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STUDIES ON THE EFFECT OF RESERPINE THERAPY ON THE FUNCTIONAL CAPACITY OF THE TRYPTOPHAN–NIACIN PATHWAY IN SMOKER AND NON-SMOKER MALES

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Abstract—The functional capacity of the tryptophan–niacin pathway was tested by analysis of eleven metabolites excreted in the urine of smoker and non-smoker males after loading with L-tryptophan (2 g) with and without vitamin B_6 supplement (120 mg). Furthermore, the excretion pattern was re-investigated after depletion of the catecholamines by applying reserpine therapy to both groups.

The results obtained were compatible with the suggestion that the modification of tryptophan metabolism by smoking may be through the catecholamines discharged by nicotine that man absorbs from smoking. Thereafter, the catecholamines react with the coenzyme (i.e. pyridoxal phosphate) to form inactive tetrahydroisoquinoline derivatives causing a rapid inactivation of the coenzyme, and thereby inhibit preferably the B_6-dependent quinolinic acid decarboxylase enzyme. It is striking, however, that this type of inhibition could not be completely overcome by a large dose of vitamin B_6 orally supplemented. It was only after reserpine therapy that both smokers and non-smokers gave excretion patterns of tryptophan metabolites which were qualitatively and quantitatively similar.

THE SUGGESTION made by Kerr and associates[1] that the mechanism of carcinogenic action of cigarette smoking on the urinary bladder by modification of tryptophan metabolism, is of particular interest since some of the metabolites along the tryptophan–niacin pathway (kynurenine pathway) are bladder carcinogens.[2–4]

Nicotine, that man may absorb from smoking or other use of tobacco, like acetylcholine, causes an increase in the release of catecholamines by the adrenals and sympathetic nerve-endings.[5] The smoking of two to three cigarettes per hour is known to increase the concentration of catecholamines in human blood.[6] It is also cited that the (nicotine-released) catecholamines react with carbonyl compounds, e.g. the coenzyme pyridoxal phosphate, to form tetrahydroisoquinoline derivatives.[7] Thus, the catecholamines as well as this coenzyme lose their pharmacological and biochemical activities.[8] If this is the case then one would expect different excretion patterns of tryptophan metabolites in smoker and in non-smoker groups since several enzymes of the kynurenine pathway, e.g. kynureninases, transaminases and quinolinic acid decarboxylase, require the participation of pyridoxal phosphate as coenzyme.[8–10]

The present work is confined, therefore, to study the excretion patterns of interrelated metabolites of the tryptophan–niacin pathway in the urine of smoker and non-smoker males before and after reserpine depletion of their catecholamines.

EXPERIMENTAL

Material

Thirty males were studied. They were classified into two groups: (a) fourteen non-smokers, and (b) sixteen smokers. They were healthy laboratory personnel varying in age between 20 and 40 years.

Defined as "smokers" were individuals who have smoked for at least 2 years and not less than 6 g of tobacco a day (1 cigarette = 1 g). These were mild smokers, since the smoking index[11] of these individuals was about 178.

All the persons were instructed to collect 24-hr basal urine specimen. After collection of these urines, each subject was given an oral loading dose of 2 g of L-tryptophan,[9] dispensed in starch cachets, and a second 24-hr urine specimen was collected (post-tryptophan urine). In all the cases, this 2-day study was repeated; in addition they were orally given 120 mg of pyridoxine hydrochloride (divided in three equal doses) and urine was again collected for a third 24-hr period (post-tryptophan supplemented with vitamin B_6 urine).

Thereafter, reserpine therapy (serpasil, Ciba, Switzerland) was given to seven subjects from the non-smokers group and to thirteen subjects from the smokers group (smoking index = 167). Serpasil (0·5 mg) was orally administered daily (divided in four equal doses) for 3 successive days. Studies with tryptophan loading were started again on the reserpine-treated smokers and non-smokers for the next 3 successive days, as mentioned above, starting from the last day of reserpine therapy.

All urine specimens were collected under toluene and refrigerated until analysed.

