

CTR GRANT NO. 346

CONFIDENTIAL PROGRESS REPORT - 1965-1966ANTHONY A. ALBANESE, PH.D.

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White Plains, New YorkEFFECTS OF NICOTINE ON PROTEIN AND AMINO ACID METABOLISM IN HUMANS

Consideration of the available information has led to the speculation that a segment of the population may be hypersensitive to the products of tobacco smoke, and that the approach of mass abstention is unsound and not likely to succeed. On this premise, we set ourselves to the task of establishing criteria of such hypersensitivity which might enable physicians and health officers to make individual recommendations on smoking habits. Prior investigations in this laboratory supported by the Council for Tobacco Research - U.S.A., indicated that cigarette smoking alters the utilization of proteins primarily via the metabolism of the aromatic amino acids, and transaminase enzyme levels (1). Investigations were therefore initiated to ascertain differences in the utilization of the aromatic acids which might be associated with cigarette smoking.

PROCEDURES AND METHODS

In the screening studies (Phase I), spot and 24-hour urine collections were tested for indigo red by the method described by Albanese (2). In Phase II studies, 24-hour urine collections were obtained on 3 successive days from subjects on self-selected diets who had been given: Day I, no amino acid load; Day II, 1 gm. L-tryptophan; and, Day III, 1 gm. L-phenylalanine. Suitable aliquots were removed from the collections for analyses of indole metabolites. Qualitative and

quantitative determinations were made for indigo red content. Indole acetic and indole propionic acids were estimated by extraction and chromatographic procedures recently developed in this laboratory by Louise A. Orto (3).

RESULTS

Indigo red tests on morning urine specimens of adult male and female subjects on normal diets revealed no traceable correlation to smoking habits (Table I).

(Insert Table I)

In a subsequent assay, indigo red measurements were made on 24-hour urine samples collected on the control day and on the following day when 1 gm. of DL-tryptophan was given by mouth. It is apparent from the indigo red readings obtained (Table II, Column b-a) that prior or present cigarette smoking was associated with an increased quantitative production or excretion of indigo red. It appears from these

(Insert Table II)

results that the indigo red test on 24-hour urine collections may prove useful in assessing the relative effects of smoking on the metabolism of tryptophan.

In the second phase of this study, we concerned ourselves with a chromatographic quantitation of the metabolites of L-tryptophan and

L-phenylalanine. The reproducibility and accuracy of the procedure are indicated by the data collected in Table III.

(Insert Table III)

Increases in the indolepropionic acid area with administration of the amino acids were greater in the smokers, M.P. and R.W. than in M.H., a non-smoker, and B.J., the heavy smoker who stopped some 3 years ago. These data are collected in Table IV.

(Insert Table IV)

SUMMARY:

Considerable time was spent in developing and validating the methods and procedures employed in this study. Preliminary results of Phase I suggest that the indigo red test of 24-hour urine collections may prove useful in screening metabolic abnormalities which are associated with smoking. It seems possible that this test may be helpful in detecting individuals who are hypersensitive to the products of tobacco smoke. The data obtained in Phase II encourages a continuation of our line of attack on the metabolic effects of smoking.

The need and desirability for extending these investigations is apparent.

REFERENCES

1. Albanese, A. A.: Confidential Report on the Effects of Nicotine on Protein and Amino Acid Metabolism in Humans. 1962-1965.
2. Albanese, A. A., and Frankston, J. E.: A Difference in the Metabolism of L- and DL-tryptophan in the Human. J. Biol. Chem. 155: 101-108 (1944).
3. Albanese, A. A., and Orto, L. A.: Unpublished Data.

TABLE I

INDIGO RED TESTS ON FRACTIONAL URINE COLLECTIONS*

SUBJECTS **	SEX	SMOKING STATUS	INDIGO RED TESTS	
			QUALITATIVE	QUANTITATIVE
				KS READING
1	♀	NS	0	986
2	♀	NS	0	98
3	♂	NS	0	22
4	♀	NS	0	569
5	♂	NS	0	338
6	♂	NS	0	148
7	♂	NS	0	235
8	♀	NS	0	704
9	♀	SMOKER - QUIT	0	74
10	♂	SMOKER - 1/2 PK/DAY	0	290
11	♀	SMOKER - 1/2 PK/DAY	0	616
12	♀	SMOKER - 1 PK/DAY	0	527
13	♂	SMOKER - 1 1/2 PK/DAY	0	125

* MORNING SPECIMENS

** AGE RANGE: 18-84 YEARS

D.S.

