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Preliminary Communication

SOME OBSERVATIONS ON THE SORBING AGENT USED IN THE ABSORBED FLUORESCENT TREPONEMAL ANTIBODY (FTA-ABS) TEST*

BY

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The FTA-200 test described by Deacon, Freeman, and Harris (1960) suffers from the disadvantage that sera have to be tested at a dilution of 1 in 200 in order to avoid non-specific results due to the presence of low-titred group anti-treponemal antibody in many normal sera. This lessens the potential sensitivity of the method, and the use of intact Reiter treponemes or blocking methods to remove group antibody was reported by Hunter (1964) and of ultrasonically disintegrated Reiter treponemes by Hunter, Deacon, and Meyer (1964). By these methods sera can be tested at a dilution of 1 in 5 with a consequent increase in sensitivity. Published reports suggest that the absorbed FTA test also has a high specificity (Deacon, Lucas, and Price (1966); Knox, Short, Wende, and Glicksman (1966); Garner, Grantham, Collins, and Roeder (1968); Johnston and Wilkinson (1968)).

The sorbing agent now in general use for the removal of group antibody from sera is a heated and concentrated culture filtrate of Reiter treponemes. This is a crude product and attempts were therefore made to determine the active principle and to study the mechanism of the reaction.

Material and Methods

To prepare the sorbate, Reiter treponemes were grown in the medium described by Rajkovic (1966) with 10 per cent. horse serum. This was dispensed in 100 ml. amounts and seeded with 10 ml. of a 3-day-old actively growing culture in the same medium and incubated for 6 to 8 days at 35°C. until maximal growth had occurred. The cultures were pooled, autoclaved at 121°C. for 15 minutes, and allowed to cool. Insoluble material was centrifuged off and the supernatant concentrated to one-tenth of the original volume by boiling. Any deposited material was removed by centrifugation and azide was added to the supernatant to a concentration of 0·1 per cent. as a bacteriostatic. The product was stored at −20°C. in aliquots, that required for current use being kept at 4–6°C. For standardization of the sorbate and for the technique of the FTA-ABS test, the methods described by the Venereal Diseases Research Laboratory (1968) were followed.

Tests were read on a Leitz Ortholux microscope using a ×54 fluorite immersion objective and ×8 oculars. A 1·5 mm. BG 12 exciter filter and 510 barrier filter were used.

Results

Tests for Completeness of Absorption of Group Antibody

FTA-ABS tests were performed on 56 sera using both Nichols and Reiter treponemes as antigens. Two batches of sorbate were examined:

(*a*) Lot 68/2, prepared in the laboratory as described above, which had a working titre of 1 in 4;

(*b*) Batch 671, kindly supplied by the Communicable Disease Center, Atlanta, Ga., which was used undiluted.

36 sera were tested with the first sorbate and twenty by the second. The results with both followed the same pattern and are combined in Table I.

TABLE I

RESULTS OF FTA-ABS TESTS ON 56 SERA AGAINST NICHOLS AND REITER TREPONEMES

Fluorescence with Nichols Treponemes	Fluorescence with Reiter Treponemes						Total
	++++	+++	++	+	±	0	
++++	·	1	3	·	·	·	4
+++	·	·	5	2	2	·	9
++	·	·	1	4	8	4	17
+	·	·	·	·	4	·	4
±	·	·	·	·	6	7	13
0	·	·	·	·	1	8	9
Total	·	1	9	6	21	19	56

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Quantitative tests with Reiter treponemes as antigen were carried out on fifteen unabsorbed sera from each of the two batches tested to estimate the amount of group anti-treponemal antibody present. A comparison of the anti-Reiter titres before absorption with the intensity of residual fluorescence of the absorbed sera against Reiter treponemes is shown in Table II.

