

STUDIES IN TOBACCO HYPERSENSITIVITY

III. REACTIONS TO SKIN TESTS AND PERIPHERAL VASCULAR RESPONSES

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THE problem of tobacco hypersensitivity has been the subject of several controversial reports. The investigations of Harkavy, Hebal, and Silbert¹ and of Sulzberger² were the first to indicate the possible allergic activity of tobacco as a cause of certain vascular diseases, especially thromboangiitis obliterans. On the other hand, reports by Trasoff and associates,³ Chobot,⁴ and Westcott and Wright⁵ failed to substantiate the conclusion that the skin reaction produced by tobacco extract is a specific response designating a sensitization process of the blood vessels.

The contradictory evidence concerning the role of allergy to tobacco as a cause of vascular disease is succinctly summarized by Lowell in a recent work edited by Wynder.⁶ An excellent and complete review on the subject of the immunologic aspects of tobacco and smoking has been published by Silvette, Larson, and Haag.⁷ The latter authors include in their summary the statement: "A well designed and properly controlled program of clinical investigation of tobacco sensitivity in relation to disease is greatly to be desired."

The divergent views and findings in this field prompted us to investigate further the possibility that certain tobacco effects are based on a specific allergic susceptibility of particular persons rather than on obligatorily toxic products in tobacco smoke. While the observations of Sulzberger, Harkavy, and others have verified the immunologic specificity of the skin reaction to tobacco, the clinical significance of these reactions is not always obvious. That serious difficulties are encountered in the interpretation of skin tests must be admitted and understood. The factors upon which success depends are the reliability of the extracts used, the method of testing, the evaluation of the reactions in a single person, and their correlation with the patient's clinical history. These precautions have been considered throughout this investigation. The extracts used in this study have been prepared by laboratory workers experienced in the field. Care was taken in the use of these extracts and in the performance and

This work was supported by grants from the Tobacco Industry Research Committee.

Presented at the fourteenth annual meeting of the American Academy of Allergy in Philadelphia, Pennsylvania, Feb. 3-5, 1958.

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Received for publication Feb. 10, 1958.

reading of the skin tests in order to eliminate or reduce to a minimum the possibility of nonspecific false positive reactions and other results of experimental errors.

MATERIALS AND METHODS

This report includes the skin reactions and historical data on 641 healthy adult volunteers and 294 children tested with tobacco extracts at the Judson Health Center in New York City. The volunteers were skin tested intracutaneously with the five different tobacco extracts. Approximately 0.02 c.c. of the excitant was injected intradermally, and the site was observed for at least twenty minutes to note the result of the test. The skin-test results were interpreted by the standards proposed by Cooke.*

In order to eliminate questionable or irritant reactions, only the moderately and markedly positive reactions were considered positive for the purposes of this study. Permanent tracings were obtained on all marked positive skin tests. Normal saline was used as a control.

The tobacco extracts were prepared by Mrs. Margaret B. Strauss, Director, Allergy Laboratory, University Hospital, New York City. Burley, Virginia, and Turkish cured tobaccos were procured from a reputable tobacconist who could assure their purity of type for the preparation of these individual tobaccos. Mixed tobacco extracts were composed of the following popular brands of cigarettes in equal parts (including the tobacco and the cigarette paper): Camel, Chesterfield, Lucky Strike, Old Gold, Pall Mall, and Philip Morris. No filter cigarettes were used.

The usual routine in preparing an extract of allergen is to treat the crude mass with an organic solvent, such as acetone, ether, toluene, or Sovasol in order to remove as much oil, fat, and coloring matter as possible before the aqueous extraction. In general, the oil and dye contain no antigenic fraction important to the inhalant type of allergies and their removal produces a superior type of extract because it is clearer and less colored. However, cigarette smoke contains certain volatile esters, aldehydes, ketones, and other organic substances, parts of which are undoubtedly soluble in organic solvents; these organic substances, which might act as allergens, would be removed if the tobacco mass were first defatted by an organic solvent.

Therefore, two types of mixed tobacco extracts were prepared. One was defatted by washing and decanting with toluene before the aqueous extraction, and the other was treated with no organic solvent before the aqueous extraction. The tobacco mass was then extracted in buffered saline (pH 7.0) for two days. After filtration, the extract was dialyzed against buffered saline for forty-eight hours and then concentrated by allowing the extract, in cellophane sausage casing, to hang in front of an electric fan. After Seitz filtration and sterility tests, each extract was standardized on a protein nitrogen basis according to the method of Stull and Cooke. The tobacco extracts used for testing were in 1,000 units phosphotungstic-acid-precipitable nitrogen strength.

