Studies on Tryptophan-Niacin Metabolism in Streptozotocin Diabetic Rats

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SUMMARY

Streptozotocin diabetic male albino rats were observed to exhibit faulty conversion of tryptophan into niacin. Streptozotocin-injected rats, kept on niacin-deficient diet and given a dose of L-tryptophan (intraperitoneal or oral) excreted comparatively less quantities of niacin and N^1-methylnicotinamide in urine than the control nondiabetic rats. Rats continued to show this effect for at least three months after streptozotocin injection. Dietary intake was found to have no influence on the excretory pattern. Streptozotocin diabetic rats were also observed to excrete greater amounts of xanthurenic acid and other tryptophan metabolites on tryptophan administration as compared to the nondiabetic rats. DIABETES *23*:977-81, December, 1974.

Diabetic condition has been observed by several workers to be accompanied by disturbed tryptophan-niacin metabolism. Kotake and Tani[1] observed xanthurenic acid and 3-hydroxykynurenine in the urine of diabetic patients. Rosen et al.[2] reported increased urinary xanthurenic acid in diabetic patients following an oral dose of tryptophan indicating an impaired tryptophan-niacin metabolism. McDaniel et al.[3] have observed decreased N^1-methylnicotinamide (NMN) after tryptophan administration (oral or intraperitoneal route) in alloxan diabetic rats as compared to normal rats. Similar observations were reported by Ginoulhiac and coworkers[4] in pancreatectomized rats. Acetoacetate induced hyperglycemic rats were reported by Shastri and Nath[5,6] to exhibit disturbed tryptophan metabolism.

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Streptozotocin,* a broad spectrum antibiotic, has been found to be strongly diabetogenic and is being increasingly used in inducing diabetes in experimental animals. Lower values of pyridine nucleotides in mouse liver[7] and rat pancreas[8] have been recently reported. In view of these reports investigations were undertaken to study the tryptophan-niacin metabolism in rats made diabetic by streptozotocin.

MATERIALS AND METHODS

Male albino rats (Wistar strain) about 200 gm. body weight were used in the present investigations. Streptozotocin* was dissolved in citrate buffer of pH 4.3 and was immediately injected intravenously at the level of 50 mg. per kilogram body weight. The control rats were injected with an equivalent volume of saline.

Some of the rats were kept on the stock laboratory diet and some were fed a niacin-deficient diet. The composition of both the diets is indicated in table 1. After two weeks, niacin-deficient rats were transferred to metabolic cages and their basal urinary excretions of niacin and NMN were determined. They were then given L-tryptophan, niacin and niacinamide at the levels indicated in table 2 and urinary excretion of niacin and NMN during the following twenty-four hours were estimated.

For the estimation of niacin and NMN, urine samples were collected under toluene and glacial acetic acid. For xanthurenic acid and other metabolites, they were collected under toluene. Niacin was estimated by the cyanogen bromide method of Swaminathan[9] and NMN was determined by the fluorometric procedure described by Huff and Perlzweig.[10] McDaniel et al.[3] have reported that the presence of glucose gives lower

*Kindly supplied by Upjohn International Inc., Kalamazoo, Mich.

TABLE 1

Composition of stock and niacin-deficient diet

Constituent	Stock diet	Niacin-deficient diet
Wheat Flour	65	——
Casein	20	——
Sucrose	——	59
Vitamin free casein	——	9
Ground nut oil	10	8
Hawk-Oser salt mixture	4	4
Vitamin mixture	1*	20†

*1 gm. of the vitamin mixture in sucrose contained: 10 mg. thiamine HCl, 20 mg. riboflavin, 10 gm. pyridoxine HCl, 40 mg. calcium pantothenate, 12.5 μg folic acid, 200 mg. choline chloride, 10 μg biotin, 5 mg. menadione, 50 mg. α-tocopherol, 50 mg. niacin, 10 μg cyanocobalamine, 2000 IU vitamin A, and 200 IU vitamin D.

