SEROLOGICAL ACTIVITY OF VARIOUS FRACTIONS OF CULTURE FILTRATES OF THE TUBERCLE BACILLUS *

TUBERCULOSIS IMMUNIZATION RESEARCH CENTRE †

SYNOPSIS

As culture filtrates of *Mycobacterium tuberculosis* have been shown by numerous workers to be antigenically heterogeneous, a series of experiments has been started to identify some of the different antigens likely to be useful in laboratory tests for measuring the antibodies in human blood. The work involves the fractionation of concentrated culture filtrates by various chemical procedures, the chemical analysis of the fractions, and their subsequent testing and cross-testing with various serological procedures.

A number of fractions made from filtrates of heated and unheated cultures have been tested for activity in the Middlebrook-Dubos haemagglutination test. As might be expected, the fractions were found to vary in potency, some showing at least a thousand times more erythrocyte-sensitizing activity than others. Cross-inhibition tests made with the most active preparations indicated the existence of at least two different substances responsible for sensitizing erythrocytes to agglutination, one being demonstrable only in certain unheated fractions.

Antibodies detected with the tannic acid haemagglutination test have a different serological specificity from those detected by the Middlebrook-Dubos test. Different heated fractions gave strong cross-reactions with each other in the tannic acid haemagglutination procedure, and little difference could be seen between them; in contrast, four active fractions from unheated filtrates were distinct from each other, showing very little cross-reaction.

Results obtained with another haemagglutination procedure, consisting of agglutination of normal erythrocytes by antiserum-antigen mixtures, showed that this method detected another antigen, not measured in the tannic acid or Middlebrook-Dubos techniques under the conditions of the tests.

The present experiments have shown that the culture filtrates and fractions thereof contain a series of distinct antigens or at least characteristic combinations of antigens that differ in serological specificity and in their ability to react in the different tests. Further experiments are necessary to determine whether the serological differences are of any special significance in relation to tuberculous disease or bacterial type specificity.

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† The Tuberculosis Immunization Research Centre was set up jointly by the Government of Denmark and the World Health Organization. The Centre was established to study experimentally various aspects of immunity in tuberculosis, particularly the cellular and humoral responses to vaccination and tuberculous infection in experimental animals and in man. The work of the Centre is carried out in collaboration with the field research studies of the WHO Tuberculosis Research Office and with the work of the Statens Serum-institut, Copenhagen, and other laboratories.

The present study was carried out and the report prepared by Stephen V. Boyden and Ernst Sorkin.

The experiments reported in the present paper were carried out primarily for the purpose of investigating, and attempting to recognize, the antigens in culture filtrates of *Mycobacterium tuberculosis* likely to play a part in laboratory tests for measuring antibodies in the blood. Emphasis has been placed on haemagglutination techniques in an effort to learn something of the different antigens, although the potentialities of other techniques are also being investigated. The work has involved fractionation of the concentrated culture filtrates by various chemical procedures, the analysis of the fractions, and their subsequent testing and cross-testing in the various serological tests. It is hoped that through such experiments a ground can be laid for large-scale studies of the immune response to vaccination and tuberculous infection in human beings as well as in laboratory animals.

That culture filtrates of *M. tuberculosis* are not antigenically homogeneous has been amply shown by Seibert and her colleagues,^{26, 27} Bevilacqua and McCarter,^{1, 13} and others (see, for example, reviews by Seibert²⁵ and Iland & Kent⁶). Seibert²⁴ has postulated the existence of three different protein components, designated A, B, and C proteins, in unheated culture filtrates. She has also described two polysaccharides in the filtrates, this being in agreement with the findings of a number of other workers.^{7, 9, 12, 15, 28}

For many years the precipitin test was the chief method used for serological examination of culture filtrates, but the recent introduction of other serological techniques has provided new tools for such studies. For instance, in 1948 workers in Australia¹⁰ showed that erythrocytes treated with polysaccharide preparations from a number of different bacteria were rendered agglutinable by the specific anti-polysaccharide sera, and suggested that the reaction might form the basis of a useful serological test.^a Middlebrook & Dubos¹⁸ found that the procedure could be used to detect antibodies formed in response to infection with the tubercle bacillus. Since its introduction, the Middlebrook-Dubos test has attracted a great deal of attention, and numerous papers (as reviewed by Gernez-Rieux & Tacquet⁵) have appeared on its possible application as an aid to diagnosis and prognosis of tuberculosis in man.

