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LEUCINE AND PELLAGRA

Pellagra has long been known as a classical nutritional deficiency disease affecting poor population groups subsisting on maize diets. The classical features of pellagra have been well-documented in numerous publications. The association of pellagra and maize consumption has been attributed, among other factors, to the low tryptophan content of maize and to the poor availability of its nicotinic acid. The disease is rare in areas where rice or wheat is the staple. On the other hand, pellagra is common in the Deccan plateau of India. Thus, it accounts for 1 per cent of all general hospital admissions and nearly 8 to 10 per cent of admissions to mental hospitals in Hyderabad. A careful examination of the dietaries of the poor segments of the population of this region, however, shows that the staple is not maize but the millet jowar (Sorghum vulgare). Practically in every case of pellagra investigated in Hyderabad, a history of regular consumption of jowar was obtained.

A comparison of the chemical composition of rice, jowar, and maize shows that the nicotinic acid content of jowar is nearly similar to that of rice. The tryptophan content of jowar varies widely, certain strains having nearly as high a content of tryptophan as rice, while certain others have somewhat lower values. Both jowar and maize have, however, one common feature with regard to their amino acid composition: a high content of leucine. Dietary supplementation of leucine at 1 per cent level was shown to cause retardation of growth in rats subsisting on low protein diets (9 per cent casein) but such growth retardation was not observed if the dietary protein was raised to 18 per cent (C. A. Elvehjem, in Some Aspects of Amino Acid Supplementation, p. 22, W. H. Cole, Editor. Rutgers University Press, New Brunswick, 1956). The average daily protein intake in the dietaries of the pellagrins investigated in Hyderabad was of the order of 45 g. daily (9 per cent protein), the protein being mainly derived from jowar. The possible role of amino acid imbalance resulting from a relative excess of leucine in the pathogenesis of pellagra, therefore, seemed to merit careful investigation.

The effects of oral administration of leucine on the metabolism of tryptophan and nicotinic acid were, therefore, investigated in pellagrins and normal subjects (C. Gopalan and S. G. Srikantia, Lancet 1, 954 (1960); B. Belavady, Srikantia, and Gopalan, Biochem. J. 87, 652 (1963)). These studies showed that both in normal subjects and in pellagrins, leucine administration brought about a significant increase in the excretion of quinolinic acid and a significant decrease in the excretion of 6-pyridone of N-methyl nicotinamide. There was also a significant decrease in the excretion of both 5-hydroxyindoleacetic acid and free indoleacetic acid. There was an increase in the urinary excretion of N-methyl nicotinamide, though this was not consistent. In the presence of a tryptophan load, these effects of leucine administration were more pronounced. To ensure that these effects were specific for leucine, the effect of oral administration of lysine to a group of subjects was investigated. This
study demonstrated that lysine administration had no effect on the urinary excretion of tryptophan metabolites.

In line with these observations on human subjects, it was found that urinary excretion of quinolinic acid and N-methyl nicotinamide were increased in both young and adult rats when L-leucine was added at 1.5 per cent level to a 9 per cent casein diet (N. Raghuramulu, B. S. Narasinga Rao, and Gopalan, J. Nutrition 86, 100 (1965)). Quinolinic acid excretion was more markedly affected in young rats, whereas N-methyl nicotinamide excretion was more affected in adult rats. Iso-leucine counteracted the effect of leucine in young rats.

While these observations pointed to the possible role of leucine in the pathogenesis of pellagra, it was felt necessary to obtain direct experimental evidence on this point. The possibility of inducing black tongue in dogs on diets containing jowar was, therefore, investigated (Belavady and Gopalan, Lancet 2, 1220 (1965)). Nine adult dogs were kept on a diet based on 65 per cent jowar. Six other animals were maintained on a similar regimen in which, however, maize was substituted for jowar. All dogs in both groups developed signs of black tongue to varying degrees. The syndrome was characterised by ulceration of the mucous membrane of the cheeks and lips, marked reduction in food intake, blood-stained diarrhea, and profuse salivation with rosy drooling from the mouth. Five animals in the jowar diet group which had developed these lesions were treated with nicotinic acid while still on the jowar diet. In these animals, signs of black tongue regressed completely. The study clearly demonstrated that classical black tongue can be induced in dogs by feeding jowar, which is not low in tryptophan and which contains fair amounts of nicotinic acid.