Quantitative estimation of metabolites

The urinary tryptophan metabolites: anthranilic acid glucuronide, free anthranilic acid, *o*-aminohippuric acid, *N*α-acetylkynurenine and kynurenine were determined by the method of Brown and Price.[12]

3-Hydroxykynurenine was estimated by the method of Brown,[13] kynurenic acid and xanthurenic acid by the method of Satoh and Price,[14] 3-hydroxyanthranilic acid by the method of Michael *et al.*,[15] and 4-pyridoxic acid by the method of Reddy *et al.*[16]

Statistical analyses were made to compare the data for smokers before reserpine therapy, as well as for both smokers and non-smokers after depletion of their catecholamines, with the data for non-smoker controls using the standard *t*-test; probability values (P) of less than 0·05 were considered almost significant.

RESULTS

The smoker and non-smoker groups have been compared with respect to the functional capacity of each subject to metabolize the tryptophan load before and after reserpine therapy. Subtraction of the basal levels of the urinary metabolites from the levels present in either the post-tryptophan or the post-tryptophan supplemented with vitamin B_6-urine, gives the metabolic response to the loading dose of tryptophan, i.e. yield I and II, respectively, expressed as the quantity, in mg/24-hr-urine, excreted in excess of the basal levels.

The functional capacity of the tryptophan–niacin pathway for the individual cases in both groups is given in Tables 1 and 2 for non-smoker controls and smokers before reserpine therapy, and for non-smokers and smokers after reserpine therapy, respectively.