Another new serological test, which, like the Middlebrook-Dubos test, can be regarded as a development of the coated-particle idea, involves the use of erythrocytes treated with tannic acid. When mixed with solutions of proteins, tanned erythrocytes adsorb protein molecules onto their surfaces so that, after washing, the cells can be agglutinated by the corresponding anti-protein sera.² When culture filtrates of tubercle bacilli are used for coating the tanned cells, the antibodies detected are of different serological specificity to those detected by the Middlebrook-Dubos test.^{6, 16, 17}

^a The term "haemosensitin" has been suggested⁴ (and will be used in this report) for bacterial substances that sensitize erythrocytes in this way.

One of the intentions of the Tuberculosis Immunization Research Centre is to use the haemagglutination reactions, as well as other techniques, to study serum antibodies against components of the tubercle bacillus. The present experiments have revealed that culture filtrates and fractions thereof contain a series of distinct antigens, or at least characteristic combinations of antigens, which differ from each other in serological specificity and in their ability to react in the different tests.

General Methods

Chemical fractionation of the filtrates

Some of the fractionation procedures were based on those used by Seibert.²⁵ Precipitation with salicylic acid and subsequent phenol extraction as described by Lamensans et al.¹¹ has also been used. Table I summarizes the methods by which the different fractions were isolated.

Middlebrook-Dubos haemagglutination test

The technique was used essentially as originally described,¹⁸ with certain slight modifications. Details of these modifications together with the technique of antigen cross-inhibition tests are to be published elsewhere.^b

Tanned erythrocyte haemagglutination test

Boyden² has previously described the principles of this test. Slight modifications have since been introduced, which are to be given, together with the antigen cross-inhibition technique, elsewhere.^c

Haemagglutination by mixtures of antiserum and antigen

The method consists essentially of mixing antigen with antiserum and subsequently adding normal sheep erythrocytes to the mixture. A positive reaction consists of haemagglutination rather similar to that seen in the Middlebrook-Dubos test. The fractions were titrated for antigens active in this method by making serial dilutions of them in buffered saline (pH 7.2) and adding four "haemagglutinating doses" of a globulin fraction of the pool of the antiserum used in the other tests. Details of the procedure are to be published elsewhere.^d

Precipitation in agar

This is, in principle, as described by Ouchterlony¹⁹ and Oudin.²⁰ Agar (1%) was used, with holes 7 mm in diameter and 7 mm apart; 0.5 ml