†20 gm. of vitamin mixture in sucrose contained: 10 mg. thiamine HCl, 20 mg. riboflavin, 10 mg. pyridoxine HCl, 40 mg. calcium pantothenate, 12.5 μg folic acid, 200 mg. choline chloride, 10 μg biotin, 5 mg. menadione, 50 mg. α-tocopherol, 10 μg cyanocobalamine, 2000 IU vitamin A, and 200 IU vitamin D.

values of NMN by this method. In preliminary investigations, the authors observed that the presence of glucose did tend to give lower values of NMN in many samples, though the decrease was only within the range of 5 to 10 per cent, whereas McDaniel et al. observed a decrease of 40 per cent in some samples. All further estimations of NMN were, therefore, performed by measuring the amount of glucose in the diabetic samples and adding an equivalent amount of glucose to the corresponding control samples. Glucose was determined by the titration method with Benedict's quantitative reagent.[11]

Xanthurenic acid was determined by the procedure of Wachstein and Gadaitis.[12] A colorimetric method described by Eckert[13] was employed to determine 'other' tryptophan metabolites by taking L-tryptophan as a standard. McDaniel et al.[3] have pointed out that tryptophan, anthranilic acid, kynurenine, etc. react in this test.

RESULTS

Table 2 indicates the results obtained with tryptophan, niacin and niacinamide administration on niacin and NMN excretion in diabetic and nondiabetic rats kept on niacin-deficient diet. It was observed that there was no significant difference between the basal excretion of niacin and NMN in diabetic and nondiabetic rats. But the increase in the excretion of these compounds after administration of various doses of L-trypotophan was significantly lower in streptozotocin diabetic rats. When niacin was administered, the average increase in NMN excretion in strep-

TABLE 2

Urinary niacin and N¹-methylniacinamide (NMN) in nondiabetic and streptozotocin diabetic rats kept on niacin-deficient diet and given supplements of L-tryptophan, niacin and niacinamide.

The values (μg/rat/day ± S.D.) are averages from twelve rats, six determinations on the pooled urine of two rats each.

Supplement*		Niacin					NMN				
		Nondiabetic		Diabetic		P†	Nondiabetic		Diabetic		P†
		Before supplement	After supplement	Before supplement	After supplement		Before supplement	After supplement	Before supplement	After supplement	
Tryptophan (50 mg./rat)	NF	8 ± 2	46 ± 8	11 ± 3	23 ± 7	< 0.05	142 ± 14	1,290 ± 276	115 ± 19	620 ± 144	< 0.01
	F	10 ± 4	60 ± 9	14 ± 5	34 ± 6	< 0.05	94 ± 17	955 ± 180	80 ± 20	612 ± 120	< 0.05
Tryptophan (100 mg./rat)	NF	34 ± 10	328 ± 28	22 ± 9	120 ± 31	< 0.01	168 ± 19	2,300 ± 402	133 ± 40	789 ± 240	< 0.001
	F	25 ± 8	351 ± 34	28 ± 7	112 ± 24	< 0.01	152 ± 20	1,940 ± 328	148 ± 34	990 ± 310	< 0.01
Tryptophan (40 mg./100 gm. body wt.)	NF	36 ± 7	210 ± 42	29 ± 8	74 ± 10	< 0.01	170 ± 30	1,830 ± 341	182 ± 32	847 ± 205	< 0.01
	F	32 ± 9	306 ± 21	22 ± 5	124 ± 16	< 0.001	154 ± 23	912 ± 210	144 ± 27	357 ± 90	< 0.01
Niacin (3 mg./rat)	NF	28 ± 10	464 ± 92	35 ± 9	460 ± 88	– NS	233 ± 28	1,695 ± 380	201 ± 50	1,220 ± 470	NS
Niacinamide (3 mg./rat)	NF	34 ± 12	358 ± 177	30 ± 11	300 ± 89	NS	210 ± 30	1,590 ± 350	182 ± 32	1,700 ± 280	NS

Average weight of nondiabetic rats: 211 gm.; Average weight of diabetic rats: 204 gm.
*L-tryptophan was injected intraperitoneally as a suspension and niacin and niacinamide were injected as solutions.
†Indicates the level of significance (Students' *t* test) between the difference in the increase in niacin and NMN excretion, after supplement in nondiabetic and diabetic rats.
Average urinary glucose excretion in diabetic rats was 2.9 gm. per day.
NF = Nonfasting; F = Fasting.