TABLE II

COMPARISON OF DEGREES OF FLUORESCENCE WITH REITER TREPONEMES AFTER TREATMENT OF SERA WITH SORBATE, WITH TITRES OF UNABSORBED SERA (30 SERA)

FTA-ABS Result	No. of Sera	Fluorescence with Reiter Treponemes after Absorption	Titre of Unabsorbed Sera against Reiter Treponemes					
			1,215	405	135	45	15	5
Reactive (++) to (++++)	18	+++	·	1	·	·	·	·
		++	1	·	6	·	·	·
		+	·	1	2	·	1	·
		±	·	·	·	4	1	1
		0	·	·	·	·	·	·
Doubtful (+)	2	±	·	·	·	1	1	·
Non-reactive (±) to 0	10	±	·	·	·	1	3	1
		0	·	·	·	·	1	4

These results indicate that treatment of sera with sorbate under the conditions of the FTA-ABS test does not necessarily remove the whole of the group anti-treponemal antibody as judged by the reactivity of the absorbed sera with Reiter treponemes. This failure of absorption was most marked with sera which were strongly reactive in the FTA-ABS test and which had titres of group antibody of 135 or above. In those sera in which the titre of group antibody was 45 or less, treatment with sorbate resulted in minimal or no reactivity with Reiter treponemes, suggesting that removal of group antibody was almost or quite complete.

Sources of Sorbing Agents

500 ml. of culture medium were inoculated with Reiter treponemes and an equal quantity of the same batch left uninoculated. Both were incubated together under the same conditions for 7 days and sorbates prepared as described above. The results of titrations of each against known positive and non-specific control sera are shown in Table III.

The results of this experiment, which were confirmed on other similar preparations, showed that the medium itself possessed sorbing activity, apparently equal in degree to the same medium in which Reiter treponemes had been grown. In a further experiment, treponemes were removed by centrifugation from an aliquot of medium after incubation and sorbates prepared from the treponeme-free supernatant, from the medium containing treponemes, and from uninoculated medium which had been incubated in parallel with the original culture. No difference in sorbing activity was found between these three preparations.

TABLE III

COMPARISON OF SORBING ACTIVITY OF MEDIUM IN WHICH REITER TREPONEMES HAD BEEN GROWN WITH UNINOCULATED MEDIUM

Serum	Dilution of Sorbate	Fluorescence with Sorbate prepared from:	
		Inoculated Medium	Uninoculated Medium
Positive	Neat	+++	+++
	1 in 2	++++	+++
	3	++++	++++
	4	++++	++++
	5	++++	++++
	7	++++	++++
	10	++++	++++
Non-specific	Neat	0	0
	1 in 2	0	0
	3	0	0
	4	±	0
	5	±	± to +
	7	+	± to +
	10	+ to ++	+

Controls with Buffered Saline: Positive ++++
Non-specific +++

The sorbing activity of the various components of the Rajkovic medium was investigated. These were dissolved in distilled water at ten times the concentration in the medium as used for culture to reproduce the effect of concentration during the preparation of a sorbate. Half of each of the solutions was autoclaved and half left unheated. Heating did not affect the pattern of results, except that the horse serum formed a solid coagulum which could not be tested. The results with the unheated reagents are shown in Table IV.

TABLE IV

SORBING ACTIVITY OF COMPONENTS OF RAJKOVIC'S MEDIUM

Component	Concentration Tested* (per cent.)	Fluorescence with:	
		Positive Serum	Non-specific Serum
Sodium chloride	2·5	+++ to ++++	+++
Glucose	5·0	++++	+++
Cysteine HCl	2·0	+	±
Yeast extract	5·0	++++	+++
Bacto casitone	30·0	++++	0
Horse serum	Undiluted	++++	+++

Controls with Buffered Saline: Positive serum ++++
Non-specific serum +++

* These concentrations represent ten times those present in the actual culture medium.

These results showed that the sorbing activity was probably associated with the casein preparation. Cysteine hydrochloride greatly reduced fluorescence with both the positive and non-specific control

sera; this was probably due to the highly acid reaction. Further tests in which Bacto casitone and Bacto casitone plus cysteine HCl were dissolved in phosphate buffered saline, pH 7·2, at the concentrations shown in Table IV showed that each had a similar sorbing effect and that the addition of cysteine did not appear to potentiate this.

39 sera were tested in FTA-ABS tests with a sorbate prepared from a heated culture filtrate of Reiter treponemes with a working dilution of 1 in 4 and with a 30 per cent. solution of Bacto casitone, in buffered saline. Identical results were obtained with 37 sera (20 non-reactive, 16 reactive, and one doubtful (+) with both preparations). One serum gave a doubtful (+) reading with the casitone but a reactive (++) reading with the standard sorbate; another was reactive (++) with casitone but non-reactive (±) with the standard sorbate.

