A preliminary study

An Investigation of Sorbing Substances in the FTA-ABS Test for Syphilis

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THE FLUORESCENT treponemal antibody-absorption (FTA-ABS) test (*1*) was developed on the basis of fundamental immunofluorescent methodology and the following three immunological premises: (*a*) avirulent and virulent treponemes have common antigens that are detectable by immunofluorescence (*2*), (*b*) the common antigens of the normal treponemal flora, such as *Treponema microdentium*, produce antibody that is detectable by immunofluorescence in 20–30 percent of presumably nonsyphilitic human serums (*2,3*), and (*c*) in a given serum the common antibody can be absorbed, or blocked, by mixing the serum with the common antigen derived from the avirulent Reiter treponeme so that only the treponemal antibody resulting from *Treponema pallidum* infection is demonstrated (*2*).

That these premises resulted in a satisfactory working hypothesis is evidenced by practical application in which the FTA-ABS test has been shown to have a high degree of sensitivity and specificity when the sorbent, or blocking agent, has been a sonicate of the Reiter treponeme (*1*) or a stable water-soluble extract from cultures of Reiter treponemes (*4*).

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No reports are available on the efficiency of soluble substances of treponemes other than the Reiter strain for the production of a satisfactory sorbent. Since other treponemal strains or species could conceivably produce more of the soluble substance, and perhaps a more satisfactory sorbent, we investigated these sources. This paper presents data pertaining to (*a*) the sorbent effect of broth media which had been used for the cultivation of 20 treponemal strains or species and two borrelial species, (*b*) uninoculated medium, (*c*) medium components, (*d*) nucleic acids, (*e*) parent and derived compounds of certain amino acids, purines, and pyrimidines, (*f*) miscellaneous compounds, and (*g*) 80 percent ethanol soluble and insoluble fractions of deproteinized yeast extract.

Materials and Methods

The following are the treponemal and borrelial species and strains which we investigated and the persons who supplied them:

Reiter strain of purported *T. pallidum* (H. Eagle, Johns Hopkins University).

English Reiter, Noguchi, Nichols, and Kazan strains of purported *T. pallidum* and *Borrelia vincentii* strain N-9 (E. G. Hampp, National Institute of Dental Research, Public Health Service).

Kazan 2, 4, 5, and 8 strains of purported *T. pallidum* (P. H. Hardy, Jr., Johns Hopkins University).

T. microdentium strains N-39, TD-2, TRRD, and 926A5 (S. S. Socransky, Forsyth Dental Institute, Boston).

T. phagedenis, T. calligyrium, T. ambigua, T. minutum, T. skoliodonta, and *T. refringens* (J. Pillot, Institut Pasteur).

T. zuelzerae (H. Veldkamp, Lab. Voor Microbiologie, Landbouwhogeschool, Wageningen, The Netherlands).

B. refringens, isolated in the Venereal Disease Research Laboratory (*5*).

The medium employed and the methods of incubating and harvesting the organisms have been described previously (*6*). The duration of incubation varied with each strain or species (4–12 days) in relation to the time required for the maximal yield of organisms that could be obtained before the development of cystlike forms. The media were preserved with 0.25 percent phenol, stored at 4° to 8° C. until autoclaved, filtered, and centrifuged as recommended for the production of FTA-ABS sorbent (*7*). Each medium was flash evaporated until it was very viscous and, finally, lyophilized.

Based on the total solids after lyophilization and the original volumes of media, aliquots were rehydrated to approximately one-third of the original volume, which is near the usual maximum effective dilution level of bulk sorbent prepared at present from the Reiter treponeme medium. Uninoculated medium was processed in the same manner to provide a control of observations of the media after cultivation of the 22 strains or species.

Media and medium components were dissolved at 10 times their usual concentration, autoclaved, and tested (undiluted, 1:2, and 1:4) for sorbent effect. The 1:4 dilution is considered near the concentration of the FTA-ABS sorbent prepared at present (*7*). The following media and medium components were investigated: NIH thioglycollate broth (Difco Laboratories, Inc., Detroit, Mich.), spirolate broth (Baltimore Biological Laboratories, Inc., Baltimore, Md.), trypticase (BBL), casitone (Difco), yeast extract (BBL and Difco), tryptone (Difco), peptone (Difco), casamino acids (Difco), dextrose, sodium chloride, sodium thioglycollate, disodium phosphate, and l-cystine.

