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A Serological Reaction Associated with Sarcoidosis.* (26614)

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At present the diagnosis of sarcoidosis is based on clinical grounds, supported by either a compatible histological pattern or a positive Kveim test(1). Since many other agents are known to produce the so-called "hard tubercle"(2) and since a potent and reliable Kveim antigen is difficult to obtain, final diagnosis is often accomplished by exclusion.

When potent Kveim antigen is available positive reactions are obtained in about 75% of cases(3). If another positive test possessing a fair degree of consistency can be found, the uncertainties resulting from diagnosis by exclusion may be somewhat reduced. Since the reported distribution of non-photochromogenic anonymous mycobacteria resembles somewhat the distribution of sarcoidosis as described in Veterans Administration studies, it seemed worth while to study sera of patients with sarcoidosis against antigens of various types of anonymous mycobacteria. If antibodies should be found they might also suggest clues as to the etiology of sarcoidosis.

The following studies were undertaken to see if serum of patients with sarcoidosis possessed antibodies detectable by diffusion against any of a number of mycobacterial and fungal antigens. Diffusion in agar was chosen as a method since Parlett and Youmans(4) have found the technic useful in mycobacterial study, while Heiner(5) has used diffusion in the study of histoplasmosis, and experience in this laboratory indicates its usefulness in other fungus infections.

Materials and methods. Sera from 32 patients with sarcodosis as determined by usual clinical criteria supported by Kveim test, biopsy or both were subjected to double diffusion in agar gel. These sera were distributed geographically as follows: Virginia 8, Wisconsin 5, Oklahoma 5, Louisiana 7, and Texas 7.

Diffusion was carried out in Petri dishes, using wells and distances as described by Crowle for slide technic(6). A 1% solution of agar was poured in the dish and allowed to harden. A second pour of 1½% ordinary bacto-agar in physiological saline was then layered on the first pour, and as it first began to harden a tooled die was placed on the surface, forming wells of a constant size and a fixed distance.

The central well was filled with undiluted serum and the peripheral wells with various antigens. In a few instances when the first

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diffusion was negative a second diffusion was carried out with the serum diluted 1:1 and 1:2 with 0.7% NaCl. All sera were studied against the antigens described below.

Mycobacterial antigens were prepared by inoculating Sauton's liquid media from fresh subcultures of strains P-1 and P-8 of Group I; P-6 and P-15 of Group II; and P-2 and P-17 of Group III. The original subcultures of these strains were prepared by Dr. Ernest W. Runyon from his strain collection and were members of the "packs" Runyon circulated for study and identification by numerous laboratories. The further technic of preparation of antigens has been described elsewhere. After the Old Tuberculin-like antigens had been concentrated by flash evaporation, the lipids were extracted with ether and discarded, and the remaining material used as antigen.

Old Tuberculin derived from H37Rv was obtained from the Texas State Dept. of Health and was extracted with ether in the same way.

Blastomyces antigens were prepared in this laboratory from an organism isolated from lung and pleura of a local patient. Antigen for *Coccidioides immitis* was furnished by Dr. C. E. Smith, Univ. of California, and is the material employed by him in complement-fixation studies. Histoplasma antigens were furnished by Furcolow and also a commercial product from Eli Lilly & Co. Antigens for Cryptococcus were produced in this laboratory from a Sabouraud broth culture of organisms isolated from a resected lesion of the lung.

When the wells were filled, the Petri dishes were covered and placed in the desiccator for 1 hour, then they were placed in an incubator at 37° for 24 hours in the case of the fungus diffusion, and 48 hours for mycobacterial diffusion. Upon removal of Petri dishes the results were read with the aid of oblique light and a hand lens when necessary (Fig. 1).

Results. Twenty-eight of 32 sera produced zones of precipitation against antigens of anonymous mycobacteria (Charts I, II). All of these diffusions resulted in 2 bands, while a few sera produced 3 or 4. The greatest number of reactions, as well as the most numerous bands, occurred against Group II antigens, while lesser numbers of reactions occurred against Groups I and III. Chart II, which presents reactions by combinations, shows that many sera produced precipitation against antigens of more than one group. The serum of a patient in Virginia produced the only reaction against any of the fungal antigens, and only 2 sera reacted with H37Rv antigen.