The sorbing activity of four brands of peptone and one each of tryptone and tryptose as 20 per cent. solutions in buffered saline was tested. All these preparations reduced or abolished reactivity with a non-specific control serum but did not significantly reduce the fluorescence given by a positive serum. In quantitative tests they did not appear more active than Bacto casitone and were not investigated further. Precipitin tests in which these preparations were overlaid with serum from an untreated case of secondary syphilis and with a rabbit anti-Reiter serum were negative.

Discussion

Removal of group anti-treponemal antibody from sera can be effectively carried out by absorption with intact or ultrasonically disintegrated Reiter treponemes. The use of intact organisms is time-consuming and the preparation of sufficient amounts of an ultrasonate for testing large numbers of sera presents technical difficulties. The concentrated, heated culture filtrate of Reiter treponemes is easy to prepare and is economical in use. It seemed probable that it owed its activity to heat-stable material from the treponemes which reacted with the group antibody. The demonstration by Cannefax, Hanson, and Skaggs (1968) that a sorbate prepared from an uninoculated culture appeared to be as effective as that made from medium in which Reiter treponemes had been grown has been confirmed in the present study. These authors also found sorbing activity present in the yeast extract present in their medium and in Bacto casitone. These observations and those of the present study raise the question whether the sorbate as currently prepared owes any of its activity to the Reiter treponemes or whether this activity can be accounted for solely by substances present in the medium. Isolation and characterization of the active principle will be necessary before this question can be answered and the mechanism of the reaction explained. If inhibition by sorbate of the union of group anti-treponemal antibody with receptors on *T. pallidum* is due to a true antigen–antibody reaction, then substances of molecular configuration similar to the corresponding antigens on the treponeme are presumably present in the protein digest products which have been studied. Alternatively, there may be differences in the stability of the group and specific antibodies to the effect of sorbate. Tests in which sorbate or Bacto casitone were applied to fixed films of *T. pallidum* for 30 minutes followed by washing and application of a non-specific serum, showed no diminution of fluorescence compared with treponemes treated with buffered saline; this suggests that the sorbate acts *via* the serum and not on the treponeme itself.

The reports on the FTA-ABS test which have been cited have shown that in practice it is both sensitive and has a high specificity. Ideally, the reagent used for absorption should remove all group anti-treponemal antibody from a serum, leaving only specific antibody free to unite with the *T. pallidum* antigen. The results described above suggest that, as the test is at present performed, this is true only of sera containing relatively low titres of group antibody as judged by their fluorescence with the Reiter treponeme. Strongly reactive sera still retain detectable amounts of group antibody after treatment with sorbate under the conditions of the test. Previous studies by Wilkinson and Rayner (1966), in which quantitative tests were performed on sera before and after absorption with Reiter treponemes to remove group antibody, showed that, except in early syphilis, group antibody tends to preponderate over that specific for *T. pallidum*. In the FTA-ABS test as performed at present, a single negative control with a non-specific serum is used to control each batch of tests. If the test is to be relied on to detect specific antibody alone, some modification of the absorption procedure with control of the completeness of absorption of group antibody from the individual sera tested would seem desirable. This could be achieved by testing sera after absorption against both Nichols and Reiter treponemes although this would add to the labour of the test. In the recommended interpretation of results (Venereal Diseases Research Laboratory, 1968), even a one plus degree of fluorescence is classed as reactive if confirmed by

repetition. The present authors prefer to class such reactions as doubtful or weakly reactive; it is felt that sera giving such results certainly merit further testing for completeness of removal of group antibody.

Summary

(1) Under the conditions of the FTA-ABS test as performed at present, all group antitreponemal antibody may not be removed by the sorbate from strongly reactive sera.

(2) Sorbates prepared from medium which has not been infected with Reiter treponemes showed a sorbing activity comparable to the same medium in which growth had taken place. This sorbing activity of the medium alone appears to be due to the casein digest in the medium used.

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Quelques observations à propos de l'agent sorbique employé dans le test fluorescent de l'anticorps absorbé du tréponème (FTA-ABS) Communication préliminaire

RÉSUMÉ

(1) Dans les conditions où le test FTA-ABS est fait en ce moment, tout le groupe d'anticorps antitréponèmes peut ne pas être retiré par le sorbique du sérum fortement réactif.

(2) Les sorbiques preparés du milieu qui n'a pas été infecté par les tréponèmes de Reiter avaient montré une activité sorbique comparable au même milieu dans lequel une culture avait eu lieu. Cette seule activité sorbique du milieu semble être due à la caséine digérée dans le milieu employé.