Parent compounds and derivatives of several amino acids, purines, pyrimidines, and certain miscellaneous compounds were tested for sorbent effect at a 1 percent concentration in phosphate buffered saline (*1*), or as saturated solutions if they were not soluble to a 1 percent concentration. Those compounds having D-structural and L-structural configurations were tested as the L-isomer or the DL-mixture. Compounds tested for sorbent effect were glycine, alpha alanine, beta alanine, cystine, cysteine (free base), cysteine HCl, leucine, isoleucine, norleucine, methionine, sarcosine HCl, serine, threonine, threonine (allo threonine free), alpha amino-n-butyric acid, gamma aminobutyric acid, valine, aspartic acid, asparagine, glutamic acid, glutamine, citruline, lysine, (mono-HCl), arginine mono-HCl, ornithine mono-HCl, phenylalanine, tyrosine, histidine mono-HCl, proline, hydroxy-L-proline, tryptophane, adenine, thymine, uracil, guanine (free base), cytosine, xanthine, hypoxanthine, 6-thio guanine, 6-mercapto guanine, 6-methyl mercaptopurine, 5-nitro uracil, spermine, spermidine phosphate, thymidine, creatinine, taurine, betaine, glutathione, glycylglycine. Commercial nucleic acids (DNA and RNA) were tested at 2 percent concentration.

Five percent aqueous solutions of yeast extract in 0.85 percent NaCl were deproteinized by shaking vigorously with a mixture of chloroform and iso-amyl alcohol (39:1 v/v) according to the method of Sevag and co-workers (*8*). Deproteinization was repeated until there was no evidence of precipitated protein at the interface of the aqueous and chloroform and iso-amyl alcohol phases. The aqueous phases were combined and fractionated into 80 percent ethanol soluble and insoluble (nucleic acid) components (*9*). Each fraction was lyophilized and rehydrated to twice the original volume. Each fraction was soluble at the concentration to which it was rehydrated. The residue of the chloroform and iso-amyl alcohol phase was a waxlike substance that could not be solubilized and investigated for sorbing effect.

Fluorescent treponemal antibody (FTA) 1:5 (*10*) and FTA-ABS (*1*) tests were performed according to the recommended techniques except that various substances replaced the FTA-ABS test sorbent. Reagents were supplied by the Reagents Control Activity of the Venereal Disease Research Laboratory (VDRL).

In a series of tests involving the investigation of several substances, or dilutions of a given substance, the test tubes were numbered ran-

domly and the person performing the tests did not know the numbering sequence.

The results of the FTA-ABS and FTA 1:5 tests represent either constantly observed intensity of fluorescence or the range (lower and upper limit) observed on each of 3 testing days based on the scale of 4+ (maximal fluorescence), 3+, 2+, 1+, ±, and N (no fluorescence). Therefore, for comparative purposes, the recorded values are the observed intensities of fluorescence rather than the customary interpretation of test results.

It should perhaps be pointed out, to those not familiar with interpreting intensities of fluorescence, that day-to-day variations occur with a constant lot of reagent, with different lots of reagents, and in the interpretation of degrees of fluorescence among several observers. To minimize variations, reagents were the same whenever possible and all tests were read by one person; to further assure validity of results, tests were performed three times, or until a comparable narrow range of results was obtained with three consecutive tests.

Results and Discussion

The efficiency of the sorbing effect of each medium, in which each of 22 spirochetal strains or species was cultivated, was compared with FTA-ABS sorbent. The FTA-ABS sorbent reduced the reactivity of a nonsyphilis control serum from a range of 3+ to 4+ with the FTA 1:5 test to N to ± with the FTA-ABS test. Media of 17 spirochetal strains demonstrated sorbing effect to the same degree as the FTA-ABS sorbent, three reduced the reactivity to the range ± to 1+, and two to a range of N to 1+. None reduced the 4+ reactivity of a syphilis control serum.