TABLE 3

Lack of influence of food intake on tryptophan-niacin metabolism in streptozotocin diabetic rats.

The values (μg/rat/day ± S.D.) are averages from five rats, five determinations, one rat each.

Treatment	Urinary Niacin				P‡	Urinary NMN				P‡
	nondiabetic		diabetic			nondiabetic		diabetic		
	before supplement	after supplement	before supplement	after supplement		before supplement	after supplement	before supplement	after supplement	
Ad lib + 100 mg. L-tryptophan*	25 ± 8	156 ± 17	30 ± 7	102 ± 15	< 0.02	154 ± 24	2,160 ± 420	139 ± 33	1,405 ± 378	< 0.01
Pair fed + 100 mg. L-tryptophan†	42 ± 12	378 ± 52	40 ± 18	167 ± 21	< 0.01	168 ± 34	9,200 ± 925	148 ± 28	4,200 ± 520	< 0.01
2 gm. diet mixed with 100 mg. L-tryptophan	28 ± 10	236 ± 42	32 ± 11	125 ± 19	< 0.01	134 ± 25	4,200 ± 450	124 ± 30	2,000 ± 380	< 0.01

*Average food intake per rat per day: nondiabetic = 15 gm.; diabetic = 25 gm. Average water intake per rat per day: nondiabetic = 28 ml.; diabetic = 43 ml.
†Amount of food given per rat per day: nondiabetic = 14 gm.; diabetic = 14 gm.
‡P values calculated as indicated in table 2.
Average weights of nondiabetic rats = 215 gm.; diabetic rats = 222 gm.

tozotocin diabetic rats was observed to be lower as compared to the corresponding control animals. But the level of significance, i.e., p value (Student's *t* test) was found to be slightly more than 0.05. There was no significant increase in excretion of niacin and NMN in rats of both groups after niacinamide injection.

Table 3 indicates the similar results obtained when L-tryptophan was given through the diet in an experiment conducted to see the effect, if any, of the food intake on tryptophan-niacin metabolism in the experimental rats. It was also noted that food intake had no influence on the tryptophan metabolism. Pair feeding gave results similar to those obtained with fasting and ad libitum feeding. Sucrose is a poor carbohydrate source of rats and it is not known whether this will have any bearing on the results obtained. Moreover, the niacin-deficient diet used in this study is rather protein limiting and could lead to protein insufficiency. That this possibility may have some bearing on the results obtained cannot be completely ruled out.

TABLE 4

Urinary excretion of xanthurenic acid (XA) and other tryptophan metabolites in streptozotocin diabetic rats kept on niacin-deficient diet and given L-tryptophan intraperitoneally (100 mg. per rat).

The values (μg./rat/day) are averages of six rats, six determinations

	Excretion before tryptophan administration		Excretion after tryptophan administration	
	XA	Other	XA	Other
Nondiabetic rats	216 ± 54	512 ± 78	1,400 ± 300	1,250 ± 315
Diabetic rats	250 ± 45	478 ± 80	2,530* ± 480	2,980† ± 450

*P <0.05
†P <0.01
Average weight of nondiabetic rats = 218 gm.
Average weight of diabetic rats = 223 gm.

Data on the excretion of xanthurenic acid and other tryptophan metabolites is presented in table 4, which indicates that the diabetic rats excreted urine with comparatively more xanthurenic acid and other tryptophan metabolites than the nondiabetic rats, following tryptophan administration. Table 5 indicates that the pattern of urinary excretion of niacin and NMN was similar in streptozotocin diabetic rats kept on stock laboratory diet and tested periodically at intervals of one month, two months, and three months after streptozotocin injection.

