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# FRACTIONATION STUDIES OF ANTIBODIES DETECTED IN SEROLOGICAL TESTS FOR SYPHILIS\*

## A PRELIMINARY REPORT

BY

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Studies employing treponemal and non-treponemal antigens in serological tests have indicated the existence of a multiplicity of antibodies in syphilis (Portnoy and Magnuson, 1955; Magnuson and Portnoy, 1956; Cannefax and Garson, 1959; McLeod and Garson, 1962; Deacon and Hunter, 1962), and suggest the probability that reagin, TPCF antibody, RPCF antibody and immobilizing antibody are separate and distinct. Tests developed to detect these antibodies as routinely performed (U.S. Public Health Services, 1959), tend to measure only one particular antibody. On the other hand, the FTA test may measure both TPCF and RPCF antibodies.

The use of fractionation of serum proteins by electrophoresis was considered as a means of obtaining additional evidence for the individuality or similarity of these antibodies. Earlier electrophoretic studies (Davis, Moore, Kabat, and Harris, 1945; Laurell, 1955) had shown the localization of reagin in the beta- and gamma-globulin fractions. More recently, using paper electrophoresis, TPI antibody was shown to be located in the gamma-globulin zone, whereas reagin was found in both the gamma globulin and beta-2 globulin zones (Laurell and Hederstedt, 1958). Because of its resolving power and yield of relatively large amounts of serum fractions, continuous flow electrophoresis (Durrum, 1951) was used in this study.

This report covers the fractionation and serological analyses of the serum of five human patients.

### Source of Specimens

These were obtained from clinically documented cases as follows:

Biologic false positive—1 case.  
Normal, presumed non-syphilitic—2 cases.  
Untreated primary syphilis, dark-field positive—1 case.

Untreated secondary syphilis, dark-field positive—1 case.  
Serum was separated by centrifugation and decantation and stored at  $-20^{\circ}\text{C}$ . until used.

### Methods

**Fractionation Procedure.**—Sera were thawed and dialysed against barbital buffer (Spinco B-2), ionic strength  $\cdot 02$ , pH 8·6, for approximately 48 hrs at about  $6^{\circ}\text{C}$ . Sera were filtered through 220  $m\mu$  Millipore filters.

The Spinco continuous flow paper electrophoresis cell, Model CP, was kept in a specially constructed chamber maintained at  $4-6^{\circ}\text{C}$ . Samples of dialysed serum, between 40 and 50 ml., were applied to the curtain at a rate of 0·5 ml./hr; a constant current of 93 milliamps was obtained, using the Beckman-Spinco Constat apparatus. The circulating buffer was completely contained in this cold chamber. Fractions were collected with an automatic fraction collector. At the end of the run, the curtain was dried and stained, using 1 per cent. bromophenol blue in methanol. On the basis of the bands observed on the stained curtain, the eluates obtained from the various drip points were pooled into the fractions indicated in Table I.

TABLE I  
CONCENTRATION OF SERUM PROTEINS ACHIEVED IN THE FRACTIONATION PROCESS USING PRIMARY SYPHILIS SERUM C-157

Protein Fraction	Serum (g./100 ml.)		Ratio Fractionated: Unfractionated
	Un-fractionated	Fractionated	
Gamma-4 globulin ..	0·24	0·33	1·4
Gamma-3 globulin ..	0·48	5·0	10·4
Gamma-2 globulin ..	0·46	7·3	15·9
Gamma-1 globulin ..	0·20	5·1	25·5
Gamma Beta globulin	0·40	3·6	9·0
Beta globulin ..	0·67	7·1	10·6
Beta Alpha-2 globulin	0·23	5·7	24·8
Slow Alpha-2 globulin	0·31	3·1	10·0
Fast Alpha-2 globulin	0·41	3·8	9·3
Alpha-1 globulin and slow albumin ..	0·71	15·0	21·1
Medium albumin ..	3·15	18·9	6·0
Fast albumin ..	0·18	21·2	
Pre-albumin ..		10·1	
	7·44		

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Pooled fractions were filtered through 220 m $\mu$  Millipore paper and dialysed at 6°C. against cold distilled water for 5 days and then lyophilized.