Uninoculated complete medium, processed in the same manner as the media in which the 22 spirochetal strains had been cultivated, reduced the reactivity of a nonsyphilis control serum from the range 3+ to 4+ to the range ± to 1+ that compared favorably with the N to ± range of the FTA-ABS sorbent with the same control serum. This observation suggested that the sorbing effect observed with the FTA-ABS sorbent prepared at present was related to medium components rather than to the common treponemal antigen (*2*) of a soluble nature derived from treponemal strains cultivated in the media (*4*). According to personal communications in 1967, Dr. K. F. Girard, Diagnostic Laboratories, Massachusetts Department of Public Health, and Miss B. E. McGrew and Joel S. Lewis of the Venereal Disease Research Laboratory have also noted the sorbing effect of uninoculated medium.

Individual medium components were tested for sorbing effect at 10, 5, and 2.5 times their concentration in NIH thioglycollate broth and spirolate broth. Pancreatic digests of casein (Bacto-casitone, trypticase) and yeast extract were the only medium components that demonstrated sorbing effect. The effectiveness of the substances was concentration-dependent, as evidenced by a reduction in sorbing efficiency with a decrease in concentration. This is also characteristic of FTA-ABS sorbent (*7*). However, the effective concentrations of the casein digests and yeast extract were higher than their concentration in the complete uninoculated medium that had shown sorbing effect. Also, there was an unaccountable variation in the sorbing effect of different lots of yeast extract and a moderate day-to-day variation with a given lot. Mixtures of casein digests and yeast extract, in the same proportion as that of the respective media, did not demonstrate greater sorbing effect than the substances tested separately.

These observations led to the supposition that certain fractions of these complex mixtures of proteins, peptides, amino acids, nucleic acids, and so forth, were more effective sorbing substances than others, and, furthermore, that the sorbing effect, although concentration-dependent, was not cumulative when two sorbing substances were combined. Yeast extract was arbitrarily chosen to determine if a relatively simple fractionation of the components would result in a fraction with a constant and reliable sorbing efficiency.

By employing the 80 percent ethanol insoluble (nucleic acids) and 80 percent ethanol soluble fractions of deproteinized yeast extracts as sorbents, it was found that both fractions were capable of sorbing the reactivity of nonsyphilis serum without affecting the 4+ reactivity of a syphilis serum control. The 80 percent ethanol

soluble fraction appeared to be a little more efficient and for that reason was selected for comparative evaluation with FTA-ABS sorbent. Before the comparative testing, the yeast fraction was tested by Joel S. Lewis of the Reagents Control Activity, VDRL, and was found to have a sorbent effect similar to that of FTA-ABS sorbent. Syphilis serum specimens were supplied by the Serum Bank and the reactive nonsyphilis specimens and FTA-ABS sorbent by the Reagents Control Activity.

Table 1 presents the results of FTA 1:5 testing and comparative testing of 30 syphilis serums with the FTA-ABS test using FTA-ABS sorbent and the yeast fraction employed as sorbent. The range of fluorescence observed on 3 different testing days with each sorbent was essentially the same with each serum. The greatest variations were with primary syphilis serum No. 32, for which the range with the FTA-ABS sorbent was 1+ to 2+ and with the yeast fraction ± to 1+, and secondary syphilis serum No. 18 with a range of 2+ to 4+ with FTA-ABS sorbent and a constant value of 2+ with the yeast fraction.

Table 1. Results of testing 30 syphilis serum specimens with the FTA 1:5 test and of comparative testing of FTA-ABS sorbent and the fraction of deproteinized yeast extract soluble in 80 percent ethanol as sorbent in the FTA-ABS test, by diagnostic category