^b Sorkin, E. & Boyden, S. V.—in preparation

^c Boyden, S. V. & Sorkin, E.—in preparation

^d Boyden, S. V. & Andersen, M. E.—in preparation

TABLE I. ISOLATION OF FRACTIONS FROM HEATED AND UNHEATED CULTURE FILTRATES

Fraction *	Precipitated by
1 h	trichloroacetic acid
4 h	salicylic acid ; phenol-insoluble (" St.")
17 h	salicylic acid ; phenol-insoluble (" St.")
33 h	salicylic acid ; phenol-insoluble (" St.")
37 unh	10% acetic acid at pH 4.1
38 unh	48% alcohol at pH 6.5
39 unh	70% alcohol at pH 6.5 ; reprecipitated by half-saturation with ammonium sulfate
40 unh	70% alcohol at pH 6.5 ; supernatant from half-saturation with ammonium sulfate
41 unh	76% alcohol at pH 6.5
55 h	10% acetic acid at pH 4.1
56 h	48% alcohol at pH 6.5
57 h	70% alcohol at pH 6.5 ; reprecipitated by half-saturation with ammonium sulfate
58 h	70% alcohol at pH 6.5 ; supernatant from half-saturation with ammonium sulfate
59 h	76% alcohol at pH 6.5
60 h	70% alcohol ; supernatant from full-saturation with ammonium sulfate at pH 5.8 ; soluble in trichloroacetic acid
61 unh	10% acetic acid at pH 4.1
62 unh	48% alcohol at pH 6.5
63 unh	70% alcohol at pH 6.5 ; reprecipitated by half-saturation with ammonium sulfate
64 unh	70% alcohol at pH 6.5 ; supernatant from half-saturation with ammonium sulfate
65 unh	76% alcohol at pH 6.5
85 unh	70% alcohol at pH 6.5 ; reprecipitated by half-saturation with ammonium sulfate
86 unh	70% alcohol at pH 6.5 ; reprecipitated by full-saturation with ammonium sulfate
87 unh	70% alcohol at pH 6.5 ; supernatant from full-saturation with ammonium sulfate
89 unh	76% alcohol at pH 6.5 ; reprecipitated by full-saturation with ammonium sulfate
90 unh	76% alcohol at pH 6.5 ; supernatant from full-saturation with ammonium sulfate
91 unh	supernatant from 76% alcohol
124 unh	76% alcohol at pH 6.5 ; reprecipitated by full-saturation with ammonium sulfate

* h = heated ; unh = unheated

antiserum RP 5 (see below) was placed in the centre hole and the antigens at 0.1 mg/ml and 1.0 mg/ml in the surrounding holes.

Antiserum

For all the studies described in this paper (except the antiserum-antigen haemagglutination test), a single pool of rabbit antiserum, referred to as RP 5, was used. It consisted of a mixture of the sera of six rabbits that had received repeated intravenous injections of phenol-killed human tubercle bacilli, strain H37 Rv.

Serological Studies

Middlebrook-Dubos test

On the supposition that Old Tuberculin may contain more than one erythrocyte-sensitizing substance, and that the value of the Middlebrook-Dubos test might be enhanced as a diagnostic aid by removing one or more of them, several workers have fractionated Old Tuberculin in the hope of obtaining a more "specific" material for the test. Pound,²² for example, precipitated material from a methanol-phosphate extract of heat-killed bacilli with different volumes of alcohol and reported the existence of three different components capable of becoming adsorbed onto erythrocytes. Other workers (e.g., Maillard & Gagliardo¹⁴) have absorbed human sera in different ways with antigens from non-virulent tubercle bacilli as well as other bacteria (e.g., staphylococci), with the intention of removing non-specific antibodies.

In this laboratory, a number of different fractions made from filtrates of heated and unheated cultures have been tested for activity in the Middlebrook-Dubos test. As might be expected, the fractions were found to vary a great deal in their potency, some possessing at least 1000-fold more erythrocyte-sensitizing activity compared with others. Some of these differences are exemplified in table II.

The preparations most active in sensitizing erythrocytes were subjected to cross-inhibition tests in order to gain information on the specificities of the sensitizing substances in the various fractions.

This technique is based on the principle that a specific antigen can react with its antibody, so that the latter is neutralized and no longer able to agglutinate cells sensitized with the homologous antigen. Thus, if twofold dilutions of two unrelated antigens are made in saline, and a given amount (e.g., four "haemagglutinating doses") of antituberculous serum is added to each tube in both rows, and erythrocytes sensitized with one of the antigens are then added to both rows, inhibition of haemagglutination occurs only in the row containing dilutions of the antigen that also coats the cells. If the two fractions were related to each other, sharing common antigens or

