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Studies in Tuberculosis. X. Skin Tests with Filterable Toxins of the Tubercle Bacillus.

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In a previous report a thermolabile toxin component and thermo-stable tuberculin element were demonstrated in Berkfeld filtrates of broth cultures of tubercle bacilli.

The present investigation is an experimental clinical test of these observations in a preliminary series of 55 tuberculous patients, and groups of non-tuberculous patients and apparently normal persons as controls.

Berkfeld filtrates were prepared from 26-day-old broth cultures of a moderately virulent human strain of tubercle bacilli incubated at a temperature of from 37° to 39° C. One-tenth cubic centimeter of a 1:20 dilution of filtrate was injected intracutaneously on the anterior aspect of one forearm, and on the other the same dose of filtrate, previously heated at a temperature of 63° to 65° C. for one hour. Controls were made with equivalent amounts of the uninoculated culture media.

Skin reactions were read after 24, 48 and 72 hours, and a note made of the time when the tests became entirely negative. Maximum reactions were observed uniformly after 48 hours, and usually showed definite infiltration and marked erythema at the sites of injection. Control material resulted negatively in all cases. Areas of reaction were approximated by measuring the extent of infiltration and reddening and recording the results in square centimeters.

The observations are summarized as follows:

The unheated Berkfeld filtrate gave the strongest and most persistent reactions as compared with those given by the heated material

Apparently normal persons, and those who had some clinical disease other than tuberculosis, gave sharp reactions of much longer duration and severity than did the tuberculous patients.

Tuberculous patients who could be classified as far advanced (50 cases) and relatively early pulmonary tuberculosis (5 cases) gave reactions which pointed to a clear-cut correlation between the type of skin reaction and the clinical course of the disease.

Analysis of the results in the group comprising 50 patients with

far advanced tuberculosis showed that slightly positive skin tests might occur after 24 hours and persist for a day or two, culminating in a ham-colored or brownish pigmentation. At other times there might be a moderately severe reaction which faded away after 24 hours. Such results occurred in a group of 12 patients who were rapidly declining in health, and who had serious tuberculous complications, such as intestinal or laryngeal involvement. In this group 3 deaths have occurred at this writing. The remaining patients of the series gave strong reactions after 24 hours, increased in severity after 48 hours, and persisted for several days. Clinical analysis of the group brought out the fact that these patients were progressing favorably and were showing definite signs of improvement.

TABLE I.
Summary of Results in Group with Far Advanced Tuberculosis.

Area of skin reaction (sq. cm.) after 48 hrs.	No. of Patients.	Condition of Patients
0-15	12	Extremely ill 9
		Dead 3
15-60	38	Arrested 14
		Improving 24

TABLE II.
Comparative Results of Unheated and Heated Berkfeld Filtrates.

Area of Skin Reaction (sq. cm.) after 48 hrs.	Unheated	Per cent	Heated	Per cent
0-20	33	66	44	88
20-40	10	20	2	4
40-60	7	14	4	8

TABLE III.
Results in Group of Females Among 50 Tuberculous Patients.

Area of Skin Reaction (sq. cm.)	Failing	Type of Reaction			Improv.
		Weak	Moderate	Strong	
0-20	8	5 0	3	3	3
20-40	1	0	1	1	1
40-60	0	0	0	4	4

Deductions from this study warrant the statement that Berkfeld filtrates of broth cultures of tubercle bacilli contain a thermolabile toxin element which gives skin reactions that can be correlated with the clinical progress of a tuberculous infection.

Definitely positive tests appear to be associated with evidence of clinical improvement, despite the presence of a tuberculous condition which might be classified as far advanced.

Skin reactions with Berkfeld filtrates are probably a measure of the resisting capacity of the patient or of normal persons, and as such are indicative of antibody content in the tissues.

The importance of evaluating the clinical course of groups of tuberculous patients must be emphasized in any attempt to interpret the significance of such skin reactions.

Further studies are now in progress with Berkfeld filtrates of tubercle bacillus cultures to determine toxicity and immunizing properties. More extensive series of tuberculous patients and normal persons are being studied along the lines indicated in this progress report.

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Thermolabile Toxin and Thermostable Tuberculin Components in Berkfeld Filtrates of Broth Cultures of Tubercle Bacilli.

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In these experiments, normal and tuberculous guinea pigs were used to determine by intracutaneous tests whether or not Berkfeld filtrates prepared from broth cultures of tubercle bacilli contain toxins which might be distinguishable from tuberculin elements. One series of tuberculous animals had been infected 3 weeks prior to the test and showed early tuberculosis with little, if any, toxemia. The other group of animals, infected 4½ months previously, showed well marked signs of advanced tuberculosis.

A human strain of virulent tubercle bacilli was inoculated into ordinary infusion broth containing 5 per cent glycerine. The Berkfeld filtrates were prepared from a 26-day old culture grown at a temperature of 38° to 40° C. One lot of the filtrate was heated at a temperature of 63° to 65° C. on a water bath. Both portions were preserved with 0.25 ether tricresol. At the time the experiments were made, the material had been stored in the ice-chest for 4 days.

The animals were injected intracutaneously with 0.03 cc. of the heated and unheated filtrates, respectively, in a dilution of 1:2 (with