The specific role of leucine in the induction of canine black tongue was demonstrated in another study wherein the effect of leucine supplementation to a non-pellagraogenic diet was investigated in pups and dogs (Belavady, T. V. Madhavan, and Gopalan, Gastroenterology 53, 749 (1967)). All pups receiving leucine supplements with a nicotinic acid-free, 21 per cent casein diet developed black tongue. In another experiment, three of five pups receiving leucine with a complete diet, including adequate nicotinic acid, developed the florid signs of nicotinic acid deficiency. The paired control animals receiving the same diet, but no leucine supplements, remained normal throughout the period of observation. The dogs which developed the lesions responded well to niacin administration.

The pathological changes observed in canine black tongue induced by jowar feeding and by leucine supplementation were found to be identical (Madhavan, Belavady, and Gopalan, J. Path. Bact. 95, 259 (1968)). Extensive degenerative changes in the small and large gut and ulceration of buccal mucosa, periportal changes in the liver, and minor changes in the spinal cord were observed in both groups.

Experimental pellagra was produced in six adult monkeys by feeding diets containing jowar (Belavady, Madhavan, and Gopalan, Lab. Invest. 18, 94 (1968)). Clinical and pathological features of this syndrome were indistinguishable from those seen in a group which developed the deficiency on maize diet. Deficiency signs included pigmentary changes in skin with loss of hair, loss of body weight, and alimentary manifestations such as anorexia and diarrhea. There was a fall in serum albumin, hemoglobin, and blood pyridine nucleotides. The pathological features were characteristic and consisted of chronic atrophic gastritis and degenerative changes in the mucosa of the small
and large intestines. Administration of niacin orally or parenterally reversed the clinical changes. Blood pyridine nucleotides showed an increase after treatment.

It has been claimed that the occurrence of pellagra on maize diets may be partly attributed to the poor availability of nicotinic acid in maize. It was demonstrated that in maize nicotinic acid was present in the bound form and that treatment with calcium oxide or with sodium hydroxide made this nicotinic acid available (E. Kodicke, R. Braude, S. K. Kon, and K. D. Michell, *Brit. J. Nutrition* 13, 363 (1959)). The nature of nicotinic acid present in the millet jowar was investigated with a view to deciding if poor availability of nicotinic acid in jowar may partly explain the occurrence of pellagra in the jowar-eaters (Belavady and Gopalan, *Ind. J. Biochem. 3*, 44 (1966)). The nicotinic acid content of jowar estimated by chemical and microbiological methods after hydrolysis by acid or alkali showed no significant differences. Acid methanol extracts of jowar on chromatographic analysis did not reveal the presence of bound nicotinic acid. Growth of rats and pups fed jowar or lime-treated jowar diets revealed that animals consuming untreated jowar grew better, giving the inference that nicotinic acid in jowar is, in fact, in the available form.

Studies were next directed to elucidating the precise biochemical mechanisms underlying the effect of leucine on nicotinic acid metabolism. Recent studies have indicated that leucine does not impair the absorption of tryptophan; in fact, the plasma tryptophan levels after leucine supplementation are elevated (R. Ghafoorunnisa, Narasinga Rao, and Gopalan, to be published). Induction of niacin deficiency signs by leucine administration may be mediated through an interference with the utilisation of nicotinic acid for the formation of pyridine nucleotides. To investigate this point, nicotinamide nucleotide synthesis *in vitro* in the erythrocytes was studied in normal subjects as well as in patients suffering from pellagra before and after administration of leucine supplements (Raghuramulu, Srikantia, Narasinga Rao, and Gopalan, *Biochem. J. 96*, 837 (1965)). It was found that the total nucleotide concentration in erythrocytes of pellagrins was not lower than that in normal subjects, but the ability of the erythrocytes to synthesise these nucleotides *in vitro* was significantly lower. Oral administration of 10 g. L-leucine daily, for five days, depressed the nicotinamide nucleotide synthesising ability of erythrocytes both in normal subjects and pellagrins. This, however, was not accompanied by changes in the actual nucleotide concentration in the erythrocytes themselves.