Subject No.	AAG		AA		*o*-AH		KA		ACK		KN		3-OHK		XA		3-OHAA		4-PA	
	YI	YII	YI	YII	YI	YII	YI	YII	YI	YII	YI	YII	YI	YII	YI	YII	YI	YII	YI	YII
1	4·0	4·0	−0·4	−0·5	6·0	7·3	25·3	32·0	0·4	1·1	13·0	10·0	4·0	3·2	2·7	3·8	2·2	2·5	−0·1	18·0
2	2·8	4·7	0·7	2·1	2·8	6·7	13·7	18·7	0·6	0·6	11·0	5·0	7·7	2·9	1·6	2·1	4·5	1·7	−0·7	17·3
3	2·0	2·7	0·1	0·9	1·9	2·7	7·0	8·5	−0·1	1·1	4·0	6·0	5·0	11·2	2·7	2·7	1·4	2·4	−0·6	21·1
4	2·5	7·9	1·7	2·6	6·0	12·0	14·3	12·6	−0·4	0·7	2·0	4·4	0·0	2·0	2·3	2·5	1·0	2·5	0·3	23·0
5	0·7	0·1	0·8	3·8	5·9	3·2	37·2	34·6	0·6	−0·4	2·4	−2·0	5·3	−4·0	5·0	4·4	—	—	−1·3	25·1
6	2·7	−2·0	3·9	−6·1	4·0	−1·3	15·4	18·8	−0·6	−0·6	15·2	−3·2	22·7	2·7	4·0	3·0	1·3	1·5	0·8	11·4
7	3·2	3·2	4·5	−0·6	8·0	21·3	18·6	28·6	0·8	0·6	26·4	12·4	9·3	0·7	4·2	4·6	—	—	−0·5	10·9
8	2·5	2·5	−1·1	0·6	5·1	4·4	21·0	16·5	1·1	−0·6	0·0	−2·0	−5·2	−8·8	1·6	1·0	0·2	0·5	−2·1	9·4
9	−3·3	−3·3	−1·3	−1·3	−3·9	−3·3	32·4	36·0	−0·7	−0·6	−2·0	−4·0	−4·0	−3·8	3·4	3·4	1·0	3·0	−0·5	25·0
10	1·3	−0·7	−0·4	−10·6	4·5	4·7	22·5	17·5	0·4	0·6	1·4	0·4	−6·8	−2·2	3·0	3·0	1·0	2·0	3·2	25·8
11	0·7	1·9	0·7	1·1	5·3	8·0	32·0	28·0	−0·3	−0·4	11·2	2·0	12·0	2·0	3·5	3·9	2·0	1·5	−0·5	24·8
12	2·0	4·7	1·5	−0·8	4·7	5·3	7·7	9·3	0·3	0·8	4·0	3·6	0·0	0·0	2·2	2·1	3·0	2·0	0·1	21·6
13	0·7	0·0	0·0	−0·3	5·3	5·3	4·2	12·0	0·3	0·3	2·0	3·2	1·3	2·0	4·2	3·6	0·8	4·0	−0·5	32·0
14	2·7	3·2	0·0	0·0	5·7	9·6	4·7	5·6	0·8	1·1	13·6	8·6	0·0	0·0	1·4	1·6	0·0	0·0	0·8	25·2
Mean	1·7	2·1	0·8	−0·7	4·4	6·1	18·3	19·9	0·2	0·3	7·4	3·2	3·7	0·6	3·0	3·0	1·5	2·0	−0·1	20·8
±S.E.	0·5	0·8	0·4	0·9	0·7	1·5	2·7	2·6	0·1	0·2	2·0	1·3	2·0	1·2	0·3	0·3	0·4	0·3	0·3	1·7
(b) THE FUNCTIONAL CAPACITY OF THE TRYPTOPHAN–NIACIN PATHWAY IN SIXTEEN MILD SMOKER MALES																				
15	3·3	3·3	−0·8	6·9	6·7	3·5	23·4	21·0	3·5	1·1	17·8	6·2	12·0	0·0	3·4	3·0	−0·6	−1·0	−0·9	15·4
16	4·3	5·3	3·0	−2·2	18·0	26·7	50·0	58·0	5·0	2·8	77·2	56·0	8·7	6·0	6·8	7·2	—	—	−0·4	11·6
17	2·4	2·8	−0·4	−0·4	3·7	4·5	5·9	7·3	3·2	3·6	19·0	11·8	3·3	2·7	2·1	3·0	—	—	−0·1	24·0
18	2·7	2·7	0·3	1·0	4·4	6·0	13·2	9·0	−2·5	−2·9	6·0	6·0	4·0	2·7	4·0	3·6	0·3	−1·0	−0·2	24·4
19	4·0	0·7	−2·0	−2·0	19·5	18·8	50·7	68·3	4·6	2·4	66·0	20·0	12·0	3·3	9·4	12·6	2·0	2·0	−0·6	17·6
20	2·0	0·9	−0·4	−0·3	1·9	1·9	19·2	11·7	0·7	0·0	7·2	4·0	5·3	2·7	4·3	3·0	1·5	0·5	−0·6	27·1
21	2·5	4·7	−0·6	−0·6	13·1	17·7	31·2	36·4	2·6	2·8	68·6	47·6	32·7	17·3	6·2	6·7	5·0	—	0·1	25·9
22	2·0	1·3	−1·1	0·4	10·7	1·3	48·6	6·4	7·6	0·3	45·0	5·0	0·0	0·0	9·8	1·4	0·7	0·5	−0·2	19·5
23	2·0	2·3	−0·8	0·8	0·3	1·7	15·8	7·4	3·3	0·8	17·6	6·8	1·3	2·0	1·8	0·1	1·5	1·5	−0·6	27·0
24	1·6	2·7	0·7	1·5	2·8	7·5	12·5	27·0	−0·6	0·0	2·6	7·6	0·0	0·0	2·1	3·0	1·5	1·5	−0·3	7·6
25	3·3	2·5	0·6	1·1	8·7	14·2	17·2	33·0	0·7	1·8	24·0	23·4	4·7	4·7	2·6	4·9	2·5	—	−2·1	17·3
26	6·0	4·7	0·5	−0·1	16·0	32·7	35·6	77·2	1·0	1·3	15·0	28·0	3·3	4·0	5·1	9·4	2·5	3·2	−1·3	18·3
27	4·5	4·5	2·8	0·5	42·0	40·0	55·6	70·0	3·5	1·0	72·4	36·4	33·3	10 0	6·8	7·2	—	—	0·0	12·5
28	3·5	1·3	2·1	2·3	13·3	7·3	13·2	10·4	0·4	0·4	23·0	6·0	16·7	2·0	2·2	0·9	4·5	1·3	−1·6	37·3
29	3·3	2·9	4·9	−0·6	26·0	20·7	25·5	18·4	0·4	0·6	31·0	14·0	12·0	0·0	2·7	2·3	7·5	4·8	−0·5	52·1
30	5·3	5·9	3·9	0·9	49·3	49·3	81·5	46·5	2·8	0·4	83·0	10·0	45·3	4·0	8·0	4·4	8·8	4·3	−0·8	32·1
Mean	3·3†	3·0	0·8	0·6	14·8†	15·9†	31·2†	31·8	2·3†	1·0	36·0†	18·1†	12·2†	3·8†	4·8†	4·5	2·9	1·6	−0·6†	23·1
±S.E.	0·3	0·4	0·5	0·5	3·4	3·6	5·0	6·0	0·6	0·4	6·8	3·9	3·3	1·1	0·7	0·8	0·8	0·5	0·1	2·7
t-test against controls																				
t	2·8	1·1	0·1	1·2	2·7	2·3	2·1	1·7	3·0	1·6	3·7	3·3	2·1	2·1	2·4	1·7	1·6	0·6	2·1	0·7
P	<0·01	>0·1	>0·5	>0·1	<0·02	<0·05	<0·05	>0·1	<0·01	>0·1	<0·01	<0·01	<0·05	<0·05	<0·05	>0·1	>0·1	>0·5	<0·05	>0·5

