr. Soc. Sth Afr. (In press.)
25. Goldsmith, G. A., Gibbens, J., Unglaub, W. G. and Miller, O. N. (1956): Amer. J. Clin. Nutr., **4,** 151.
26. Harper, A. E. (1961): *Op. cit.*[21], p. 225.
27. Berg, C. P. and Rose, W. C. (1929): J. Biol. Chem., **82,** 479.
28. Geiger, E. (1947): J. Nutr., **34,** 97.
29. Henderson, R. and Harris, R. S. (1949): Fed. Proc., **8,** 385.
30. Pearson, W. N. and Phornphiboul, B. (1961): *Ibid.*, **20,** 7.
31. Goldsmith, G. A. (1956): J. Amer. Diet. Assoc., **32,** 312.
32. Bressani, R. and Navarrete, D. A. (1959): Food Res., **24,** 344.
33. Goldsmith, G. A., Miller, O. N., Unglaub, W. G. and Kercheval, K. (1953): Proc. Soc. Exp. Biol. (N.Y.), **102,** 579.
34. Carpenter, K. J. and Kodicek, E. (1956): Biochem. J., **46,** 421.
35. Orr, M. L. and Watt, B. K. (1957): *Amino Acid Content of Foods.* Washington, D.C.: U.S. Dept. of Agriculture.
36. Cantoni, G. *in* Colowick, S. P. and Kaplan, N. O. eds. (1955): *Methods in Enzymology*, vol. 2. p. 257. New York: Academic Press.
37. Gassman, von B. and Scheunert, A. (1958): Pharmazie (Berlin), **13,** 515.
38. Truswell, A. S. (1962): S.Afr. Med. J., **36,** 943.