Effect of leucine at different levels of pyridoxine on hepatic quinolinate phosphoribosyl transferase (*EC* 2.4.2.19) and leucine aminotransferase (*EC* 2.6.1.6) in rats

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1. Effects of incorporating 30 g leucine/kg into diets on quinolinate phosphoribosyl transferase (QPRT; *EC* 2.4.2.19) activity and leucine aminotransferase (*EC* 2.6.1.6) activity were studied in groups of rats receiving 5, 30 and 60 μg of pyridoxine/10 g diet.

2. The results indicated that 30 g leucine/kg diet significantly reduced the QPRT activity when the diets provided 5 μg pyridoxine/10 g and that the effect was only marginal when the diet included 30 μg pyridoxine/10 g. The inhibitory effect was completely absent when the diet provided higher amounts of pyridoxine (60 μg/10 g).

3. These results suggest that additional amounts of pyridoxine are necessary to counteract the effects of excess of leucine in the diet.

4. Leucine aminotransferase activity was increased in rats given diets containing higher amounts of pyridoxine; supplementary leucine also increased the enzyme activity.

Dietary excess of leucine affects the tryptophan-nicotinamide adeninedinucleotide (NAD) pathway in man and one of the effects is an increased excretion of quinolinic acid in urine (Belavady, Srikantia & Gopalan, 1963; Raghuramulu, Narasinga Rao & Gopalan, 1965). In the rat, excess dietary leucine has been shown to lead to a decreased activity of quinolinate phosphoribosyl transferase (*EC* 2.4.2.19, QPRT) in the liver (Ghafoorunissa & Narasinga Rao, 1973). It has been postulated that excess leucine in the diet would result in a decrease in NAD formation from tryptophan, thus leading to the disease pellagra in subjects inadequately provided with preformed niacin. Another factor which can influence the tryptophan-NAD pathway is dietary vitamin B_6 and it has been reported that vitamin B_6 deficiency in man can lead to increased excretion of quinolinic acid in urine after a tryptophan load (Brown, Yess, Price, Linkswiler, Swan & Hankes, 1965; Kelsay, Miller & Linkswiler, 1968; Rose & Toseland, 1973). Recently Krishnaswamy, Bapurao, Raghuram & Srikantia (1976) observed that in normal human volunteers, simultaneous administration of pyridoxine orally with leucine could counteract some of the metabolic effects of excess leucine, i.e. increased quinolinic acid excretion, decreased in vitro nicotinamide nucleotide synthesis in erythrocytes and abnormalities in 5-hydroxytryptamine metabolism. Since the enzyme QPRT is concerned with the metabolism of quinolinic acid and since pyridoxine could counteract the effects of leucine on the increase in quinolinic acid excretion, it was considered possible that pyridoxine may modify the activity of QPRT in leucine-fed animals.

In view of these observations, the effect of leucine when administered to rats fed on diets containing different levels of pyridoxine on QPRT activity and also on leucine aminotransferase (*EC* 2.6.1.6) activity, a pyridoxal phosphate-dependent enzyme, were studied. The results of these investigations are reported here.

Table 1. *Composition of diet fed to rats* (g/kg)*

Casein	110
Peanut oil	50
Vitamin mix	10
Salt mix	40
Maize starch	790
L-Cystine	2
Choline chloride	1

* Bapurao, Raghuram & Krishnaswamy (1975).

Table 2. *Details of supplements of pyridoxine and leucine added to casein-based* (110 *g/kg*) *diets* fed to groups of rats and the activities of quinolinate phosphoribosyl transferase* (*QPRT*, EC 2.4.2.19) *and leucine aminotransferase* (*LAT*, EC 2.6.1.6) *in livers of these animals*

(Mean values and their standard errors of 8 animals)

Level of pyridoxine (μg/10 g diet)	Enzyme assays: QPRT† Leucine** −	QPRT† Leucine** +	LAT‡ Leucine** −	LAT‡ Leucine** +
5	$1{\cdot}21 \pm 0{\cdot}100^{a}$	$0{\cdot}86 \pm 0{\cdot}088^{a, c}$	$2{\cdot}25 \pm 0{\cdot}146^{d, e}$	$3{\cdot}24 \pm 0{\cdot}135^{d, g, h}$
30	$1{\cdot}13 \pm 0{\cdot}117$	$0{\cdot}93^{***} \pm 0{\cdot}070^{b}$	$2{\cdot}94 \pm 0{\cdot}136^{e, f}$	$3{\cdot}83^{***} \pm 0{\cdot}247^{f, g}$
60	—	$1{\cdot}21 \pm 0{\cdot}094^{b, c}$	—	$4{\cdot}56 \pm 0{\cdot}301^{h}$

Mean values with the same superscript letters are statistically significantly different: a, b, g $P < 0{\cdot}05$; c $P < 0{\cdot}02$; e, f, h $P < 0{\cdot}01$; d $P < 0{\cdot}001$.

* For details, see Table 1.

** Leucine was added to the diets at 30 g/kg.

*** Mean value of seven animals since one animal died during the period of study.

† Expressed as nmol of product formed/h per mg protein.

‡ Expressed as nmol of α-ketoisocaproate formed/mg protein per 15 min.