The values recorded represent the increase or decrease in the excretion of various metabolites (in mg/24-hr-urine) after the ingestion of 2 g L-tryptophan (post-tryptophan values and post-tryptophan supplemented with B_6 values minus the basal values i.e. yield I and II, respectively). The abbreviations used are AAG, anthranilic acid glucuronide; AA, free anthranilic acid; *o*-AH, *ortho*-aminohippuric acid; KA, kynurenic acid; ACK, acetylkynurenine; KN, kynurenine; 3-OHK, 3-hydroxykynurenine; XA, xanthurenic acid; 3-OHAA, 3-hydroxyanthranilic acid; 4-PA, 4-pyridoxic acid; YI, yield I; YII, yield II; ±S.E., standard error of the mean and † indicates that this value is significantly different from the corresponding value for non-smoker controls before reserpine therapy († $P < 0.05$).

TABLE 2. THE FUNCTIONAL CAPACITY OF THE TRYPTOPHAN–NIACIN PATHWAY AFTER DEPLETION OF THE CATECHOLAMINES WITH RESERPINE*

(a) IN SEVEN CASES OF THE NON-SMOKER MALES (TABLE 1a)

Subject No.	Metabolites determined‡																			
	AAG		AA		*o*-AH		KA		ACK		KN		3-OHK		XA		3-OHAA		4-PA	
	YI	YII	YI	YII	YI	YII	YI	YII	YI	YII	YI	YII	YI	YII	YI	YII	YI	YII	YI	YII
1	2·0	0·0	−1·0	−0·5	10·7	0·1	28·0	0·4	1·1	0·0	16·4	2·0	3·3	−0·7	4·3	−0·1	0·2	0·2	−0·3	12·0
2	0·5	0·3	−2·0	0·8	4·1	5·5	9·2	10·2	0·8	0·1	4·0	3·4	0·0	2·0	2·1	2·9	—	—	−0·1	10·4
3	0·7	0·7	0·7	−3·5	1·6	1·6	6·5	4·7	0·3	0·8	2·0	3·0	0·0	0·0	3·5	2·2	0·0	2·4	0·3	21·0
4	1·9	1·2	−0·2	−0·8	6·0	7·3	8·0	18·8	1·0	0·4	2·0	3·0	1·3	0·0	0·8	3·2	−1·0	−2·8	0·8	19·3
5	2·7	2·0	1·1	−0·2	23·3	26·6	27·3	27·5	0·7	0·7	9·0	4·0	5·3	2·0	4·1	3·2	1·2	0·7	0·2	6·4
6	4·7	6·0	2·0	2·0	7·3	7·3	12·7	14·6	0·0	0·0	9·0	2·0	5·3	0·0	1·0	1·2	−0·5	0·0	−0·6	6·1
7	6·7	6·7	2·3	2·5	37·3	33·3	42·4	36·0	3·1	0·8	37·0	17·0	16·7	0·0	4·6	4·6	3·0	2·3	−0·8	6·2
Mean	2·7	2·4	0·7	0·0	12·9†	11·7	19·2	16·0	1·0†	0·4	11·3	4·9	4·6	0·5	2·9	2·5	0·5	0·5†	−0·1	11·6†
±S.E.	0·8	1·0	0·4	0·7	4·5	4·5	4·8	4·4	0·4	0·1	4·3	1·9	2·0	0·4	0·6	0·5	0·5	0·7	0·2	2·2
t-test against controls																				
t	1·1	0·3	0·1	0·5	2·4	1·4	0·2	0·8	2·3	0·4	0·9	0·7	0·3	0·1	0·1	0·9	1·5	2·2	0·1	3·1
P	>0·1	>0·5	>0·5	>0·5	<0·05	>0·1	>0·5	>0·1	<0·05	>0·5	>0·1	>0·1	>0·5	>0·5	>0·5	>0·1	>0·1	<0·05	>0·5	<0·01