**Physical, Chemical, and Serological Analyses of Fractions.**—The dried protein fractions were dissolved with small volumes of TPCF buffer (U.S.P.H.S., 1959). Insoluble matter was removed by centrifugation.

Protein concentration of the fractions was determined by the trichloroacetic acid (TCA) method (U.S.P.H.S., 1959).

Serological tests were performed in accordance with the Manual of Serologic Tests for Syphilis (1959).

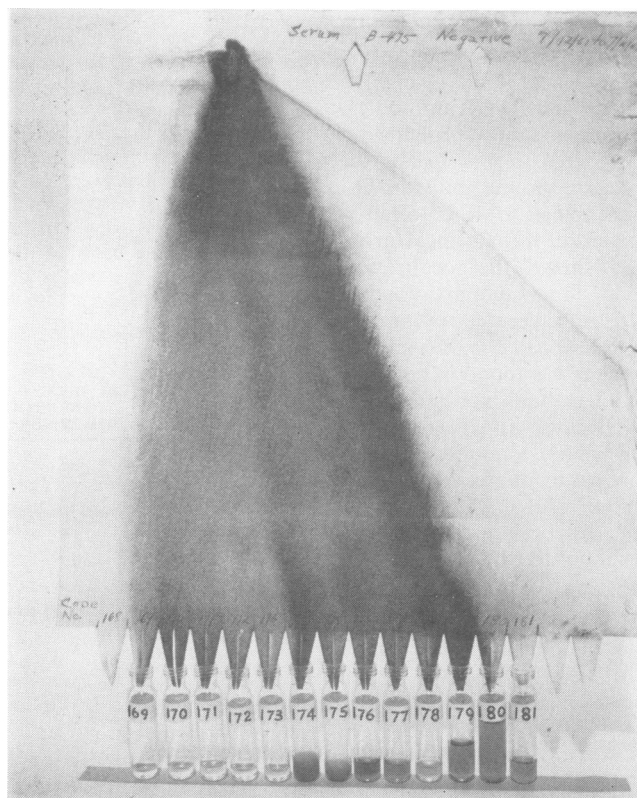
The mobility characteristics of the isolated fractions were determined using the Spinco vertical paper electrophoresis apparatus (Durrum cell). A sample volume of .008 ml. was applied on S & S paper 2043 Agl. Barbitol buffer, pH 8.6, ionic strength .075, was used. The cells were run for 16 hrs at room temperature using a constant current of 3 milliamps. Scanning was accomplished with the Beckman-Spinco Analytrol at 1.5 mm. slit-width, balanced cam No. 5,500. m $\mu$  filter.

## Results

**Separation and Identification of Fractions.**—The Figure illustrates the results obtained with the fractionation process. Superimposed against the stained curtain of normal serum B475 are the concentrated solutions resulting from the complete fractionation process. The isolated fractions were examined by vertical paper electrophoresis and their characteristic mobilities were similar to the unfractionated serum.

The magnitude of the concentrations of serum fractions achieved by the method employed is illustrated in Table I, which presents the results obtained for the primary syphilis serum C-157.

**Serological Activity associated with Fractions.**—The fractions were tested with quantitative VDRL slide and one-fifth volume Kolmer complement-fixation tests with cardioliipin (KCCF) and Reiter



FIGURE

## ANTIBODIES DETECTED IN SEROLOGICAL TESTS FOR SYPHILIS

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protein antigen (RPCF). The fluorescent treponemal antibody (FTA) test was performed qualitatively, using the original technique (1 : 5 serum dilution) (Deacon, Falcone, and Harris, 1957), and the newer technique (1 : 200 serum dilution) (Deacon, Freeman, and Harris, 1960). The treponema pallidum immobilization (TPI) test was performed only qualitatively.