These apparently paradoxical results would suggest that distribution of different nucleotide fractions of erythrocytes in pellagrins may be different from that in normal subjects. Fractionation of different nucleotides was, therefore, carried out to investigate this possibility (Srikantia, Narasinga Rao, Raghuramulu, and Gopalan, *Am. J. Clin. Nutrition in press*). Erythrocytes of pellagrins were found to contain higher amounts of NMN and lower amounts of NAD and NADP compared with normals when expressed in absolute amounts or in comparison with total nucleotides. NMN was present in very small amounts in a few of the normal subjects and averaged 2.5 per cent of total nucleotides. The ratio of NAD to NADP was similar in both the groups. Administration of nicotinic acid brought about a reduction in NMN concentration and an increase in the NAD and NADP levels. Fractionation of the nucleotides formed during *in vitro* incubation of erythrocytes indicated that almost all the newly syn-
themsised nucleotide fraction was NAD both in normals and pellagrins.

Mental symptoms form an important part of the clinical manifestations of pellagra and have been reported from several parts of the world, where the disease occurs in endemic form. It was demonstrated to 20 to 30 g. of L-leucine daily, for a period of seven days, produced a deterioration of the mental condition in pellagrins and that this could be reversed with the discontinuance of leucine and administration of nicotinic acid (Gopalan and Srikanthia, Lancet 1, 954 (1960)). A study of the electroencephalographic pattern in 29 adult male subjects suffering from pellagra showed that in 22 subjects, excess delta activity and sometimes delta activity were present as compared with such patterns in only two of the 18 non-pellagrins (Srikanthia, M. V. R. Reddy, and K. Krishnaswamy, Electroenceph. 25, 386 (1968)). All the six pellagrins who had obvious clinical and mental changes had abnormal patterns while several of them who had abnormal patterns had apparently normal mental status. These abnormal EEG patterns were completely reversed following therapy with nicotinamide. Administration of 10 g. L-leucine daily for six to ten days to pellagrins brought about a deterioration in their mental status accompanied by alterations in their EEGs. This abnormality in EEGs in these subjects reverted to the pre-supplementation pattern once leucine was stopped. Administration of 10 g. L-leucine to normal subjects, however, was not associated with any changes either in their mental status or in their EEG pattern.

The biochemical mechanisms underlying the mental changes in pellagra have yet to be elucidated. Among the various factors that may be involved, metabolism of serotonin may also be concerned. Recent observations in the Nutrition Research Laboratories, Hyderabad, have indicated that the levels of platelet serotonin are significantly lower in pellagrins with mental depression as compared with normal subjects (Krishnaswamy, P. S. V. Ramanamurthy, and Srikanthia, to be published). Rats fed a 10 per cent casein protein diet to which 3 per cent and 8 per cent leucine were added, showed significantly lower levels of brain serotonin as compared with control animals. Rats fed diets based on jowar showed lower levels of brain serotonin than those fed casein diets. Studies using the precursor amino acid 5-hydroxytryptophan and MAO inhibitor indicate that synthesis of serotonin from the precursor as well as the rate of breakdown of formed serotonin could be influenced by leucine feeding (Ramanamurthy and Srikanthia, to be published).

Studies reviewed above provide strong evidence of the role of leucine in the pathogenesis of pellagra in jowar-eaters. In view of the high leucine content of maize, the possible role of leucine in the causation of pellagra among the maize-eaters also requires consideration.

Pellagra is still one of the major public health problems in some parts of the world. Any preventive approach which involves a radical alteration in the dietary habits or the agricultural practices of the populations would not seem feasible. Fortunately, the leucine contents of different strains of maize and jowar show wide variations. Thus, the Opaque-2 strain of maize is not only high in lysine but also low in leucine. Identification of low-leucine strains of these millets and the selective propagation of such strains would be the logical approach to the prevention of pellagra among the poor population groups.

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