(b) IN THIRTEEN CASES OF THE SMOKER MALES (TABLE 1b)

18	0·1	0·7	−0·1	1·1	2·5	−0·8	11·9	11·1	0·0	0·0	6·8	4·0	0·0	1·3	2·2	4·5	−0·2	2·0	−1·5	36·6
19	2·0	4·8	0·2	1·2	18·7	34·0	45·8	80·2	1·1	2·8	11·0	38·4	−2·0	−2·0	8·0	13·8	0·0	1·3	−1·3	7·1
20	−0·9	0·5	0·3	1·1	0·7	1·3	2·6	13·4	−0·3	−0·1	2·4	3·2	0·0	0·0	0·2	2·7	0·5	0·1	−2·8	16·6
21	0·0	13·3	0·5	15·5	3·2	14·5	12·0	2·0	0·6	2·6	6·0	6·3	6·7	6·7	2·1	0·8	−2·0	−0·1	−0·9	−2·2
22	0·7	1·9	0·5	−0·3	6·1	3·5	15·7	10·1	0·3	0·3	3·0	0·0	3·3	0·0	3·1	2·5	4·2	2·2	−1·3	15·4
23	0·0	1·3	0·0	−0·6	0·7	3·3	0·0	11·1	0·0	0·6	1·4	6·0	0·0	2·0	−0·4	2·0	2·3	2·3	−0·1	11·6
24	3·9	1·2	1·3	−0·3	5·9	0·5	12·0	0·0	0·3	0·3	3·0	0·0	0·0	0·0	3·0	1·3	3·4	1·5	−0·6	−0·7
25	1·3	2·7	0·0	0·4	10·7	12·7	15·2	21·8	0·4	0·3	15·0	15·0	4·7	8·0	2·0	3·0	1·3	2·8	−0·1	31·5
26	−3·3	6·0	0·6	0·0	17·3	29·3	39·6	59·6	1·4	0·0	43·0	16·0	8·0	3·3	6·2	8·2	1·3	2·0	−0·9	22·4
27	4·0	5·3	3·6	5·2	48·0	50·0	49·6	49·6	1·8	0·8	58·0	14·0	14·0	6·0	8·0	6·8	0·8	−1·0	−1·0	2·7
28	0·9	0·0	−1·3	−1·3	3·3	0·0	2·8	0·0	−0·4	0·0	2·8	2·8	1·3	−2·7	0·9	−0·3	0·8	0·3	−2·6	−2·2
29	2·7	4·7	1·9	2·7	22·7	44·7	26·2	49·4	28·4	0·0	43·0	39·0	17·3	8·0	1·4	3·9	0·0	3·2	−2·5	6·3
30	5·7	7·9	6·6	1·2	51·2	55·9	67·8	56·8	4·0	0·8	64·0	23·0	18·0	5·3	7·1	6·2	11·5	9·6	−0·3	18·9
Mean	1·8	3·9	1·1	2·0	14·7†	19·2†	23·2	28·1	2·9	0·6	20·0	12·9†	5·5	2·8	3·4	4·3	1·8	2·0	−1·2†	12·6†
±S.E.	0·5	1·0	0·5	1·2	4·6	5·6	5·6	7·2	2·1	0·3	6·2	3·6	1·9	1·0	0·8	1·0	0·9	0·7	0·2	3·2
t-test against controls																				
t	0·1	1·4	0·5	1·8	2·2	2·3	0·8	1·1	1·3	1·1	1·9	2·6	0·7	1·4	0·5	1·2	0·3	0·03	2·8	2·3
P	>0·5	>0·1	>0·5	>0·05	<0·05	<0·05	>0·1	>0·1	>0·1	>0·1	>0·05	<0·02	>0·1	>0·1	>0·5	>0·1	>0·5	>0·5	<0·02	<0·05

* 0·5 mg serpasil was given daily for three successive days.
† $P < 0·05$.
‡ Abbreviations as in Table 1.

