

A STUDY OF THE CUTANEOUS REACTIONS TO OIDIOMYCIN, TRICHOPHYTIN, AND MUMPS SKIN TEST ANTIGENS IN PATIENTS WITH SARCOIDOSIS*

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A decrease in the reaction to intracutaneous injection of tuberculin or its purified protein derivative is generally accepted as a feature of the disease syndrome sarcoidosis. The phenomenon has aroused considerable interest, and many discussions of the various possible underlying mechanisms have appeared in the literature.^{9, 10, 11} A few detailed investigations have been conducted, but there is no information on the delayed reactions of individuals with this disease to commonly encountered antigens other than tuberculin. Reported here are the results of a study of this problem.

METHODS

The reactions to the intracutaneous injection of several antigens were studied in cases of sarcoidosis and in a group of control patients. First and second strength purified protein derivative was used to determine the reaction of each individual to tuberculin. Tests were made at the same time with three other unrelated antigens which produce tuberculin-like reactions: mumps virus, oidiomycin, and trichophytin. The mumps antigen was a preparation of chick embryo allantoic fluid, normal allantoic fluid serving as control test material.⁹ *Candida albicans* extract (oidiomycin) was used in a dilution of 1:100 and *Trichophyton gypseum* extract (trichophytin) was used in a dilution of 1:30.‡ The antigens were injected in 0.1 ml. doses and the reactions read after 45-50 hours. In reading the tests, the largest diameter of the area of edema was measured. Erythema is also an indication of a positive reaction, but this was not used because it is frequently obscured in dark-skinned individuals. At the time of the skin tests each individual also received a subcutaneous injection of 1.0 ml. of a pneumococcus vaccine.§ Serological studies on serum obtained at that time and 21 days later will be the subject of a later report.

Tests were carried out on 86 individuals, of whom 64 were controls and 22 were cases of sarcoidosis.§ This diagnosis was considered established if clinical, laboratory,

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‡ The mumps skin test antigen was furnished by the Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York, through the kindness of Dr. Victor Cabasso, Section of Viral and Rickettsial Research. The oidiomycin and trichophytin were also a product of Lederle Laboratories.

§ Pneumococcus polysaccharide vaccine A., E. R. Squibb & Sons, Inc., New Brunswick, New Jersey. This preparation contains polysaccharides of types 1, 2, 3, 5, 7, and 8.

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or roentgenological studies revealed evidence of a disseminated disease process, biopsy of one or more sites revealed characteristic epithelioid granulomata, and attempts to establish a specific etiologic diagnosis were not successful. Such studies included stain, culture, and animal inoculation of sputum, and stain for the tubercle bacillus on all biopsy material. The chief clinical features of the cases of sarcoidosis are summarized in Table 1.

TABLE 1
CLINICAL FEATURES OF THE CASES OF SARCOIDOSIS INCLUDED IN THIS STUDY*

	Case																					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Lung		x	x		x		x	x	x	x		x				x	x	x	x		x	x
Lymph nodes	x	x	x	x	x	x		x	x	x	x	x			x	x	x	x	x	x	x	x
Liver	x						x	x		x	x	x		x	x		x	x	x	x		x
Spleen				x				x				x		x	x			x				x
Skin		x						x	x	x												x
Iridocyclitis	x																	x				
Salivary gland											x			x								
Fever								x	x					x	x							
Arthralgia										x												x
Respiratory symptoms			x			x					x											x
Other	x							x	x					x				x	x	x		
Increased serum globulin	x	x	x	x			x	x	x		x	x		x	x	x		x	x			x
1st PPD	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.5	0	0	0	2.0	1.5
2d PPD	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.0	..	3.0	1.5	2.0	0	0

* The marks in boldfaced type indicate that biopsy from that particular site revealed the presence of characteristic epithelioid granulomata.

The control group included 29 individuals who presented no evidence of disease, and 35 suffering from a variety of chronic systemic diseases. Cachectic patients, and those acutely or severely ill, were excluded from the study because of the known decrease in skin reactivity in such states. There were at least as many female and Negro subjects in the control as in the experimental group, although the proportionate numbers were smaller in the former. The mean age of the controls was 33 years, and of the sarcoidosis patients 36. Of the controls, 17 per cent were younger than 25 years of age and 14 per cent were older than 44. Corresponding figures for the experimental group were 14 and 23 per cent, respectively. The tuberculin negative control and experimental groups resembled each other more closely in mean age and range of age distribution than did those in the groups that were tuberculin positive. In the control group there were no appreciable differences in diameter or frequency of positive reactions between whites and Negroes, between normal individuals and those with disease, or between those of different ages. However, there was a difference between males and females in the frequency of reactions to trichophytin. Seventy-two per cent of the males and 29 per cent of the females reacted to this antigen. Males and females did not differ, however,

in the mean diameter of positive trichophytin tests, nor did they differ in frequency or mean diameter of reactions to oidiomycin or the mumps antigen. Therefore, in analyses of data involving the frequency of reactions to trichophytin, males and females were considered separately. Statistical analysis was carried out whenever data showed apparent differences, using the t test when mean values were being compared, and the chi-square test when the data were concerned with the frequency of positive tests. Differences which were statistically significant are indicated in the tables by inclusion of the results of the analysis.*