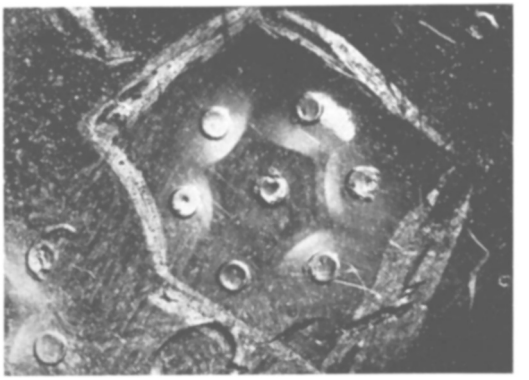
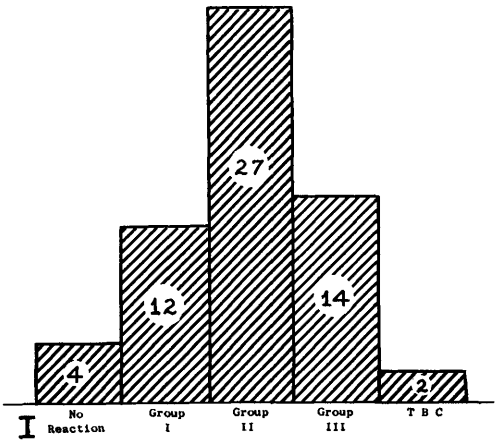


FIG. 1. Agar diffusion pattern obtained with serum of sarcoidosis patient. Antigens clockwise from top:
Photochromogen (P-1, P-8)
Scotochromogen (P-6, P-15)
Non-chromogen (P-2, P-17)
H37Rv antigen
P-6 alone
P-1 "

Three local patients are of particular interest in that sera obtained from each of these at the time they originally presented for diagnosis were uniformly negative. As these patients were followed and roentgenographic improvement was obvious the sera of all 3 produced multiple bands in from 5 to 9 months after the original study.

Discussion. Group II anonymous mycobacteria, which are regarded as the least pathogenic for man, elicited the most numerous and extensive reactions. In general the distribution of antibody reactions to antigens of Groups I and III corresponds to the reported geographic predominance of the groups. However, multiple antigen reactions may with equal probability represent identity of antigen components, or separate mycobacterial experiences.

Control sera have been obtained from medical students in the first 2 years, from a



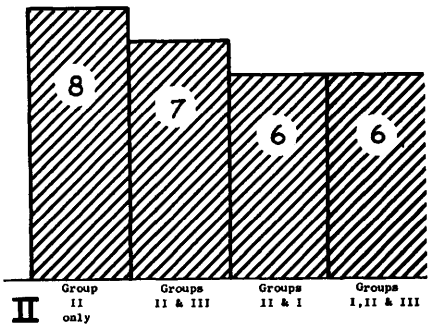


CHART I. Number of sera reacting to mycobacterial antigens according to group (Runyon system).

CHART II. Number of sera showing reactions against antigens of Group II alone, and against antigens of Group II and one or more other groups.

series of ambulant allergy patients, and from a series of patients in a tuberculosis hospital who first were found to have human tubercle organisms in the sputum and later produced various strains of anonymous mycobacteria. Table I presents the results of diffusion studies against the same antigens as used in the cases with sarcoidosis. From 49 allergy patients 5 sera produced reactions with the antigens of mycobacteria but only one of these produced more than a single band. From 111 medical students 28 sera formed bands against the antigens, but again only 4 sera resulted in more than one band, while 15 of the 28 positive sera produced reactions against O.T. as compared with 2 of the 32 sarcoidosis patients.

TABLE I. Diffusion Reactions in Sera from Ambulant, Non-hospitalized Individuals.