TABLE II. ACTIVITIES OF FRACTIONS OF CULTURE FILTRATES IN DIFFERENT SEROLOGICAL TECHNIQUES

Fraction *	Middlebrook-Dubos haemagglutination	Tanned erythrocyte haemagglutination	Haemagglutination by antiserum- antigen mixture	Precipitation in agar number of bands
1 (PPD) h	+++	+++++	++	—
37 unh	++	+++++	±	—
38 unh	++	++++	+	1
39 unh	+++	++++	+++	2
40 unh	++++	±	+	—
41 unh	+	++++	+	2
55 h	++	+++++	+	—
56 h	—	—	—	—
57 h	+++	+++++	—	—
58 h	++++	±	—	—
59 h	++	++	—	—
85 unh	++++	+++++	+++	—
87 unh	+++++	—	—	trace
91 unh	—	—	—	—
124 unh	±	+++++	+++++	1

* h = heated ; unh = unheated

KEY : ++++++ indicates activity at 0.002 mg/ml

+++++ " " " 0.008 "

++++ " " " 0.03 "

+++ " " " 0.125 "

++ " " " 0.5 "

+ " " " 2.0 "

± indicates trace of activity at 2.0 mg/ml

— indicates inactive at 2.0 mg/ml

determinant groups, then some inhibition could be expected in both rows.

The inhibition results with eight of the fractions made from heated cultures are shown in table III. Whichever of the eight preparations was used for sensitizing the erythrocytes, they all fell in the same order with regard to their ability to inhibit the haemagglutination by the antiserum. Thus, whether the erythrocytes were sensitized with fraction 17 or fraction 1 (PPD), fraction 17 had the higher inhibition titre, and fraction 1 (PPD) the lower. A number of other heated preparations have since been tested, including Old Tuberculin (Lederle, 4×international standard), and the results all conform to this arrangement.

This evidence indicates that the material (tentatively called the α haemo-sensitin) in each of the heated fractions responsible for sensitizing erythro-

TABLE III. CROSS-INHIBITION RESULTS WITH SOME HEATED FRACTIONS IN THE MIDDLEBROOK-DUBOS TEST

Preparation tested for inhibition	Preparation used for sensitizing erythrocytes							
	17	33	4	58	59	57	1	55
17	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++
33	+++++	+++++	+++++	++++	++++	++++	+++++	++++
4	++++	++++	++++	++++	++++	++++	+++	+++
58	++	+++	+++	++	++	++	++	+++
59	++	++	+	+	++	+	++	++
57	+	++	+	+	+	+	+	+
1	+	+	+	±	+	+	+	+
55	±	+	+	±	+	±	±	+

KEY : +++++ = Inhibition of haemagglutination to titre of 1/128
 ++++ = " " " " " " " 1/64
 +++ = " " " " " " " 1/32
 ++ = " " " " " " " 1/16
 + = " " " " " " " 1/8
 ± = " " " " " " " 1/4
 - = " " " " " " " 1/2 or less

cytes to agglutination, under the conditions of the experiments, was of the same specificity. Our work does not provide any evidence for the existence of more than one specific haemosensitin in the heated culture filtrates that we have used, although it does not completely exclude the possibility of it.

The picture seen with similar cross-inhibition tests with unheated culture filtrates was quite different (table IV). Different inhibition results were obtained according to which fraction was used for sensitizing the cells. There is evidence in these unheated fractions of at least two substances of different serological specificity. One of them, the active haemosensitin of preparations 63 and 87, seems to be the same as the α haemosensitin contained in PPD and the other heated fractions.

The agglutination of cells treated with fractions 39, 41, 65, and 86, however, is not inhibited at all by the fraction (87) most active in inhibiting the α haemagglutination. Instead, the agglutination is most inhibited by fractions 39, 41, and 65, which have a relatively low inhibition titre against the α haemosensitin. It thus appears that the main haemosensitin (tentatively called the β haemosensitin) in these fractions is different from that in the heated fractions. Judging from its inhibition titres, fraction 86 has a particularly high content of both α and β haemosensitins.