DISCUSSION

Results presented in tables 2, 3 and 5 indicate that the conversion of tryptophan into niacin is impaired in streptozotocin diabetic rats and that this effect is fairly prolonged. It is relevant here to note that Rakieten et al.[14] have found rats remaining in diabetic condition

TABLE 5

Urinary niacin and NMN excretion following L-tryptophan administration (100 mg. per rat intraperitoneally) in streptozotocin diabetic rats kept on stock laboratory diet for three months.

The values are averages from twelve rats, six determinations on the pooled urine of two rats each (μg./rat/day ± S.D.).

Period of analysis after streptozotocin injection	Niacin					NMN				
	nondiabetic		diabetic		P	nondiabetic		diabetic		P
	before tryptophan	after tryptophan	before tryptophan	after tryptophan		before tryptophan	after tryptophan	before tryptophan	after tryptophan	
Initial	82 ± 15	174 ± 22	70 ± 16	102 ± 18	< 0.05	450 ± 50	1,800 ± 150	405 ± 45	704 ± 65	< 0.01
One month	48 ± 10	300 ± 40	39 ± 12	172 ± 25	< 0.02	248 ± 30	2,095 ± 280	228 ± 40	1,060 ± 102	< 0.01
Two months	71 ± 8	214 ± 25	79 ± 16	105 ± 20	< 0.01	420 ± 62	1,250 ± 120	388 ± 50	605 ± 82	< 0.05
Three months	64 ± 12	329 ± 32	52 ± 20	110 ± 19	< 0.01	312 ± 34	2,260 ± 240	344 ± 30	1,310 ± 115	< 0.02

Average weights at the time of streptozotocin injection: nondiabetic rats = 204 gm.; diabetic rats = 201 gm.
Average weights one month after streptozotocin injection: nondiabetic rats = 212 gm.; diabetic rats = 195 gm.
Average weights two months after streptozotocin injection: nondiabetic rats = 225 gm.; diabetic rats = 210 gm.
Average weights three months after streptozotocin injection: nondiabetic rats = 240 gm.; diabetic rats = 231 gm.

for 206 days after streptozotocin injection.

Increased xanthurenic acid excretion (table 5) indicates that, in streptozotocin-injected rats, the tryptophan-niacin metabolism may be shunted, to some extent, towards xanthurenic acid. Similar results have been obtained in alloxan diabetic rats[3] and in diabetic patients.[2] Later studies performed by Mehler and coworkers[15] on the liver enzymes involved in tryptophan-niacin pathway, however, indicated that only picolinic carboxylase activity was abnormally high in alloxan diabetic rats as compared to normal control. Tryptophan oxygenase activity was also increased to some extent in the experimental animals. Picolinic carboxylase is involved in the conversion of the oxidation product of 3-hydroxyanthranilic acid into picolinic acid and, therefore, increase in its activity will divert the tryptophan metabolism towards picolinic acid instead of niacin, resulting in decreased urinary excretion of niacin and NMN. Korbitz et al.[16] have postulated that an alternate pathway exists for xanthurenic acid biosynthesis and it is possible that the stimulation of this pathway is a contributing factor in the high excretion of xanthurenic acid in diabetic condition including streptozotocin-induced diabetes.

As far as the excretion of the so-called "other" tryptophan metabolites is concerned, it has been reported that the method employed measures kynurenine amongst other metabolites. Tenconi[4] observed greater excretion of kynurenine after tryptophan load in pancreatectomized rats as compared to normal animals. This was suggested to be due to increased activity of tryptophan oxygenase in the livers of pancreatectomized rats.[17]

In the light of these observations, it seems that though the present paper indicates an impairment of tryptophan-niacin metabolism in streptozotocin diabetic rats, the exact mechanism of action of streptozotocin will be better understood after the various intermediates and enzymes involved in the tryptophan-niacin metabolism are studied. Investigations on these lines are in progress.

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