Serum No.	Range of fluorescence observed on 3 different days		
	FTA 1:5	FTA-ABS sorbent	FTA-ABS (yeast fraction)
	Primary syphilis		
1	1–2	N–±	N–±
4	2–4	3–4	3–4
8	3–4	3–4	3–4
13	4	3–4	3–4
17	2–4	2–4	2–3
21	4	3–4	4
25	2–4	N–1	N–1
29	3–4	2–4	3–4
32	3–4	1–2	±–1
36	3–4	3–4	3–4
	Secondary syphilis		
2	4	3–4	3–4
6	4	4	4
11	3–4	3–4	3–4
15	3–4	3–4	3–4
18	3–4	2–4	2
21	4	3–4	4
26	4	4	4
30	4	3–4	4
33	4	4	4
38	4	3–4	3–4
	Latent syphilis		
3	3–4	4	3–4
7	3–4	3–4	3–4
12	3–4	3–4	3–4
16	3–4	3–4	4
19	3–4	4	3–4
24	3–4	3–4	3–4
28	3–4	4	4
31	4	3–4	3–4
34	3–4	3–4	3–4
40	3–4	3–4	3–4

Table 2 presents the results of comparative testing of 21 reactive nonsyphilis serums and two serums used as controls for the titration of FTA-ABS sorbent. There were no instances of disagreement in ranges of observed fluorescence in comparisons between FTA-ABS sorbent and yeast fraction. The comparative testing of these samples of 30 syphilis and 23 reactive nonsyphilis serums demonstrated that the yeast fraction had essentially the same sorbent efficiency as the lot of FTA-ABS sorbent to which it was compared.

Among the miscellaneous compounds listed in the "Materials and Methods" section (many of which are known to be present in casein digests and yeast extracts), apparent sorbing effect was observed with 1 percent concentrations of glutathione, cysteine HCl, lysine HCl, sarcosine HCl, and 2 percent commercial RNA and DNA. These compounds, although dissolved in phosphate buffered saline at pH 7.2, were found to have a pH range of 4.1–4.7. When the pH values were adjusted to 7.0, none demonstrated sorbing effect. Although many of these compounds are considered hydrolytic products present in casein digests and yeast extracts and therefore might be contributory, the lack of sorbent effect was expected, a priori, since the compounds, with the exception of the nucleic acids, are dialyzable, and prolonged dialysis had previously demonstrated the sorbent effect of the FTA-ABS sorbent to be nondialyzable, according to a personal communication from Dr. D. S. Kellogg, Jr., Venereal Disease Research Laboratory. Sorbent effect of yeast fractions also was subsequently found to be nondialyzable.

Table 2. Results of testing 23 reactive nonsyphilis serum specimens with the FTA 1:5 test and of comparative testing of FTA-ABS sorbent and the fraction of deproteinized yeast extract soluble in 80 percent ethanol as sorbent in the FTA-ABS test

Serum No.	Range of fluorescence observed on 3 different days		
	FTA 1:5	FTA-ABS sorbent	FTA-ABS (yeast fraction)
9	2–3	N–±	N–±
10	2–4	N–±	N–±
23	1–2	N–±	N–±
35	2–3	N–±	N–±
529	2–3	N	N
612	1–2	N–±	N–±
672	3	N	N
784	2	N	N
803	3	N	N
857	3	N	N
008	1–2	N	N
018	2	N	N
246	1–2	N	N
251	1	N	N
254	1	N	N
255	1	N	N
256	1	N	N
264	2–3	N	N
312	1–2	N–±	N
390	1	N	N
391	1	N	N
643 (control)	3–4	N	N
671 (control)	3–4	±–1	±–1

The observation that nontreponemal substances in casein digests and yeast extracts are capable of sorbing or blocking nonsyphilis reactivity (if concentrated enough, syphilis reactivity as well) poses the obvious question, "What components provide the sorbing, or blocking, effect?" Dr. T.A. Nevin of the Venereal Disease Research Laboratory, according to a personal communication in 1967, has isolated a peptide from the Reiter treponeme and from *T. zuelzerae* which has a blocking effect with reactive nonsyphilis serums. Indirectly and theoretically, the data of this study, coupled with the known diminishing molecular complexity of hydrolytic products of proteins, led us to suspect peptide as the sorbing substance. The hydrolytic decomposition of protein molecules to molecules of successively lesser complexity is considered to progress through several stages to the component amino acids: protein → protean → metaprotean → proteoses → peptones → peptides → amino acids (*11*).