TABLE II

INDIGO RED TESTS ON 24 HOUR URINE COLLECTIONS

SUBJECTS #	SEX	SMOKING STATUS	CONTROL DAY (a) INDIGO RED TESTS		TRYPTOPHAN LOAD DAY (b) INDIGO RED TESTS		
			QUALITATIVE	QUANTITATIVE	QUALITATIVE	QUANTITATIVE	(b-a)
				KS READINGS		KS READINGS	
A	♂	NS	0	204	0	224	20
B	♀	NS	0	265	+	337	72
C	♀	NS	0	94	+	174	80
D	♀	NS	0	818	+	539	-279
E	♀	NS	0	587	+	258	-329
F	♂	HEAVY SM. - QUIT	0	577	0	928	351
G	♂	HEAVY SM. - QUIT	0	468	+	10460	9992
H	♀	SMOKER - 1/2 PK/DAY	0	549	+	9115	8566
I	♂	SMOKER - 1 PK/DAY	0	136	+	640	504
J	♂	SMOKER - 1/2 PK/DAY	0	149	0	1179	1030

* ONE GRAM DL-TRYPTOPHAN ADMINISTERED 12 HOURS BEFORE COLLECTIONS.
 ** AGE RANGE: 24-84 YEARS.

TABLE III

SAMPLE	AMT.	Rf					PEAK HEIGHT				
		1	2	3	4	Average	1	2	3	4	Average
INDOLEACETIC ACID	3.0	43	43	47	45	45	4.4	3.7	3.6	5.0	4.2
INDOLEPROPIONIC ACID	3.0	26	27	27	27	27	5.3	5.0	6.7	5.8	5.7
TRYPTOPHAN	3.0	41	46	44	44	44	4.0	3.5	2.9	3.0	3.4

SAMPLE	PEAK HGT.
INDOLEACETIC ACID	cm / 4
INDOLEPROPIONIC ACID	1.9
TRYPTOPHAN	1.1

TABLE IV

NAME, DIAGNOSIS	AGE	SEX	SMOKING STATUS	TEST PERIOD	AROMATIC AMINO ACID DATA		
					RF	PEAK HT.	Δ
M. P. SYMPLECTOMY	54	♂	S 1/2 pack/day	CONTROL	31	2.4	
				" + TRYPT. MARKER	45	3.0	
				" + BALAN. MARKER	33	2.0	
				A.A.T.*	34	3.0	+25
				" + TRYPT. MARKER	47	3.5	
				" + BALAN. MARKER	42	1.9	
				A.A.P.**	34	2.1	+12
				" + TRYPT. MARKER	43	2.5	
" + BALAN. MARKER	36	1.3					
R. W. PEPTIC ULCER	52	♂	S 1/2 pack/day	CONTROL	29	2.3	
				" + TRYPT. MARKER	36	2.2	
				" + BALAN. MARKER	20	1.4	
				A.A.T.	33	3.3	+50
				" + TRYPT. MARKER	38	4.0	
				" + BALAN. MARKER	36	3.7	
				A.A.P.	33	3.8	+73
				" + TRYPT. MARKER	33	2.7	
" + BALAN. MARKER	28	2.6					
B. J. POST PNEUMONIA	58	♂	HEAVY SMOKER 6 PKGS/DAY QUIT 3 YRS AGO	CONTROL	37	5.0	
				" + TRYPT. MARKER	33	6.4	
				" + BALAN. MARKER	35	5.0	
				A.A.T.	37	4.8	+4
				" + TRYPT. MARKER	36	6.3	
				" + BALAN. MARKER	38	5.9	
				A.A.P.	37	5.4	+8
				" + TRYPT. MARKER	35	6.8	
" + BALAN. MARKER	36	5.8					
M. H. HERNIORRHAPHY	77	♂	NS	CONTROL	32	3.3	
				" + TRYPT. MARKER	33	3.7	
				" + BALAN. MARKER	34	3.9	
				A.A.T.	35	2.9	-12
				" + TRYPT. MARKER	35	3.2	
				" + BALAN. MARKER	32	4.2	
				A.A.P.	34	3.9	+18
				" + TRYPT. MARKER	33	4.1	
" + BALAN. MARKER	37	3.5					

* A.A.T. = 1GM. L-TRYPTOPHAN LOAD FED ; ** A.A.P. = 1GM. L-BALANINE LOAD FED