*Original wheal—negative reaction—0.2 to 0.3 cm. wheal. Slight reaction—0.4 to 0.6 cm. wheal. Moderate reaction—1 to 1.5 cm. wheal. Marked reaction—2 plus cm. with pseudopodia.

Before skin testing, each subject was thoroughly interrogated as to any personal and familial history of allergy and was questioned specifically about peripheral vascular symptoms and pertinent smoking habits. Each subject was carefully questioned as to the following specific points:

On Allergy—Personal or familial occurrence of seasonal coryza, bronchial asthma, urticaria, food sensitivity, and drug sensitivity.

On Peripheral Vascular Disease—Evidence of numbness of extremities and fainting spells.

On Symptoms Directly Related in Time to Tobacco Smoking.—Coughing, dizziness, headaches, effect on appetite, and cold, numbness, and tingling of the extremities.

Eighty healthy adults who had been skin tested with tobacco extract were studied in the New York University Research Service at Goldwater Memorial Hospital. The vascular responses of these persons were tested by automatic recording of surface temperature and by large-limb venous occlusion plethysmograph in a room with constant temperature and humidity under environmental conditions set at 20° and 25° C. with 55 per cent humidity. Measurements were taken before the subjects smoked a cigarette, and at 2-, 6-, and 20-minute intervals following the onset of the smoking procedure. Changes in blood flow greater than 2 c.c. per 100 ml. of tissue per minute and changes in surface temperature of at least 2° C. were considered significant. Ballistocardiograms and electrocardiograms were also obtained on these volunteers, and they were interpreted by the standard criteria. The cardiovascular measurements were recorded before, during, and after the smoking of special cigarettes composed of a mixture of tobaccos from all the above-mentioned commercial cigarettes.

RESULTS

Cutaneous Reactions to Tobacco Extracts.—Six hundred forty-one healthy adult volunteers, both smokers and nonsmokers, were skin tested with five different tobacco extracts. Table I shows the number and percentage of cutaneous reactions, ranging from negative to marked, to Burley, Virginia, Turkish, mixed (not defatted), and mixed tobaccos in a group of 641 adults. Of the persons

TABLE I. PERCENTAGE OF VOLUNTEERS TESTED BY REACTIONS TO EACH OF FIVE TOBACCO EXTRACTS BY SMOKING HISTORY

EXTRACT	SMOKERS					NONSMOKERS				
	TOTAL	NEGA-TIVE	SLIGHT	MOD-ERATE	MARKED	TOTAL	NEGA-TIVE	SLIGHT	MOD-ERATE	MARKED
Burley	379	71.5	15.3	5.0	8.2	261	73.6	15.7	5.4	5.4
Virginia	378	70.4	14.3	6.1	9.3	261	73.6	14.6	3.8	8.0
Turkish	379	71.0	15.6	4.0	9.5	261	75.5	12.3	3.8	8.4
Mixed	341	68.3	12.6	5.3	13.8	234	73.5	14.5	2.1	9.8
Mixed (de-fatted)	340	70.3	13.5	7.4	8.8	232	73.7	13.8	5.6	6.9
Total*	380	63.2	12.4	6.1	18.4	261	65.5	15.3	5.4	13.8

*Each person classified by most severe reaction to any of five extracts. No statistical difference between smokers and nonsmokers.

tested, 18.4 per cent of the smokers showed markedly positive reactions to one or more of the tobacco extracts, as compared with 13.8 per cent of the nonsmokers. The difference between the percentages of smokers and nonsmokers reacting to each extract was not found to be statistically significant. Table I also indicates that there were more reactions to the mixed brand of tobacco extract that was not defatted. The defatting process would remove an appreciable quantity of esters, aldehydes, and other organic compounds ordinarily thought to be volatilized in the smoke of the cigarette. This mixed extract also contains, besides tobacco, unrelated allergens and irritating chemicals. It was noted that in the positively reacting group, some persons reacted to only one of the extracts, some to two, some to three, some to four, and some to all five extracts. By the skin-test criterion, a person who was skin sensitive to tobacco could therefore be sensitive to only one tobacco and the tobacco product of one provenance and not be sensitive to any other type.

No attempt was made to classify the smokers as occasional, moderate, or heavy smokers, as it was found that such classification would be impossible or would more likely be misleading than instructive. Many of the subjects smoked different amounts at different times, some ranging from one or two cigarettes daily during certain periods of their lives to a pack or more at other periods. If it had been possible for us to make an accurate quantitative breakdown of smoking habits, this might have been informative. We do not consider it essential, however, since the incidence of positive skin tests was not significantly greater in the entire group of smokers as compared with entire group of nonsmokers.