	No.	I	II	III	O.T.
Allergic patients	49	1	3	2*	10
Medical students	111	12	12	6†	37

* 1 patient reacted with a single band each to Group I and III.

† 2 sera reacted to more than one group antigen.

Roman numerals in this table refer to anonymous mycobacterial groups. Arabic numerals refer to No. of sera presenting reactions. More than a single band against one of the group antigens was encountered once among the allergy patients and 4 times among medical students.

The sera of 23 patients in a tuberculosis hospital were subjected to the same diffusion studies. These patients had all had sputum cultures positive for human strains of *M. tuberculosis,* but had subsequently had positive cultures for certain of the anonymous mycobacteria. All of these formed zones of precipitation (one, as many as 5) against antigens of H37Rv. Against anonymous antigens, 15 sera produced bands, inconsistent single zones only in the case of the individuals producing Group I or Group III organisms, but more consistent reactions in the case of Group II.

The distribution of reactions among patients with sarcoidosis is different from that of the 3 control groups, mostly nearly resembling the reactions of individuals who produce Group II organisms. Three possibilities can be considered in relation to the presence of mycobacterial antibodies in sera of patients with sarcoidosis: 1) The sarcoidosis patient, as a result of severe stimulus to the reticuloendothelial system, releases a great many antibodies not directly related to the agent. The results of immunization experiments in sarcoidosis patients and the absence of fungal antibodies in the group under study make this explanation unlikely. 2) The antibodies against anonymous mycobacteria may represent cross-reacting antibodies against an agent not represented in the diffusions. 3) The demonstrated antibodies may be specific. In this case their demonstration is of some diagnostic value, if this work can be confirmed, and may have etiological implications.

Conclusions. 1. A serological reaction has been obtained between sera of patients with sarcoidosis and antigens from anonymous

mycobacteria. using agar diffusion technic. 2. The reaction has occurred in sera of 28 of 32 patients, 3 of which changed from negative to positive as lesions improved. 3. The data permit no conclusion as to specificity of this reaction, but suggest its value as an additional diagnostic method in sarcoidosis.

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Chemical Interaction of S^{35}-6-Mercaptopurine and Ribonucleic Acids.* (26615)

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The question as to whether S^{35}-labelled 6-mercaptopurine. (S^{35}-6 MP) is incorporated into nucleic acids has not been settled. Elion *et al.*, injected mice with S^{35}-6 MP and within 4 hours found the label associated with RNA and DNA isolated from visceral organs(1). Brockman has recently questioned this observation as indicating incorporation of 6-mercaptopurine and states that one would not expect 6-MP to be incorporated into nucleic acid because it is an analog of hypoxanthine and not an analog of adenine or guanine(2). 6-MP, however, is converted to thioxanthine and thiouric acid by mouse tissue and by bacteria(2). It is possible that small amounts of a given dose of 6-MP administered to rats might be converted to thioguanine. Lepage has reported that thioguanine is incorporated into nucleic acid(3). In view of these considerations it was thought worthwhile to examine the nature of incorporated S^{35}-6 MP in rat liver RNA.

In vivo incorporation of S^{35}-6 MP into rat liver RNA was accomplished as follows: Three groups (3 rats per group) of adult white male rats were injected intraperitoneally with 2 mg S^{35}-6 MP. After 4 hours the rats were killed; the livers were removed and frozen in dry ice. They were homogenized in a Waring blender and RNA was isolated by the Kirby phenol extraction method(4). The liver RNA from each of the rats in the first group was assayed for radioactivity in an open window gas flow well counter. Fifty mg liver RNA from each of the rats in the second group were dissolved in 5 ml H_2O and treated with an equal volume of 10% trichloracetic acid at 5°C. The precipitate was collected by centrifugation, washed with 95% EtOH and the radioactivity per mg RNA was measured. Fifty mg liver RNA from each of the rats in the third group were dissolved in 5 ml of water containing 100 μg unlabelled 6-MP and aerated with H_2S gas for one hour at room temperature. The

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