TABLE IV. CROSS-INHIBITION RESULTS WITH SOME UNHEATED FRACTIONS IN THE MIDDLEBROOK-DUBOS TEST

Preparation tested for inhibition	Preparation used for sensitizing erythrocytes						
	1 (PPD)	63	87	39	41	65	86
1 (PPD)	++	++	++	+	+	+	—
63	+++	++	+++	++	++	+	+
87	+++++	+++++	+++++	—	—	—	—
39	++	+	±	++	++	+	++
41	±	—	—	++++	++++	+++	+++
65	+	+	—	++++	++++	+++	+++++
86	++++	++++	+++	+++++	+++++	+++++	+++++

KEY : +++++ = Inhibition of haemagglutination to titre of 1/128
 ++++ = " " " " " " " 1/64
 +++ = " " " " " " " 1/32
 ++ = " " " " " " " 1/16
 + = " " " " " " " 1/8
 ± = " " " " " " " 1/4
 — = " " " " " " " 1/2 or less

Many other unheated fractions have been tested in the same way, and the majority have fallen into one of the two groups (α or β). A few have been found that do not behave as representing either α or β , but whether this is because of the presence in them of other sensitizing components, or whether they are mixtures of α and β is not yet clear. Old Tuberculin (Lederle, 4 $\times$ international standard) does not contain any of the β substance, as judged by its inability to inhibit agglutination of cells sensitized with fractions 41, 65, etc.

It has long been suspected that the important erythrocyte-sensitizing substance in the Middlebrook-Dubos test is polysaccharide in nature,^{17, 18} and there is considerable evidence to support this view, at least in the case of the α haemosensitin.^{6, e} The chemical nature of the β haemosensitin is not yet known.

Tannic acid test

It has been shown previously by absorption and cross-inhibition tests that when PPD (trichloroacetic acid precipitated) is used for coating the erythrocytes in both the tannic acid and Middlebrook-Dubos haemagglutination tests, the two techniques measure different antibodies.^{3, 6, 16}

^e Sorkin, E., Boyden, S. V. & Rhodes, J. M.—in preparation

As in the case of the Middlebrook-Dubos method, different fractions have been found to vary considerably in their potency in the tannic acid technique (table II). The relative potencies of the fractions in the two methods varied independently.

The results of cross-inhibition tests on various fractions active in the tannic acid test are given in table V. The fractions included three preparations of PPD^f (trichloroacetic acid precipitated from filtrates of heated cultures) and fractions made by a method of alcohol precipitation similar to that of Seibert²⁵ from filtrates of heated (fractions 55, 57, 59) and unheated (fractions 37, 38, 39, 41) cultures.

The cross-reactions of the three PPD's are striking: although they were prepared at different places and from different strains of bacilli, they are very similar to each other in specificity as far as the tannic acid test is concerned. The three heated fractions (made by acetic acid and alcohol precipitation from a single culture filtrate) also react strongly with each other and with the PPD's.

Four of the five unheated fractions made by alcoholic precipitation were active in the test (table II), and the results of the cross-inhibition tests show that each was antigenically distinct from the others. Fraction 37 is noted for its cross-reactions in both directions with most of the heated fractions and the PPD's. Fractions 38, 39, and 41 not only differ from each other and from fraction 37 but also show very little cross-reaction with any of the heated fractions.

Subsequent work has shown that it is relatively easy to obtain fractions from fresh unheated culture filtrates that cross-react strongly with either fraction 37 or fraction 41. The intermediate fractions (analogous to fractions 38 and 39) are less easily reproducible and are often less distinct than in the first separation, tending to react more with each other and with fraction 37 (although not to the same extent as the heated fractions). Other methods of fractionation, however, have produced fractions that behave similarly in cross-inhibition tests to each of these.