Sex and age did not seem to play any role in the incidence of the reactions to tobacco in the various groups tested.

Relationship of Peripheral Vascular Symptoms to Cutaneous Tobacco Reactions.—Table II sets forth the percentage of general vascular symptoms in a group of 377 smokers tested with Virginia tobacco extract. It can be seen from these statistics that peripheral vascular symptoms were approximately twice as

TABLE II. PERCENTAGE OF VOLUNTEERS WITH HISTORY OF PERIPHERAL VASCULAR SYMPTOMS AND REACTIONS TO THE VIRGINIA TOBACCO EXTRACT

TOTAL NUMBER	SKIN TEST	NUMBNESS OF EXTREMITIES (PER CENT)	MUSCULAR CRAMPS OF EXTREMITIES (PER CENT)	COLD EX- TREMITIES (PER CENT)	TINGLING OF EXTREMITIES (PER CENT)
Smokers (377)	Positive (57)	28	26	30	30
	Negative (320)	14	11	13	15
Nonsmokers (260)	Positive (31)	16	19	16	10
	Negative (229)	14	13	13	11

A significant association was found between smokers with positive skin reactions to tobacco and:

Numbness of the extremities— $\chi^2 = 6.0$; $n = 1$; $P = <0.02$.

Cold extremities— $\chi^2 = 8.6$; $n = 1$; $P = <0.01$.

Muscular cramps of the extremities— $\chi^2 = 11$; $n = 1$; $P = <0.01$.

Tingling of the extremities— $\chi^2 = 6.6$; $n = 1$; $P = <0.01$.

The above results tend to show that among the nonsmokers there appeared to be no association of any of the four symptoms with the positive skin tests to tobacco.

common in the smokers with positive skin tests than in the smokers with negative skin reactions. In the nonsmokers, the incidence of peripheral vascular symptoms was about the same, whether the skin-test reaction to tobacco was positive or negative.

Relationship of the Allergic State to Cutaneous Tobacco Reactions.—Table III lists the percentage of volunteers with manifestations of personal allergy and the reactions to the Virginia tobacco extract. This table shows the increased incidence of positive skin reactions to tobacco among the allergic persons, both smokers and nonsmokers. Over twice as many persons with positive reactions reported having seasonal rhinitis, bronchial asthma, or urticaria as compared with the subjects with negative skin reactions. In the group of smokers reporting a history of allergic manifestations, 53.4 per cent were found to react in a positive manner to one or more of the tobacco extracts. In contrast, only 18.7 per cent of the normal nonallergic smokers reacted to the tobacco extract. Among the nonsmokers, 40.9 per cent of the allergic persons were found to give positive reactions, and 14.7 per cent of the normal nonsmokers reacted positively to the tobacco extract. This finding would seem to indicate that the reaction to the tobacco extract may be evidence of multiple sensitization characteristic of the allergic state. A skin sensitivity to tobacco could well be more readily acquired by the generally allergic person than by the generally less allergic one on intimate or casual contact with tobacco smoke and tobacco products.

TABLE III. PERCENTAGE OF VOLUNTEERS WITH MANIFESTATIONS OF PERSONAL ALLERGY AND REACTIONS TO THE VIRGINIA TOBACCO EXTRACT

TOTAL NUMBER	SKIN TEST	SEASONAL RHINITIS (PER CENT)	BRONCHIAL ASTHMA (PER CENT)	URTICARIA (PER CENT)	OTHERS* (PER CENT)
Smokers (377)	Positive (57)	7	18	26	37
	Negative (320)	4	4	5	18
Nonsmokers (260)	Positive (31)	19	19	16	13
	Negative (229)	7	4	7	16

*Food sensitivity, drug sensitivity, and rashes.

Among smokers there was no significant association between positive skin tests to tobacco and seasonal rhinitis.

Among smokers a significant association was found between positive skin tests to tobacco and:

Bronchial asthma— $\chi^2 = 12.9$; $P = <0.01$.

Urticaria— $\chi^2 = 26.2$; $P = <0.01$.

Among nonsmokers no significant association was noted between the positive skin test reaction to tobacco and the manifestations of personal allergy.

Symptoms Related to Cigarette Smoking and the Cutaneous Reactions to Virginia Tobacco Extract.—In Table IV it is noted that 24 per cent of the smokers with positive tests to tobacco gave evidence of coughing spells directly related to the act of smoking, as compared with 12 per cent of the smokers who had negative skin reactions. Symptoms of cold, numbness, and tingling of the extremities associated with smoking were reported by 8 per cent of the smokers with positive tests and by 2 per cent of those with negative reactions. There

was no apparent difference in incidence of dizziness, headache, and effect on appetite among the smoking group, whether they reacted in a positive or negative manner to the tobacco extract.

