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Biochem. J. (1963) **87**, 652

The Effect of the Oral Administration of Leucine on the Metabolism of Tryptophan

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(*Received* 21 *November* 1962)

The occurrence of pellagra in an endemic form in a population subsisting mainly on the millet jowar (*Sorghum vulgare*) has been briefly reported by Gopalan & Srikantia (1960). Both jowar and maize have one common feature with regard to their amino acid composition, namely a high content of leucine. The possible role of amino acid imbalance resulting from a relative excess of leucine in the pathogenesis of pellagra was therefore suggested. In the present investigation the effects of the oral administration of leucine on the metabolism of tryptophan and nicotinic acid have been studied.

METHODS

Experimental procedure. Six patients suffering from pellagra (pellagrins) and six apparently normal male adults, between the ages of 25 and 45 years, formed the subjects for the study. They were kept in hospital and put on a standard diet, based on rice, wheat flour, dhal and vegetables, providing them with approximately 2300 kcal., 50 g. of protein and 50 g. of fat/day. The protein was derived entirely from vegetable sources, and constituted about 8% of the total calories. Preliminary studies had indicated that the urinary excretion of some nicotinic acid metabolites usually became stabilized on this diet by the third or fourth day. Therefore 24 hr. collections of urine were made on the fifth, sixth and seventh days of the standard diet. The subjects were then given 10 g. of pure L-leucine daily, in a single dose, taken with one of the main meals of the day, for five days. Then 24 hr. urine collections were again made on the second, third, fourth and fifth days of the supplementation with leucine. The supplement of leucine was then withdrawn, but the collection of urine samples was continued for 4 more days. The samples of urine were collected over 15 ml. of acetic acid and analysed without delay.

In four other normal volunteer adults the effects of simultaneous ingestion of (*a*) tryptophan and leucine, and of (*b*) nicotinic acid and leucine, on the urinary excretion of tryptophan metabolites were studied. Subjects were maintained on the standard diet described above, and 24 hr. urine samples were collected on the fifth, sixth and seventh days. On the morning of the eighth day, at 9 a.m., 5 g. of DL-tryptophan was given orally, and the urine was collected till 9 p.m. At 9 p.m. another load at 5 g. of DL-tryptophan was given, and urine was collected for the next 12 hr. Samples (24 hr.) were collected from the morning of the ninth day for the next 3 days. The subjects were then given 5 g. of leucine twice a day for 5 days; on the fifth day they received 5 g. of tryptophan in addition to the leucine. Urine collections were made in the manner described above. For the load test with nicotinamide the experimental procedure was identical except that 100 mg. of nicotinamide/dose was used instead of tryptophan.

Analytical methods. Tryptophan was determined by the method of Albanese & Frankston (1945). Nicotinic acid was determined by the cyanogen bromide method of Swaminathan (1939), after the removal of interfering substances by the method described by Nandi & Banerjee (1949). Quinolinic acid was measured as the increase in nicotinic acid, after autoclaving at 15 lb./sq.in. for 60 min. and calculation by the method of Sarett (1951). *N*-Methylnicotinamide was measured by the fluorimetric method of Carpenter & Kodicek (1950). The 6-pyridone of *N*-methylnicotinamide was determined by the method of Rosen, Perlzweig & Handler (1948) and Rosen, Perlzweig & Leder (1949). Indican was determined by the colorimetric method after development of the colour with Obermeyer's reagent in the presence of thymol and then extracted with chloroform. Indolylacetic acid, both free and total, was measured by the method described by Weissbach, King, Sjoerdsma & Udenfriend (1959). 5-Hydroxyindolylacetic acid was determined by the modified method of Macfarlane and co-workers, as described by Dalgliesh (1958).

RESULTS

The amounts of some metabolites of tryptophan and nicotinic acid excreted in 24 hr. in the urine of normal subjects and of pellagrins are shown in

Table 1. The values for the urinary excretion of tryptophan, quinolinic acid, nicotinic acid, *N*-methylnicotinamide, the 6-pyridone of *N*-methylnicotinamide, 5-hydroxyindolylacetic acid and indolylacetic acid are lower, and the excretion of indican is higher, in pellagrins than in normal subjects. In normal subjects the administration of leucine caused no significant change in the excretion of tryptophan, nicotinic acid or *N*-methylnicotinamide (Table 1). There was, however, a significant increase in the excretion of quinolinic acid and a significant decrease in the excretion of the 6-pyridone of *N*-methylnicotinamide after the administration of leucine in normal subjects. There was also a significant decrease in the excretion of both 5-hydroxyindolylacetic acid and free indolylacetic acid. In pellagrins a significant depression in the excretion of tryptophan occurred after the administration of leucine. Notable features in pellagrins and in normal subjects were the marked increase in the excretion of quinolinic acid and the marked decreases in the excretion of the 6-pyridone of *N*-methylnicotinamide and 5-hydroxyindolylacetic acid after the administration of leucine. The changes, however, were smaller in pellagrins than in normal subjects. In both normal subjects and pellagrins the withdrawal of leucine was followed by a reversal of the above changes.

