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From the Department of Medicine (Head: V.-M. Anttonen, M. D.), Central Hospital, Kuopio, and the Central Laboratory (Head: F.-E. Krusius, M. D.), Kivelä Hospital, Helsinki, Finland

Metabolism of Tryptophan in Diabetes Mellitus

By

MARTTI OKA and V. V. E. LEPPÄNEN

Some previous studies indicate that diabetes mellitus is one of those diseases in which a disorder of tryptophan metabolism is present. Rosen et al. (7) found that diabetic patients excreted on the average significantly greater quantities of xanthurenic acid after an oral test load of 10 g of DL-tryptophan than did non-diabetic controls. Kotake and Tani (3) detected xanthurenic and 3-hydroxykynurenic acids in the urine of diabetics, while paper chromatography did not reveal these substances in that of normal subjects. Conflicting results were obtained, however, by another group of workers (10). Their diabetic subjects were found to excrete a subnormal proportion of the administered load of 4 g of L-tryptophan in the form of tryptophan, kynurenine, anthranilic acid and xanthurenic acid, but normal amounts of 5-hydroxyindoleacetic acid.

According to a paper chromatographic study by the authors (6), tryptophan metabolism is deranged in diabetes mellitus. Eleven out of 23 diabetics showed abnormal indole chromatograms. Deviations from the normal occurred irrespective of the treatment the patients had received.

In this investigation an attempt has been made further to clarify the metabolic pattern of tryptophan in diabetes mellitus. Quantitative studies have been made of the main indole derivative of tryptophan, 5-hydroxyindoleacetic acid, and of the following derivatives of the kynurenine pathway: kynurenine, anthranilic acid, 3-hydroxyanthranilic acid and xanthurenic acid.

Material and methods

The material comprised 10 diabetics — 4 females and 6 males. Their ages varied from 10 to 68 years (average 41.8). Seven of the cases were treated with insulin and 3 by dietetic means only. The control group consisted of 12 hospitalized subjects with no known disease. Their ages varied from 19 to 61 years (average 32.8).

All medication except insulin was discontinued one day before the experiment. The determination of tryptophan metabolites was

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Table I. An abbreviated scheme of the metabolism of tryptophan by the kynurenine pathway

	Tryptophan
	↓
Kynurenic acid ←	Kynurenine → Anthranilic acid
	↓
	3-hydroxykynurenine → Xanthurenic acid
	↓
	3-hydroxyanthranilic acid
	↓
	Nicotinic acid

performed on two consecutive days from 24-hour urine. The first test day was a control day. On the morning of the second day a dose of 2 g of L-tryptophan (9.8 mM) was administered. The collection of urine was controlled by creatinine estimations and the possible forbidden use of interfering drugs by p-aminobenzoic acid determinations. Toluene was used as preservative.

Creatinine excretion varied in the diabetics from 0.37 to 1.60 g/24 hours (average 0.85) and in the controls from 0.69 to 1.75 g/24 hours (average 1.12). Urinary excretion of p-aminobenzoic acid ranged in the diabetics from 4 to 14 μM/24 hours (average 7.5) before tryptophan and from 5 to 37 μM/24 hours (average 13.2) after the tryptophan load. The corresponding figures in the controls were as follows: before tryptophan 7—31 (average 12.4) and after the tryptophan load 9—49 (average 19.0).

5-hydroxyindoleacetic acid was estimated by the method of Hanson and Serin (1), kynurenine, anthranilic acid, p-aminobenzoic acid and 3-hydroxyanthranilic acid according to the methods of Tompsett (9), and xanthurenic acid by the method of Rosen et al. (8).

Results

A derangement of tryptophan metabolism in diabetes mellitus was observed in this study. The most impressive abnormality was the markedly increased urinary excretion of xanthurenic acid both before and after the administration of the loading dose of L-tryptophan. In the diabetics the basal xanthurenic acid excretion exceeded the normal range in all of the cases and the mean excretion was about five times higher than in the control group. The ratio did not alter after tryptophan loading when 8 cases out of 10 presented values above the upper limit of the normal range.

Another abnormality of tryptophan metabolism found in the diabetics was the lowered urinary excretion of kynurenine after tryptophan loading, during which the excretion of kynurenine was on average 2.6 times lower than in the controls. (The difference is statistically significant, $u = 2.92$ in the Wilcoxon test, $P < 0.01$).

The urinary excretion of anthranilic acid and 3-hydroxyanthranilic acid in diabetes mellitus did not differ significantly from that of the controls.

The urinary excretion of 5-hydroxyindoleacetic acid seemed to be slightly higher in the diabetics than in the control group. Two of the cases presented abnormally high values before trytophan loading and 6 cases after it. (The difference is statistically significant after the tryptophan load. $u = 3.00$, $P < 0.01$). However, no markedly elevated individual values were recorded (tables I and II).