TABLE 2
MEAN DIAMETER OF RESULTS WITH OIDIOMYCIN, TRICHOPHYTIN, AND MUMPS ANTIGEN
IN ALL CASES

	Number tested	Mean diameter of reactions				Difference and standard error*
		Oidiomycin	Trichophytin		Mumps	
			Males	Females		
Tuberculin negative	Sarcoidosis	0.49 cm. ①	0.32 cm. ③	0.00 cm.	0.24 cm. ④	① 1.79 ± 0.58
	15	②				② 1.74 ± 0.78
	Controls	2.28 cm. ①	1.00 cm. ③	0.14 cm.	1.28 cm. ④	③ 0.68 ± 0.33
	20					④ 1.04 ± 0.33
Tuberculin positive	Sarcoidosis	2.23 cm. ②	0.76 cm.	(1 case only)	0.24 cm. ⑤	⑤ 0.85 ± 0.39
	7					
	Controls	2.50 cm.	1.18 cm.	0.50 cm.	1.09 cm. ⑤	
	44					

* The encircled numbers relate the results of the statistical analyses to the corresponding values in the table.

Reactions to the normal allantoic fluid material used with the mumps test occurred in 12 of the individuals tested. In no instance was there a reaction to normal allantoic fluid in the absence of a reaction to that containing virus. Of those in whom the reactions to virus and control material were of nearly equal size, none had a history of mumps infection. Of five individuals in whom the diameter of the reaction to the virus was larger than 1.0 cm. and at least three times as large as that of the control, the history of mumps was positive in four. Because of these findings, and the experience of others that positive and negative tests can be distinguished even when the control gives a reaction,² these five reactions were accepted as specific positive tests. There were five reactions to the virus that were less than 0.5 cm. in diameter, and because the normal allantoic fluid gave no reaction, these were accepted as positive tests. With oidiomycin and trichophytin a total of three tests gave reactions of less than 0.5 cm. in diameter. Whether these are specific or non-specific reactions cannot be determined, but their inclusion does not alter the significance of the observed differences.

RESULTS

In Table 2 are shown the degree of reactivity to each of the three antigens in the tuberculin negative and positive sarcoidosis cases and

* The statistical analyses were carried out by Dr. M. H. Quenouille, Department of Biostatistics, Yale University.

corresponding controls. These values are expressed as the means of all individuals in each group, whether positive or negative.

The mean diameter of the oidiomycin tests in tuberculin negative sarcoidosis was significantly smaller than it was in either tuberculin negative controls or tuberculin positive sarcoidosis. In the male subjects the diameter of the trichophytin tests in tuberculin negative sarcoidosis was significantly smaller than the tuberculin negative controls. Trichophytin reactions in females with sarcoidosis and in the tuberculin positive males with sarcoidosis tended to be smaller than corresponding controls, but these differences were not significant. Reactions to the mumps antigen were significantly smaller in both tuberculin reactors and non-reactors with sarcoidosis than in the controls.

TABLE 3
THE MEAN DIAMETER OF TESTS IN CASES REACTING TO OIDIOMYCIN,
TRICHOPHYTIN, AND MUMPS

	Number tested	Mean diameter of reactions			Difference and standard error*
		Oidiomycin	Trichophytin	Mumps	
Tuberculin negative	Sarcoidosis 15	0.91 cm. ①	0.95 cm.	0.90 cm.	① 1.50 ± 0.72
	Controls 20	2.41 cm. ①	1.31 cm.	1.60 cm.	② 1.14 ± 0.51
Tuberculin positive	Sarcoidosis 7	2.60 cm.	1.06 cm.	0.57 cm. ②	
	Controls 44	2.69 cm.	1.55 cm.	1.71 cm. ②	

* See footnote, Table 2.

It was of interest to determine whether similar differences could be demonstrated using data from which the factor of frequency of positive tests had been eliminated. This was done by calculating the mean diameter of all positive tests (Table 3). The difference between tuberculin negative sarcoidosis and controls in the mean diameter of positive oidiomycin tests was statistically significant, and this was also true for reactions to mumps antigen in tuberculin positive sarcoidosis and controls.

The frequency of positive tests in the various groups is shown in Table 4. The same trends that have been evident in previous tabulations are apparent here. In respect to oidiomycin and mumps antigen, the differences between tuberculin negative sarcoidosis and tuberculin negative controls were significant.