It is evident from the data given in yield I (Table 1b) that the group of smoker males excreted significant amounts of anthranilic acid glucuronide, *o*-aminohippuric acid, kynurenic acid, acetylkynurenine, kynurenine, 3-hydroxykynurenine and xanthurenic acid as compared to the group of non-smoker males. Free anthranilic acid is equally excreted in both groups, whereas 4-pyridoxic acid is significantly less excreted in the smokers than in the non-smokers group.

The pattern given by the smokers group reflects, therefore, vitamin B_6 deficiency as evidenced by the accumulation of 3-hydroxykynurenine, acetylkynurenine, kynurenine and xanthurenic acid,[17, 18] and by the finding that yield I of 4-pyridoxic acid, the metabolic end product of vitamin B_6,[8, 17] is significantly less excreted (Table 1b). Moreover, the vitamin B_6-supplemented tryptophan load reduced the difference between the basal and the post-tryptophan level of some metabolites to those of the control levels. These metabolites are anthranilic acid glucuronide, kynurenic acid, acetylkynurenine, xanthurenic acid and 4-pyridoxic acid. However, some other metabolites are not reduced by vitamin B_6 supplementation to those of the control levels e.g. *o*-aminohippuric acid, kynurenine and 3-hydroxykynurenine (yield II, Table 1b). The reason for this will be discussed later.

The B_6 deficiency encountered in the smokers group affects either the B_6-dependent enzyme 3-hydroxykynureninase leading to the synthesis of 3-hydroxyanthranilic acid, or the quinolinic acid decarboxylase[10] leading to the synthesis of nicotinic acid. There is evidence, however, which excludes the first possibility. Thus if the kynureninase enzyme activity is the B_6-dependent enzyme which suffers inhibition by smoking, then one would expect: (a) a decrease in the formation of anthranilic acid from kynurenine and eventually a low production of anthranilic acid glucuronide and *o*-aminohippuric acid, and this is not the case (yield I, Table 1b), and (b) that the 3-hydroxyanthranilic acid level would be significantly less than that of the non-smoker controls, and instead its level is slightly higher than the corresponding control level. An augmented excretion of kynurenine and its acetyl derivative, 3-hydroxykynurenine and 3-hydroxyanthranilic acid is to be expected, therefore, in presence of inhibition to the quinolinic acid decarboxylase enzyme. The overflow of these metabolites takes place in the direction of kynurenic acid and xanthurenic acid through the transaminase enzymes and in the direction of anthranilic acid glucuronide, *o*-aminohippuric acid and 3-hydroxyanthranilic acid through the kynureninase enzymes.

It is of particular interest, therefore, to compare between the excretion patterns of smokers and non-smokers after reserpine depletion of their catecholamines, specially because the smoking habit was not interrupted throughout the whole investigation. In contrast to the data given in Table 1, the excretion patterns of smokers and non-smokers are qualitatively and quantitatively similar to each other and to that of the non-smoker controls given in Table 1a. However, minor differences do exist e.g. some metabolites are more while some others are less excreted by both groups than by the non-smoker controls. The former metabolites include yield I of *o*-aminohippuric acid in both groups, acetylkynurenine in the non-smokers group as well as yield II of *o*-aminohippuric acid and kynurenine in the smokers group; while yield II of 3-hydroxyanthranilic acid is significantly less in the non-smokers group after than before reserpine therapy.

It is noteworthy that the patterns given by both groups do not reflect vitamin B_6 deficiency after reserpine depletion of their catecholamines as is found before depletion,

although the excretion level of yield II of 4-pyridoxic acid is significantly less excreted (yield II, Table 2). This finding will be interpreted later.