Whether each, or any, of the dissimilar fractions represents a single protein antigen or a characteristic mixture or combination of antigens active in the tannic acid test cannot yet be stated. The relative ease with which fractions similar in reaction to fractions 37 and 41 can be reproduced suggests that, at least in these two instances, the activity of each may be due to a single component. On the other hand, analytical-chemical, paper-electrophoretic, and agar-precipitation studies show that these fractions are still far from being homogeneous.

It can be safely concluded from the results that different antibodies can be detected by the tannic acid haemagglutination test depending on which

^f PPD RT XXII was kindly supplied by Dr. K. Tolderlund and Mr. M. Magnusson of the Statens Seruminstitut, Copenhagen. PPD (Weybridge) was kindly supplied by Dr. H. H. Green and Mr. A. B. Paterson of the Ministry of Agriculture Veterinary Laboratory, Weybridge, England.

chemical fraction is used to sensitize the tanned cells. The differences between the various unheated fractions are much more marked than those between the heated fractions.

Haemagglutination by antiserum-antigen mixtures

Mixtures of certain rabbit antituberculous sera and certain fractions of culture filtrates of the tubercle bacillus can cause the agglutination of normal sheep erythrocytes, or their haemolysis in the presence of guinea-pig complement. This phenomenon was encountered accidentally during antigen haemagglutination inhibition studies,⁸ and experiments have been carried out to investigate its mechanism. The results clearly show that the specific substance in the culture filtrates responsible for the reaction, as observed in this laboratory, is not responsible for sensitization of erythrocytes in the Middlebrook-Dubos test. The reaction is due to an affinity, even in the presence of antibody, of the bacterial substance for the cell surface.

Middlebrook¹⁷ has referred briefly to a similar observation, and assumed that the reaction was due to the erythrocyte-sensitizing polysaccharide. This was not the case in the reaction observed in our laboratory.

The evidence available at present suggests that the specific material responsible for this haemagglutination reaction is distinct from the antigens detected by the other haemagglutination methods, thus providing further evidence of the complex heterogeneity of the culture filtrates.

Other serological procedures

The emphasis in our serological studies has been on haemagglutination techniques, as they have some special advantages for our purposes. Haemagglutination techniques, however, do not work for all antigens, and it was thought that with other serological procedures further light might be thrown on the antigenic composition of some of the fractions.

Table II, column 4, summarizes results of precipitation in agar of some of the fractions. Clearly, none of the heated fractions showed any zone of precipitation in the gel, although some of them had high activity in the other tests. Many of the unheated fractions showed one or more distinct lines of precipitation, and these fractions (e.g., 41) were also active in haemagglutination tests, but the data are not sufficient to show whether any one of the lines corresponds to a particular erythrocyte-sensitizing antigen.

Fractions 56 and 91 showed no reaction with the rabbit antiserum either in the haemagglutination tests or in the agar-precipitin technique. Both fractions, however, fixed complement in the presence of the rabbit serum, and fraction 91 gave two bands of precipitate with horse antituberculous serum in agar. Similarly, paper-electrophoretic studies with fraction 91

⁸ Boyden, S. V. & Andersen, M. E.—in preparation

revealed the presence of two proteins of different electrophoretic mobilities (for further details, see Rhodes & Sorkin²³).

It is thus clear that antigens are present in the culture filtrates that are not active in any of the haemagglutination tests but give a reaction with antiserum in complement fixation, agar precipitation, or paper electrophoresis.

Discussion

The results of the present experiments indicate that different antibodies can be measured depending upon (1) the serological test used and (2) the fraction tested in a given procedure.

In the Middlebrook-Dubos test there is evidence of the existence in the culture filtrates of at least two different haemosensitins, one being demonstrable only in certain unheated fractions. Different heated fractions reacted strongly with each other in the tannic acid haemagglutination procedure, and little difference could be seen between them; in contrast, four active fractions from unheated filtrates were distinct from each other, showing very little cross-reaction.

Evidence for the existence of another specific activity was provided by haemagglutination by antiserum-antigen mixture; the specific activity is probably due to protein and is not recognized as active in the Middlebrook-Dubos or tanned erythrocyte methods, under the conditions of the experiments.