The following three products, of a single manufacturer, representing successively greater hydrolytic decomposition were studied for sorbing effects: Bacto-peptone (relatively low concentration of peptides in relation to other nitrogeneous components), Bacto-casitone (moderate concentration of peptides in relation to other nitrogeneous components), and Bacto-casamino acids (relatively low concentration of peptides, principally amino acids). Only Bacto-casitone demonstrated sorbing effect. It does not seem likely that amino acids, singly or in combination, are responsible for the sorbent effect since a natural mixture (Bacto-casamino acids) and the investigated individual acids, or certain derivatives, did not demonstrate sorbing effect. Also, these compounds are dialyzable. and the sorbing effect is not.

From a practical point of view, the sorbent prepared at present for the FTA-ABS test provides a test reagent of adequate reliability. There are, however, variations in required concentration (30–40 percent) among several lots of sorbent, and the effect rather than the responsible substance is being measured. The isolation and characterization of the sorbing component could conceivably contribute to a better understanding of the kinetics of the reaction, as well as result in a more defined and reproducible test reagent. Studies of this nature are in progress.

Summary

An investigation of sorbing substances in the fluorescent treponemal antibody-absorption (FTA–ABS) test demonstrated that pancreatic digests of casein and yeast extract used as components of media for the cultivation of spirochetes are capable of sorbing, or blocking, the reactivity of nonsyphilis serums observed in the FTA 1:5 test when used in place of the usual sorbent of the FTA-ABS test. Ethanol (80 percent) soluble and insoluble fractions of deproteinized yeast extract also sorbed readily. The ethanol soluble fraction had sorbing efficiency equivalent to FTA-ABS sorbent when comparative testing was done with 30 syphilis serums and 23 reactive nonsyphilis serums. Peptides or peptide present in yeast extracts and hydrolytic decomposition products of casein are suspected to represent the sorbing substances or substance.

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To Study Adaptability of Eskimos

The ability of Eskimos to thrive in the earth's most hostile environment will be the object of a 5-year study by U.S., Canadian, Danish, and French scientists under the auspices of the International Biological Program (IBP), a 50-nation study of the biological basis of productivity and human welfare.

The remarkable success of Eskimos in adapting to difficult circumstances is reflected in their geographic distribution. Over the centuries, they have migrated around a large sector of the circumpolar world so that they and their close relatives, the Aleuts, occupy the longest linear distance of any group in the world.

The U.S. research effort will be concentrated near the origins of the Eskimo wanderings of Wainwright, Alaska, a village 90 miles from Point Barrow, with 300 residents. The Canadians will work near the center of the circumscribed migration route at Igloolik, a remote settlement in the Northwest Territories, while Danish and French scientists will investigate Eskimo adaptation at Upernavik in northeastern Greenland, one of the farthermost points in the Eskimo migratory pattern.

The U.S. portion of the Eskimo study was developed by the Human Adaptability Subcommittee of the US. National Committee for the IBP which is in the Division of Biology and Agriculture of the National Research Council. The major objective of this investigation is to determine the ways in which an Eskimo community on the Arctic coast of Alaska has successfully perpetuated itself under severe climatic conditions with relatively meager resources.

A multidisciplinary approach to the Wainwright study will be used, since it is believed that the complex processes of survival and population expansion in a harsh environment can only be understood if treated as a whole. As a result, scientists of various disciplines from the Universities of Wisconsin, Chicago, Indiana, Oregon, California at Los Angeles, the State University of New York at Buffalo, and the Wisconsin State Laboratory of Hygiene will participate in the American effort. Logistic support will be supplied by the Naval Arctic Research Laboratory in Point Barrow. The researchers will concentrate on aspects of genealogy and demography, genetic markers, growth and development, epidemiology, nutrition, environmental physiology, behavior, and population history and prehistory.

Data gathered by U.S. researchers will be coordinated with those collected in the Canadian and Danish-French studies to give an overall picture of Eskimo adaptation. In addition, all health-related information gathered in the American survey will be made available to government agencies in Alaska so that it may be used to better understand and treat Eskimo health problems.