The effects of the administration of leucine in the presence of a tryptophan load in normal subjects are presented in Table 2. The most noticeable changes were a marked increase in the excretion of quinolinic acid and a decrease in the excretion of the 6-pyridone of *N*-methylnicotinamide.

The effects of the administration of leucine in the presence of a nicotinamide load in normal subjects are presented in Table 3. A significant increase in the excretion of quinolinic acid after the administration of leucine was observed in this instance also. This may be the effect of the administration of leucine itself. However, the decrease in the amount of the 6-pyridone of *N*-methyl-

Table 1. *Effect of leucine and lysine on the urinary excretion of tryptophan and some of its metabolites in normal subjects and in pellagrins*

Experimental details are given in the text. The results are given as the means ± S.E. of six subjects from each group with leucine and from three subjects with lysine.

	Excretion (mg./24 hr.)					
	Normal subjects		Pellagrins		Normal subjects	
Metabolite	Initial	After administration of leucine	Initial	After administration of leucine	Initial	After administration of lysine
Tryptophan	181·0±12·79	146·7±13·88	113·5±9·96	70·4±8·37*	—	—
Quinolinic acid	9·4±2·35	32·2±4·58†	5·2±0·80	9·1±1·37*	8·6±1·32	8·0±1·12
Nicotinic acid	6·0±0·78	6·7±0·87	4·9±0·83	4·6±0·67	6·5±0·81	6·2±0·73
N-Methylnicotinamide	6·1±0·22	6·9±0·40	3·7±0·30	3·2±0·20	5·9±0·18	6·5±0·19
6-Pyridone of *N*-methylnicotinamide	4·6±0·30	1·7±0·28†	3·2±0·52	1·8±0·43*	4·4±0·27	4·7±0·31
5-Hydroxyindolylacetic acid	28·7±3·52	18·0±2·19*	12·2±1·23	6·8±0·80†	—	—
Indican	18·3±1·68	13·5±1·12*	27·5±2·86	20·7±2·32	—	—
Indolylacetic acid						
Total	26·6±3·49	24·8±2·58	13·9±2·01	13·4±2·01	—	—
Free	12·9±1·52	9·3±0·85*	7·7±1·15	6·5±0·96	—	—

* Significant difference: $P < 0.05$. † Significant difference: $P < 0.001$.

Table 2. *Effect of the administration of leucine after dosage with tryptophan*

Experimental details are given in the text. The results are given as the means ± S.E. for four subjects.

	Excretion (mg.)			
	0–12 hr. period after the dose		12–36 hr. period after the dose	
Metabolite	Control	With leucine	Control	With leucine
Tryptophan	1345·9±61·74	868·1±115·3*	632·3±81·25	533·3±60·82
Quinolinic acid	8·1±2·24	18·2±3·98*	8·8±3·71	30·7±1·97†
Nicotinic acid	6·9±0·88	3·8±0·64*	8·3±2·35	11·5±2·57
N-Methylnicotinamide	5·3±0·61	9·8±1·92*	11·7±2·36	11·9±1·31
6-Pyridone of *N*-methylnicotinamide	5·8±0·75	2·3±0·31†	6·6±2·46	3·2±0·42

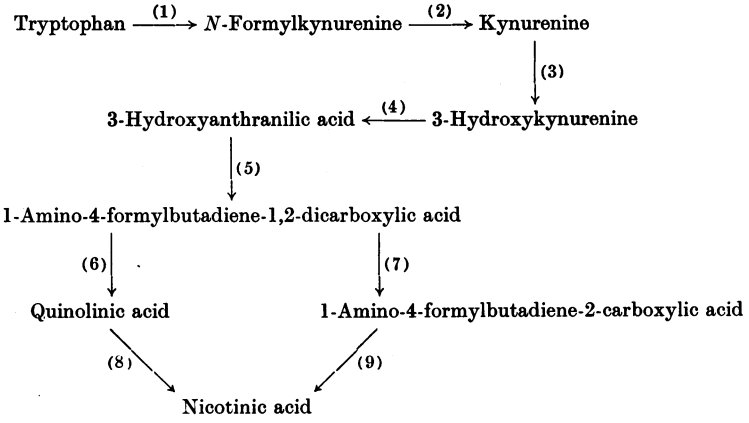
* Significant difference: $P < 0.05$. † Significant difference: $P < 0.001$.