Some of the fractions that proved inactive in the haemagglutination tests gave clear precipitates with antiserum in agar and showed more than one protein constituent in paper electrophoresis.

These results stress the great complexity of the culture filtrates of the tubercle bacillus. Except perhaps in the case of what we have tentatively called the α and β haemosensitins of the Middlebrook-Dubos test, no attempt has been made to attribute any of the serological activities of the various fractions described to any one single substance. Although a single substance may in fact be responsible for the reaction in some instances, there is not enough evidence at present to prove it: there has possibly been too great a tendency in the past to describe different fractions as different proteins or polysaccharides without sufficient supporting evidence. The work, for example, of Pope et al.²¹ on purified diphtheria toxin has emphasized the need for caution in this respect. Much more work will be necessary, and more chemical, physico-chemical, and serological criteria will have to be satisfied before we can say that, with a certain test and a certain fraction of a filtrate, we are measuring antibodies against a single antigen.

Whether the serological differences between the different antigenic constituents of the culture filtrates are of any special significance in relation to tuberculous disease or bacterial type specificity are questions yet to be answered.

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RÉSUMÉ

De nombreux chercheurs ayant montré que les filtrats de cultures de *M. tuberculosis* sont antigéniquement hétérogènes, une série d'expériences a été entreprise pour l'identification de certains des différents antigènes susceptibles d'être utiles dans des épreuves de laboratoire destinées à déterminer la teneur du sang humain en anticorps. Ce travail consistait à fractionner, par divers procédés chimiques, les filtrats de culture concentrés, à faire l'analyse chimique des fractions obtenues et à étudier ensuite leur comportement quand on les soumet, en recourant à diverses techniques, à des épreuves sérologiques directes ou croisées.

On a étudié, par l'épreuve d'héماغglutination de Middlebrook-Dubos, l'activité d'un certain nombre de fractions obtenues à partir de filtrats de cultures chauffés et non chauffés. Comme on pouvait s'y attendre, les fractions se sont montrées dissemblables, certaines d'entre elles ayant une activité sensibilisatrice au moins 1.000 fois plus forte que d'autres à l'égard des érythrocytes. Les épreuves d'inhibition croisée auxquelles on a soumis les préparations les plus actives ont révélé que deux substances au moins sont responsables de la sensibilisation des érythrocytes à l'agglutination, l'une d'elles n'étant décelable que dans certaines fractions non chauffées.

Les anticorps mis en évidence par l'épreuve d'héماغglutination à l'acide tannique ont une spécificité sérologique différente de ceux que permet de détecter l'épreuve de Middlebrook-Dubos. Avec la technique d'héماغglutination à l'acide tannique, on a observé de fortes réactions croisées entre différentes fractions chauffées, et l'on n'a pu constater entre elles qu'une légère différence; en revanche, quatre fractions actives provenant de filtrats non chauffés se sont montrées nettement distinctes les unes des autres, ne permettant d'obtenir qu'une très faible réaction croisée.

Les résultats obtenus avec une autre technique d'héماغglutination — consistant à agglutiner des érythrocytes normaux au moyens de mélanges antisérum/antigène — ont montré que cette méthode révélait la présence d'un autre antigène, que ni la technique de Middlebrook-Dubos ni celle à l'acide tannique ne permettaient de déterminer dans les conditions de l'épreuve.

La présente expérimentation a montré que les filtrats de culture et les fractions que l'on en a séparées renferment une série d'antigènes distincts ou, du moins, des combinaisons caractéristiques d'antigènes qui diffèrent par leur spécificité sérologique et aussi par leur aptitude à réagir à diverses épreuves. De nouvelles expériences sont nécessaires pour qu'on puisse déterminer si ces différences sérologiques présentent une signification particulière en ce qui concerne la tuberculose ou la spécificité des types bactériens.

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