TABLE IV. PERCENTAGE OF SMOKERS WITH HISTORY OF TOBACCO SYMPTOMS AND REACTIONS TO THE VIRGINIA TOBACCO EXTRACT

TOTAL NUMBER	SKIN TEST	COUGHING (PER CENT)	DIZZINESS (PER CENT)	HEADACHES (PER CENT)	EFFECT ON APPETITE (PER CENT)	COLD, NUMBNESS, AND TINGLING OF EXTREMITIES (PER CENT)
57	Positive	24	10	6	8	8
320	Negative	12	10	2	6	2

A significant association was found between the positive skin test to tobacco and the incidence of:

Coughing when smoking— $\chi^2 = 5.2$; $P = < 0.02$.

Cold, numbness, and tingling of the extremities— $\chi^2 = 5.5$; $P = < 0.02$.

Relationship of Allergy, Peripheral Vascular Symptoms, and Cutaneous Reactions to the Tobacco Extracts.—Table V is presented in an attempt to correlate allergic manifestations, peripheral vascular symptoms, and the skin reactions to tobacco extract. It was found that 33 per cent of ninety-six smokers with positive skin tests to tobacco gave a history of allergic manifestations and reported having symptoms of peripheral vascular disturbances. Only 10 per cent of 291 smokers with negative skin tests gave evidence of both allergy and peripheral vascular symptoms. Among the nonsmokers, whether they gave positive or negative reactions, no significant difference in incidence of allergy and vascular symptoms was noted.

TABLE V. NUMBER OF SMOKERS WITH PERSONAL ALLERGY AND PERIPHERAL VASCULAR SYMPTOMS AND SKIN REACTIONS TO TOBACCO EXTRACTS

	POSITIVE SKIN TEST	NEGATIVE SKIN TEST	TOTAL NUMBER
Positive allergy, positive peripheral vascular symptoms	32 (33%)	30 (10%)	62
Positive allergy, negative peripheral vascular symptoms	17 (18%)	71 (25%)	88
Negative allergy, positive peripheral vascular symptoms	13 (14%)	35 (12%)	48
Negative allergy, negative peripheral vascular symptoms	34 (35%)	155 (53%)	189
Total number	96	291	387

$\chi^2 = 30.0$; $n = 3$; $P = < 0.001$.

Vascular Responses to Smoking and Cutaneous Reactions to Tobacco Extracts.—Tables VI and VII list the skin temperature and blood flow changes in eighty healthy adults, with their skin-test reactions to tobacco extracts. From this study, it was learned that blood flow and skin temperature changes appear to be independent, since few persons gave reactions to both of these vascular responses. With respect to changes in blood flow, no significant difference was found between those giving positive and those giving negative skin tests to tobacco extracts. On the other hand, skin temperature changes

were noted in 28 per cent of the smokers giving positive skin-test reactions and in only 4 per cent of those giving negative reactions. The difference is statistically significant.

TABLE VI. CHANGES IN SKIN TEMPERATURE AND BLOOD FLOW ON EIGHTY SMOKERS AND SKIN REACTIONS TO TOBACCO EXTRACTS

	BURLEY		VIRGINIA		TURKISH		MIXED		MIXED DEFATTED	
	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE
T+ B+	1		1		1		1		1	
T+ B-	6	4	5	5	5	5	4	6	5	5
T- B+	9	11	7	13	7	13	9	11	7	13
T- B-	8	41	9	40	10	39	12	37	8	41
Total	24	56	22	58	23	57	26	54	21	59

T = Skin temperature.
B = Blood flow.

+ = Positive.
- = Negative.

The electrocardiographic and ballistocardiographic recordings were not included in this discussion, since so few volunteers presented any significant changes and since skin tests with tobacco extracts did not appear to be correlated with the results obtained.

TABLE VII

	POSITIVE REACTORS	NEGATIVE REACTORS
T+ B+	1	0
T+ B-	8	2
T- B-	11	9
T- B+	12	37
Total	32	48

T = Skin temperature.
B = Blood flow.

+ = Positive.
- = Negative.

Positive reactors to the tobacco extracts are more likely to show changes in skin temperature after smoking. $\times 2 = 2.6$; $n = 1$; $P = < 0.01$.