Table 3. *Effect of the administration of leucine after dosage with nicotinamide*

Experimental details are given in the text. The results are given as means ± S.E. for four subjects.

	Excretion (mg.)			
	0–12 hr. period after the dose		12–36 hr. period after the dose	
Metabolite	Control	With leucine	Control	With leucine
Tryptophan	115·2±9·54	112·5±16·22	203·8±10·45	205·1±36·19
Quinolinic acid	6·2±1·70	21·8±6·41*	4·3±4·52	10·7±3·98
Nicotinic acid	5·5±0·78	5·1±0·66	8·1±2·60	8·1±1·11
N-Methylnicotinamide	31·6±4·93	26·9±5·56	10·2±1·05	12·0±1·89
6-Pyridone of *N*-methylnicotinamide	9·8±0·49	8·5±0·67	7·5±0·41	8·1±0·84

* Significant difference: $P < 0.001$.



Scheme 1. Probable route for the conversion of tryptophan into nicotinic acid in animals and fungi.

nicotinamide after the administration of leucine in the presence of a nicotinamide load was not marked.

To determine whether the changes in the urinary excretion of quinolinic acid and of the 6-pyridone of *N*-methylnicotinamide after the oral administration of leucine were specific to leucine, or whether they were non-specific responses to an excess of any essential amino acid, the effects of the oral administration of 10 g. of lysine were studied in three normal subjects. The results showed that the excretion neither of quinolinic acid nor of the 6-pyridone of *N*-methylnicotinamide was significantly influenced (Table 1).

DISCUSSION

Gopalan & Srikantia (1960) showed that the administration of leucine brought about an increase in the urinary excretion of *N*-methylnicotinamide in two normal subjects and in three pellagrins. Examination of a larger series of subjects in the present investigation has shown that the increase in the urinary excretion of *N*-methylnicotinamide after the administration of leucine was not significant. The increase in the excretion of *N*-methylnicotinamide after the administration of leucine in the presence of a tryptophan load, however, appeared to be significant.

The main finding is that the oral administration of leucine brings about a significant increase in the urinary excretion of quinolinic acid both in normal subjects and in pellagrins. This is associated with a significant decrease in the urinary excretion of the 6-pyridone of *N*-methylnicotinamide. The probable route of conversion of tryptophan into nicotinic acid in animals and fungi is indicated in Scheme 1; thus the increase in the excretion of quinolinic acid brought about by the oral administration of leucine may be due to (*a*) a relative block in the conversion of quinolinic acid into nicotinic acid (reaction 8), or to (*b*) a relative block in the alternative pathway of conversion of 3-hydroxyanthranilic acid into nicotinic acid, which does not lie through the quinolinic acid stage (reactions 7 and 9), or to (*c*) a stimulation of the conversion of 3-hydroxyanthranilic acid into quinolinic acid (reaction 6).

The marked depression in the urinary excretion of the 6-pyridone of *N*-methylnicotinamide in the presence of a normal or higher-than-normal ex-

cretion of *N*-methylnicotinamide requires explanation. A comparison of the response to the oral administration of leucine after dosage with tryptophan or nicotinamide shows that, in the presence of nicotinamide, the depression in the excretion of the 6-pyridone of *N*-methylnicotinamide is not significant. The relationship, if any, between the mechanisms involved in the increased excretion of quinolinic acid and the decreased excretion of the 6-pyridone of *N*-methylnicotinamide also requires investigation. It is possible that, in the presence of increased amounts of quinolinic acid, the conversion of *N*-methylnicotinamide into its pyridone derivative may be retarded. This retardation may be partly overcome by using an increased concentration of nicotinic acid.

SUMMARY

1. The oral administration of leucine increased the urinary excretion of quinolinic acid and decreased the excretion of tryptophan, the 6-pyridone of *N*-methylnicotinamide and 5-hydroxyindolylacetic acid, both in normal subjects and in patients suffering from pellagra.

2. When a tryptophan load was given, leucine had similar effects on the urinary excretion of tryptophan, quinolinic acid and the 6-pyridone of *N*-methylnicotinamide.

3. In the presence of a nicotinamide load, however, there was a much smaller decrease in the urinary excretion of the 6-pyridone of *N*-methylnicotinamide observed with leucine.

4. The oral administration of lysine had no effect on the urinary excretion of nicotinic acid, quinolinic acid and the 6-pyridone of *N*-methylnicotinamide.

The authors thank Mr F. Dewar and Mr K. Vivekanandan for technical assistance.

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