It was of some interest to consider the reactions of individuals to all three antigens in addition to the separate results with each. The data presented in Table 5 show the number of individuals reacting to at least two of the three antigens, the number reacting to less than two, and the mean number of positive tests per person. In the control group there were few individuals, whether they reacted to tuberculin or not, who did not react to at least two of the other three antigens. In the experimental group, those who reacted to tuberculin usually reacted to two or more of the other materials, whereas those not reacting to tuberculin seldom reacted to more than one. This

TABLE 4
THE FREQUENCY OF REACTIONS TO OIDIOMYCIN, TRICHOPHYTIN, AND MUMPS ANTIGEN IN SARCOIDOSIS AND CONTROLS

<i>Number tested</i>		<i>Number of tests positive</i>								<i>P*</i>
		<i>Oidiomycin</i>		<i>Trichophytin</i>				<i>Mumps</i>		
				<i>Males</i>		<i>Females</i>				
		<i>No.</i>	<i>%</i>	<i>No.</i>	<i>%</i>	<i>No.</i>	<i>%</i>	<i>No.</i>	<i>%</i>	
Tuberculin negative	Sarcoidosis 15	8 ①	53	2/6	33	0/9	0	4 ②	27	① < 0.02 ② < 0.01
	Controls 20	19 ①	95	11/15	73	1/5	20	16 ②	80	
Tuberculin positive	Sarcoidosis 7	6	86	5/6	83	0/1	0	3	43	
	Controls 44	41	93	25/35	71	3/9	33	28	64	

* See footnote, Table 2.

difference was highly significant for the male but not for the female group. The mean number of positive tests per person was significantly smaller in the tuberculin negative sarcoidosis cases than in the tuberculin negative controls for both the male and female groups. Males with tuberculin negative sarcoidosis differed significantly from those who were tuberculin positive, but the latter group was similar to the two groups of controls.

DISCUSSION

The reactions to these antigens in patients with sarcoidosis were both quantitatively and qualitatively less than in the controls. This effect was most marked in cases of sarcoidosis with negative tuberculin reactions. Patients with sarcoidosis and positive tuberculin tests had reactions to these antigens which were intermediate between the tuberculin negative cases of sarcoidosis and the controls. It appears that the mechanism

responsible for this altered state of reactivity was most pronounced in the tuberculin negative cases.

Various explanations have been proposed for the decreased tuberculin reactivity in sarcoidosis. The most popular point of view has been the theory of so-called "positive tuberculin anergy" which assumes that the decreased reactivity is to tuberculin alone. This implies that the reaction of such individuals to other antigens to which they may have been previously exposed would be unaltered. The data that have been presented here indicate that the reaction to the other antigens is altered, and that the phenomenon is not specific for tuberculin. Proponents of the theory that the tubercle

TABLE 5
NUMBER OF POSITIVE TESTS PER PERSON IN SARCOIDOSIS AND CONTROLS

Number tested		Number positive						P*
		Males			Females			
		>1	<2	Mean	>1	<2	Mean	
Tuberculin negative	15 Sarcoidosis	1 ①	5 ①	0.83 ②	2	7	1.00 ④	① < 0.01
	20 Controls	14 ①	1 ①	2.43 ②	4	1	2.00 ④	② < 0.01
								③ < 0.05
Tuberculin positive	7 Sarcoidosis	5	1	2.17 ③	..	1	④ < 0.05
	44 Controls	30	5	2.26	6	3	2.00	

* See footnote, Table 2.

bacillus is the etiologic agent of sarcoidosis have advanced this "specific tuberculin anergy" as an argument in support of their view.^{8,11} The decreased tuberculin reactivity has also been cited by those opposed to this theory.^{6,9} The evidence presented here indicates that the phenomenon carries no implication in either direction.

Recently it has been shown that in the United States most individuals with sarcoidosis are of southern rural origin, and the suggestion has been made that the decreased incidence of tuberculin sensitivity may be due to a decreased exposure to tuberculosis.⁷ If this were true, reactions to the mumps antigen and perhaps also trichophytin might be similarly affected. Although there is no evidence to suggest that infection with *Candida albicans* is less frequent in rural areas, there was a lower frequency of reactions to this antigen among the tuberculin negative members of the experimental group than among the controls. Many of the cases studied here did come originally from rural areas, but most of them had lived in urban areas

for many years, and it seems probable that the effect of rural isolation would have been minimized. Several of the individuals who did not react to any antigens had always lived in urban surroundings. For these reasons it does not seem likely that the differences in frequency of positive tests can be attributed to this factor. Certainly the smaller mean diameter of positive reactions in the sarcoidosis cases cannot be explained on epidemiological grounds.

The results of these tests seem to fit only with the concept of a mechanism which would suppress delayed cutaneous reactions to all antigens. There is actually little to indicate that the abnormality is primarily concerned with immunological reactions rather than the reactivity of tissue to a variety of stimuli. This problem is now under study.⁴

These results also have some practical importance. The tests may be useful in determining the ability of an individual to react, particularly in cases of suspected sarcoidosis. It is apparent that cutaneous sensitivity reactions of the delayed type should not be expected to give reliable information in specific etiologic studies in sarcoidosis.

SUMMARY AND CONCLUSIONS

In a study of the reactions of patients with sarcoidosis to mumps virus and extracts of *Candida albicans* and *Trichophyton gypseum*, it was found that there was a generalized depression of reactivity as compared with a group of controls. This was greatest in those with negative tuberculin reactions. The implications of these findings in regard to the various etiologic and immunologic theories of the disease are discussed. Certain practical applications of such tests are mentioned.

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