Cutaneous Reactions to Tobacco Among Allergic and Nonallergic Children.—

A total of 294 children between the ages of 1 and 5 years were skin tested with the five tobacco extracts. Of this total, 11.5 per cent were found to react in a markedly positive manner to one or more of the tobacco extracts. Thorough questioning of the parents of 269 of the children revealed no evidence of any allergic manifestations. Therefore, these children were considered normal for purposes of this study. Of these normal children, 6 per cent reacted positively to tobacco on skin testing. Twenty-five of the children were found to be allergic and, of these, 64 per cent gave evidence of skin sensitivity to the tobacco extract on skin testing. None of these children had ever smoked, but they had had the same exposure to tobacco smoke and other tobacco products as other nonsmokers. Practically all of these children were exposed to one or more smokers within the immediate family at home. Here again, as in the adult group, it would seem that the generally allergic disposition of the person renders him very receptive to the development of multiple skin sensitization, including

sensitization to tobacco, on casual or intimate exposure to the allergen. There was no difference in the incidence of positive skin reactions to tobacco in the various age groups. Female subjects reacted in the same proportions as male subjects.

SUMMARY AND INFERENCES

A group of 641 healthy volunteers were questioned concerning personal allergies, smoking history, symptoms presumed to be connected with smoking, and peripheral vascular disturbances.

These persons were skin tested by the intradermal method with five different tobacco extracts: Burley, Virginia, Turkish, and mixed tobaccos. The mixed tobacco extract was made by combining tobaccos from six popular cigarettes.

It was found that about 15 per cent of 641 adults reacted in a positive manner to one or more of the tobacco extracts. The per cent reacting was essentially the same in the smokers as in the nonsmokers.

It might have been argued that the heavier smokers, by exposing themselves to more tobacco smoke, became skin reactive to tobacco extracts more frequently. This argument scarcely appears valid, however, in view of our finding that smokers did not react to skin tests with tobacco extracts significantly more often than nonsmokers.

An increased incidence of peripheral vascular symptoms was noted in the smokers with positive reactions to the tobacco, as compared with the smokers who did not react on skin testing with the tobacco.

It might also be argued that excessive smoking could lead to increased incidence of skin-test reactions to tobacco and that the amount of smoking might be an indication of the degree of the patient's anxiety and tension.

In view of the absence of any difference between smokers and nonsmokers in incidence of skin reactions to tobacco, however, it appears extremely unlikely that the degree of anxiety and tension could be a factor in producing skin-test reactions. Moreover, in similar fashion, since the increased smoking could not be proved responsible for increased skin reactions to tobacco extracts, increased anxiety and nervousness could not explain the increased incidence of peripheral vascular symptoms in the smokers with positive skin tests to tobacco as compared to those with negative skin tests.

Volunteers with positive tests to the tobacco extracts reported a history of nontobacco-related allergic manifestations more frequently than the group with negative reactions.

Of the smokers with positive skin reactions to tobacco extracts 33 per cent gave a personal history of allergic manifestations and peripheral vascular symptoms, as compared with 10 per cent of the smokers who did not react to the skin tests with tobacco extracts.

Eighty of these persons were investigated further for circulatory disturbances by means of plethysmographic measurements of blood flow to the lower extremities and automatic recording of surface temperature. These

tests were performed before, during, and after the smoking of a mixed-tobacco cigarette. These experiments indicated that, on smoking the mixed-tobacco cigarette, 23 per cent of the smokers with positive skin tests to tobacco had changes in peripheral circulation indicated by skin temperature as compared with the smokers with negative skin tests, of whom 4 per cent had changes in skin temperature.

In a group of 294 children between the ages of 1 and 5 years tested with the tobacco extracts, 11.5 per cent reacted in a positive manner to one or more of the tobacco extracts. In the small group of allergic children tested, 64 per cent gave evidence of skin sensitivity to the tobacco extracts as compared with 6 per cent of a nonallergic control group.

The close and consistent parallelism between the positive cutaneous reactions to tobacco extract and symptoms of peripheral vascular disturbances, tobacco symptoms, and personal history of allergic manifestations might possibly serve as a clue to the interpretation of the tobacco reactions that we obtained. The findings indicate that our positive skin-test reactions to tobacco extracts were based on an allergic (that is, immunologic) mechanism and were associated in more than normal incidence with hypersensitivity to other allergens as well as with fall of peripheral temperature on smoking and with certain clinical peripheral vascular symptoms. It is felt that, although the number of volunteers tested is small, there is a suggestion that the skin test with tobacco may be helpful as a "screening" test to aid in determining the possible importance of tobacco as an etiological factor in certain forms of peripheral vascular symptomatology.

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