There appeared to be no difference in the urinary excretory pattern of tryptophan metabolites between the diabetics receiving insulin and those treated by dietetic means alone.

Discussion

Disorders of tryptophan metabolism are not uncommon. Abnormal metabolic patterns have been found in several conditions including bladder cancer, porphyria, scleroderma, disseminated lupus

Table II. Urinary excretion of tryptophan metabolites by patients with diabetes mellitus and healthy controls

	No. of cases	Before (B) or after (A) trypto-phan	Urinary tryptophan metabolites (μM/24 hrs)				
			5-hydroxy-indoleacetic acid	Kynurenine	Anthranilic acid	3-hydroxy-anthranilic acid	Xanthurenic acid
Diabetes mellitus	10	B	39.5 16—60	9.8 5—16	6.9 4—14	44.7 29—60	72.4 34—141
		A	53.1 23—95	15.2 7—27	9.7 5—18	64.3 49—86	128.9 58—265
Controls.....	12	B	28.8 12—57	14.7 7—31	10.4 1—21	37.1 11—82	14.8 4—26
		A	35.0 25—47	39.3 11—89	13.3 3—24	56.5 27—134	28.4 10—65

erythematosus, pregnancy (Price 1958), rheumatoid arthritis, tuberculosis, renal diseases and cancer of varying sites (5, 6). Tryptophan metabolism may also be altered by drugs with antipyridoxine activity (Price). The metabolic patterns found (quantitative or paper chromatographic) have not proved specific for any condition except the carcinoid syndrome, in which large quantities of 5-hydroxyindoleacetic acid and other indole derivatives are excreted, and certain rare congenital errors of metabolism (Hartnup's disease, phenylketonuria). The same holds true for the various metabolites of tryptophan. No really abnormal or specific metabolites have yet been demonstrated in any disease.

The previous studies on tryptophan metabolism in diabetes mellitus have indicated an abnormality (6, 7, 10). However, the results obtained by Rosen et al. and on the other hand by Kalant et al. were quite different. Rosen's group studied only the 24-hour excretion of xanthurenic acid, which appeared to be increased. Kalant et al. measured the urinary excretion of tryptophan, xanthurenic acid, kynurenine, anthranilic acid and 5-hydroxyindoleacetic acid. They used a 2-hour basal urine specimen and after oral administration of tryptophan a 6-hour specimen. This might be the reason for the differing results. We feel that 24-hour urine collection is essential in diabetics, in whom the rate of urine excretion varies widely.

Our studies confirm the results of Rosen et al. We also found an increased xanthurenic acid excretion in diabetics. Both basal xanthurenic acid excretion and that after the loading dose of L-tryptophan were markedly elevated. This was the most noteworthy abnormality in the excretory pattern of tryptophan metabolites observed in our study. In addition, the excretion of kynurenine was diminished compared to normal and the 5-hydroxyindoleacetic acid excretion slightly enhanced after tryptophan loading.

It is generally accepted that an increased urinary excretion of xanthurenic acid is a sign of pyridoxine deficiency. Vitamin B_6 in the form of pyridoxal phosphate is required for the metabolic breakdown of tryptophan at several metabolic steps (as the coenzyme of both kynureninase and 5-hydroxytryptophan decarboxylase). A deficiency of this coenzyme is followed by increased formation of quinoline compounds — xanthurenic and kynurenic acids. Thus the derangement of tryptophan metabolism observed in our study might be connected with a deficiency of vitamin B_6 in diabetes mellitus. The possibility that vitamin B_6 deficiency and consequently disordered tryptophan metabolism might contribute to the development of certain diabetic complications deserves further study.

It should also been borne in mind that tryptophan has been shown to be involved in the regulation of blood sugar as the precursor of a competitive inhibitor of insulinase (4), and that hyperglycemia can be induced in rabbits by 5-hydroxytryptophan, which is the precursor of 5-hydroxytryptamine (2).

Summary

The metabolism of tryptophan was studied in 10 patients with diabetes mellitus and 12 control subjects by determining the urinary excretion of 5-hydroxyindoleacetic acid, kynurenine, anthranilic acid, 3-hydroxyanthranilic acid and xanthurenic acid before and after a loading dose of 2 g of L-tryptophan.

A disorder of tryptophan metabolism was observed. This appeared as a markedly increased excretion of xanthurenic acid both before and after the loading dose of L-tryptophan. The excretion of kynurenine was diminished compared to normal and that of 5-hydroxyindoleacetic acid slightly enhanced after the tryptophan load.

It is suggested that the metabolic derangement observed is at least partially due to a deficiency of vitamin B_6.

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