MATERIALS AND METHODS

Forty weanling rats (inbred strain of the National Institute of Nutrition, Hyderabad) of both sexes were randomly assigned to one of five groups of eight animals each. Details of the supplementation of leucine to the groups and diets are indicated in Table 2. The composition of the diet given in Table 1 was similar to that reported earlier by Bapurao, Raghuram & Krishnaswamy (1975). The pyridoxine content of the casein used was 1·3 μg/10 g diet (estimated microbiologically using *Saccharomyces carlsbergensis*) and is comparable with the results of Orr (1969).

Animals which received 30 g leucine/kg diet and 5 μg pyridoxine/10 g diet were fed *ad lib.* and depending on their daily food intake, the other animals were pair-fed. Animals were fed on these diets for 6–7 weeks. At the end of the experimental period, they were killed and the livers immediately removed and weighed. QPRT activity was assayed by the microbiological procedure described by Nishizuka & Nakamura (1970) using *Lactobacillus arabinosus* (ATCC 8014) as the test organism. The activity of leucine aminotransferase was assayed by the procedure described by Taylor & Jenkins (1966). Protein was estimated by the method of Lowry, Rosebrough, Farr & Randall (1951). Statistical analyses were done using Student's *t* test.

RESULTS

The results of the experiment are presented in Table 2.

There were no significant differences in body-weights and liver weights of animals given different levels of pyridoxine in the diet.

Incorporation of 30 g leucine/kg diet significantly decreased the QPRT activity when the diet provided 5 μg pyridoxine/10 g and a similar trend was observed in animals which received 30 μg pyridoxine/10 g diet though the differences were not statistically significant. However, additional leucine in the diet did not alter the enzyme activity when the diet provided 60 μg pyridoxine/10 g.

The mean QPRT activity in livers of animals which received 5 μg pyridoxine/10 g diet ($1{\cdot}21 \pm 0{\cdot}100$) was similar to that obtained for livers of animals which received 30 μg pyridoxine/10 g diet ($1{\cdot}13 \pm 0{\cdot}117$) indicating that pyridoxine had little influence on enzyme activity.

The activity of leucine aminotransferase, however, was significantly higher when the diet contained more pyridoxine (Table 2, $2{\cdot}25 \pm 0{\cdot}246$ as against $2{\cdot}94 \pm 0{\cdot}136$). Also excess dietary leucine significantly increased the activity of leucine aminotransferase, the values being 3·24 and 3·83 units respectively compared with 2·25 and 2·94 units respectively.

DISCUSSION

Incorporation of 30 g leucine/kg in the diet significantly reduced the activity of the enzyme QPRT when the diets provided marginal amounts of pyridoxine (5 μg/10 g diet). However, the effect of leucine was not statistically significant when the diet had adequate amounts of pyridoxine (30 μg/10 g diet) and the enzyme activity in animals given 60 μg pyridoxine/10 g diet was similar to that in controls, indicating that pyridoxine had modified or reversed the effect of leucine on the enzyme QPRT. This, however, may not necessarily indicate that pyridoxine directly influences QPRT activity, since the enzyme activity is not influenced by higher pyridoxine levels in the diet. In fact QPRT has not been shown to be a pyridoxal phosphate-dependent enzyme (Gholson, Ueda, Ogasawara & Henderson, 1964; Nishizuka & Nakamura, 1970; Taguchi & Iwai, 1974).

Leucine aminotransferase activity, a pyridoxal phosphate-dependent enzyme was increased in leucine-fed animals indicating substrate induction of the enzyme. These findings are similar to those of Knox & Greengard (1965) who found that administration of tyrosine and tryptophan resulted in an increase in tyrosine aminotransferase (*EC* 2.6.1.5) and tryptophan oxygenase (*EC* 1.13.1.12) respectively.

Leucine aminotransferase activity has been shown to be significantly low in animals given a vitamin B_6-deficient diet (Shiflett & Haskell, 1969). This is similar to observations made in the present study that the activity of the enzyme was higher when the diets provided 30 and 60 μg pyridoxine/10 g as compared with the diet which provided only 5 μg pyridoxine/10 g. The increase in activity may be due to more availability of pyridoxal-5-phosphate.

It is evident that dietary excess of leucine induces the enzyme, leucine aminotransferase which requires pyridoxal phosphate as coenzyme, thus modifying the requirements for vitamin B_6. This is in keeping with the earlier observations that excess leucine in the diet brings about a significant reduction in the activity of liver kynureninase (*EC* 3.7.1.3) when the diet is low in vitamin B_6 (Bapurao *et al.* 1975).

Results of the present study suggest that on diets containing higher amounts of pyridoxine, the enzyme activities are so modified that leucine is capable of being metabolized at a faster rate. As a result, it may be expected that the effects of excess dietary leucine on tryptophan-

NAD pathway would be less pronounced. These results suggest that in endemic pellagra arising as a result of high intakes of leucine from jowar (*Sorghum vulgare*), the vitamin B_6 content of the diet may be an important determinant of the extent of impairment of the tryptophan-NAD pathway leading to a decrease in NAD. It is relevant here to point out that the vitamin B_6 content of jowar is only half that of wheat and less than that of rice (Bapurao, 1975).

Further studies are in progress to evaluate the therapeutic effects of vitamin B_6 in cases of pellagra. Also leucine-tolerance studies are being conducted in pellagra patients to study the effect of pyridoxine.

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