3215

The Presence of Two Different Antigens in Alcohol Extract of the Tubercle Bacilli.

L. DIENES AND J. FREUND.

From the Von Ruck Research Laboratory for Tuberculosis, Asheville, N. C.

The first thorough investigator of this subject, Meyer,[1] supposed that in the lipoid extract of the tubercle bacillus two different antigens are present and are characterized, beside serological differences, by their different solubility in ether and alcohol. The slight serological differences described by Meyer did not seem to one of us to be sufficient ground for this supposition, all the more as the solubility of the specific substance was found different from the solubility of the preparations of Meyer.[2] Our present observations show that considerable serological differences can be found between certain fractions of the extract, although we are not able to ascertain the true significance of these differences. As was found by Meyer, the serological differences are connected with different solubility of the preparations.

A methyl alcohol extract of the tubercle bacillus strain 597, obtained after previous acetone, ether, and ethyl alcohol extraction, was evaporated and dissolved in ether. The ether soluble part was precipitated twice with four volumes of acetone at 37° C. The acetone precipitate was extracted three times with large amounts of ethyl alcohol under thorough grinding of the precipitate with a glass rod. The alcohol insoluble part was dissolved in ether. When the smallest amount of the alcohol and ether soluble substance giving positive complement fixation reaction was determined with two tubercle bacillus sera, one obtained with the fast growing strain 597, the other with the freshly cultivated strain R, from rabbits, considerable difference was found between the two preparations. The ether solution reacted with one fourth the amount of serum R than with serum 597. The alcohol soluble substance reacted in two and a half times the amount with serum 597 than with serum R. The figures for the alcohol soluble substance in the table were obtained when the potency of the preparation was considerably diminished. The same difference is systematically found with the preparations obtained in the way described, and with the derivatives of them,

not only with the tubercle bacillus, but in the case of a smegma bacillus strain examined, and with different sera obtained with the same strains.

When the ether solutions of the two substances described are shaken with distilled water, and centrifuged, three layers are formed; an ether solution, an inseparable jelly (formed of ether solution and water) and water solution. When the shaking with distilled water is repeated several times all the specific substance and all the dissolved lipoids are taken out from the ether solution, the largest part of them being found in the jelly. From a non-purified extract, the specific substance is taken out in this way from an ether solution, the non-specific lipoids remaining in the ether. Treating the alcohol non-soluble fraction of the methyl alcohol extract (in ether solution as described) with water, the specific substance is found nearly undiminished in the jelly and in the water solution, both reacting better, before water treatment, with serum R than with serum 597. The specific substance soluble in alcohol is in large measure destroyed by the described procedure, and it can be found only in the jelly. The water solution, although it contains considerable amounts of organic substance, has little or no antigenic effect.

The water solution, obtained in the described way from the alcohol non-soluble part of the methyl alcohol extract, shows usually in higher concentration an inhibition of the specific reaction. In one instance with 0.025 to 0.001 cc. the reaction was negative. It was positive only with .0005 to .0001 cc. The alcohol soluble antigen of the tubercle bacillus usually shows no inhibition even when high concentration, 500 to 1,000 antigenic units, can be examined. The addition of the watery solution described to other antigens not showing inhibition of the reaction in higher concentration, inhibits to a great extent the complement fixation with the serum R, and does not inhibit at all with the serum 597.

With the absorption experiment we were not able to differentiate the two antigens. The suspension of one, absorbed the antibodies also of the other. But it might be that the antigens in the preparation used were not sufficiently separated from each other.

The observations, except the absorption experiment, favor the conception that the antigen, non-soluble in alcohol and reacting better with serum R, is different from the alcohol soluble substance reacting better with serum 597. But there is an observation which appears to be in contrast with this supposition.

The preparation, described in a former note in these PROCEEDINGS,[3] which is probably separated from the lipoids, reacts better with serum 597 than with serum R, although so far it has been obtained only from preparations corresponding to the alcohol non-soluble substance reacting better with serum R. It is not yet decided whether or not it is the derivative of this substance or of the alcohol soluble substance contaminating it.

We have not yet studied the individual behavior of numerous sera. But the following observation might be of use for the interpretation of the difference found between different fractions of the lipoid extract: Four rabbit sera obtained with the strain 597, which we used extensively, gave indistinguishable reactions with the different preparations. But the sera, immediately after they are taken from the rabbit, give positive reactions with 5 to 10 times smaller amounts of the preparations than when examined a few weeks later. Not the antibody titer of the sera, but the avidity of the antibodies, diminishing rapidly in the first weeks, decides with how small an amount of antigen we will obtain positive reaction. It might be that the cause of the differences described in this paper between the different fractions of the extract is that the avidity of the antibodies is different in different sera toward the same specific antigen in different chemical binding.

TABLE I.

	Antigen Unit with Serum R	Antigen Unit with Serum 597
597 tubercle bacillus methylalcoholic extract, alcohol non-soluble fraction	.00018 mgm.	.0007 mgm.
The wash water of the ether solution of the former	.00025 mgm.	.0006 mgm.
597 tubercle bacillus methylalcoholic extract, alcohol soluble fraction	.0018 mgm.	.0008 mgm.
The wash water of the ether solution of the former	.06* mgm.	.06* mgm.
The specific substance probably separated from the lipoids, alcohol solution	.06 cc.	.008 cc.
Smegma bacillus methylalcoholic extract, alcohol non-soluble fraction	.00065 mgm.	.0005 mgm.
Smegma bacillus methylalcoholic extract, alcohol soluble fraction	.0015 mgm.	.0001 mgm.

* No reaction.

[1] Meyer, K., *Z. Immunitätsforsch.*, 1912, xv, 245.

[2] Dienes, L., and Schoenheit, E. W., *J. Immunol.*, 1925, x, 631.

[3] Dienes, L., and Schoenheit, E. W., PROC. SOC. EXP. BIOL. AND MED., 1925, xxii, 106.