DISCUSSION

The results of this study on the functional capacity of the tryptophan–niacin pathway, revealed that smoker males suffer from pyridoxine deficiency as compared to non-smoker males. This was evidenced by the inhibition of the B_6-dependent quinolinic acid decarboxylase enzyme[10] (Table 1b). The observation that anthranilic acid glucuronide, *o*-aminohippuric acid, kynurenic acid, xanthurenic acid and 3-hydroxyanthranilic acid were excreted in amounts equal to or larger than those of the non-smoker controls (yield I, Table 1b) may be interpreted as the overflow of kynurenine and 3-hydroxykynurenine occurring via the kynureninase and transaminase enzymes. Therefore, quinolinic acid decarboxylase activity was apparently preferentially depressed over that of kynureninase and transaminase enzymes of the kynurenine pathway in smokers as compared to non-smoker males. The preferential depression of some enzyme activities over that of other enzymes along the same pathway, may be a demonstration of the variation in sensitivity of specific B_6-requiring enzymes to pyridoxine deficiency and to pyridoxine antimetabolites, as pointed out by Holtz and Palm.[8] The abnormal tryptophan pattern excreted in the urine of smoker males should, therefore, be understood in terms of induced alteration of vitamin B_6-dependent physiological reactions which result from vitamin B_6 deficiency.[8, 9, 18]

Vitamin B_6-dependent enzymic reactions in which the cofactor is functionally inhibited by a mechanism such as that postulated above would be expected to be restored after therapy for three successive days with the norepinephrine-depleting agent, reserpine,[19, 20] and indeed, such therapy has been shown to restore the excretion pattern of tryptophan metabolites of smokers to that of non-smoker males, although the smoking habit was not interrupted throughout the whole investigation (Table 2b). It is striking, however, that this type of inhibition could not be overcome completely by a large dose (120 mg) of vitamin B_6 orally administered (yield II, Table 1b). This is not extraordinary, since it has been shown that pyridoxine alone failed to correct the abnormal tryptophan metabolism seen in pregnant women.[21] This finding may be attributed to the inadequacy of the oral administration of the vitamin to meet the increasing requirements for vitamin B_6 of the smokers to metabolize the 2 g tryptophan load plus the dietary protein, or/and the tryptophan metabolism might have returned to normal with a daily intake of vitamin B_6 if continued for a long period of time.[22] However, after reserpine therapy, smokers and non-smokers excreted significantly less 4-pyridoxic acid after loading with vitamin B_6 (yield II, Table 2) indicating a retention of pyridoxine as a result of an increased requirement during reserpine therapy. Furthermore, this retention did not manifest itself by any inhibition to the B_6-dependent enzymes along the kynurenine pathway. This finding may indicate that the available amount of vitamin B_6 can cope with the released amounts of catecholamines after treatment for 3 successive days with reserpine in both groups.

The results of this study concerning the excretion pattern of tryptophan metabolites of smoker as compared to that given by non-smoker males, were generally in agreement with the results given by Kerr *et al.*[1] However, comparison of these results with those obtained in our study is difficult because some of the methods applied in the determination of the metabolites as well as some experimental factors differed e.g. the

L-tryptophan loading dose was given on the basis of 50 mg per kg body weight, whereas in the present study the L-tryptophan load was administered in a single oral dose of 2 g.[9]

It can be concluded, therefore, that the overall excretion pattern of tryptophan metabolites found in smoker males is probably the result of both vitamin B_6 inactivation and endocrine changes associated with cigarette smoking. Thus, the probable inhibitory effect of the smoking-released catecholamines on the B_6-dependent quinolinic acid decarboxylase may be due to the reaction of the catecholamines with its coenzyme i.e. pyridoxal phosphate to form inactive tetrahydroisoquinoline derivatives.[7] This reaction is readily demonstrable with catecholamines possessing an unsubstituted amine group,[7, 23] but apparently it also occurs slowly with *N*-alkylated catecholamines.[24, 25] This interpretation may be further substantiated by the finding that the inhibition of 5-hydroxytryptophan decarboxylation in rat kidney homogenates by norepinephrine was due to a mechanism of this type and the demonstration that the corresponding tetrahydroisoquinoline derivative was formed nonenzymatically during the incubation.[23]

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