The results obtained in these tests are given in Table II. To conserve space, particular tests were left out of the tabulation if negative results were obtained with all fractions tested.

*Primary Syphilis.*—Reagin was located in the broad area covering the gamma and beta globulins. Antibody detected by the RPCF was confined to the gamma-3 globulin zone. Antibody detected by the FTA 1/5 test was found over a broad area covering the gamma and beta globulins, whereas the antibody detected by the FTA 1/200 was confined to the gamma-3 and gamma-2 globulins. No antibody was detected in the TPI test.

*Secondary Syphilis.*—Reagin as measured by the VDRL slide test was confined to the gamma globulin area, whereas that detected by the Kolmer cardioli-  
pin complement-fixation test was located in a wide zone covering gamma and beta globulins; Reiter antibody was found in all gamma globulin fractions as well as in an interzone between beta and gamma globulins. Antibody as measured by the FTA 1/5 was broadly spread from the gamma-4 to the slow alpha-2 globulin; antibody measured by the FTA 1/200 was found in all gamma globulin plus the beta-gamma globulin fractions. TPI activity was confined to the gamma-3 and gamma-2 globulins.

*Normal Sera.*—In one of these VDRL slide activity was demonstrated in the fast alpha-2 globulin, and Kolmer cardioli-  
pin activity was found in the gamma-2 globulin. In the other normal serum several of the fractions showed activity with the FTA test 1/5.

*Biologic False Positive.*—This serum showed a broad spread of activity covering all gamma globulin

TABLE II  
SEROLOGICAL ACTIVITY OF ISOLATED FRACTIONS\*

Clinical Category	Test‡	Un-fractionated Serum	Globulins										Albumins
			Gamma-4	Gamma-3	Gamma-2	Gamma-1	Beta Gamma	Slow Beta	Fast Beta	Beta Alpha-2	Slow Alpha-2	Fast Alpha-2	
Normal (B-475)	VDRL ..	0	0	0	0	0	0	0	0	0	0	250	0
	KCCF ..	0	0	0	90	0	0	0	0	0	0	0	0
Normal (B-355)	FTA 1/5 ..	0	0	0	+ †	+	0	+	0	0	0	0	0
Primary Syphilis	VDRL ..	47	16	62	23	64	45	89†	0	0	0	0	0
	KCCF ..	19	8	8	6	130	23	22	0	0	0	0	0
	RPCF ..	190	0	8	0	0	0	0	0	0	0	0	0
	FTA 1/5 ..	+	0	+	+	+	+	+	+	0	0	0	0
	FTA 1/200	+	0	+	+	0	0	0	0	0	0	0	0
Secondary Syphilis	VDRL ..	6	11	6	12	14	0	0	0	0	0	0	0
	KCCF ..	5	3	2	3	7	14	14	290	0	0	0	0
	RPCF ..	22	3	8	12	14	220	0	0	0	0	0	0
	FTA 1/5 ..	+	+	+	+	+	+	+	+	+	0	0	0
	FTA 1/200	+	+	+	+	+	+	0	0	0	0	0	0
	TPI ..	+	0	+	+	0	0	0	0	0	0	0	0
Biologic False Positive	VDRL ..	24	34	24	32	20	56	0	0	0	64	0	0
	KCCF ..	18	4	0	32	0	0	0	0	0	0	0	0
	FTA 1/5 ..	+	0	+	+	+	+	0	0	0	0	0	0

+ Positive (Reactive).

0 Negative (Non-reactive).

\* Values expressed as smallest amount of fraction in g./100 ml., giving a positive (reactive) result, multiplied by 100 to avoid use of decimal point.

† Where value appears between two fractions, individual fraction not obtained.

‡ The absence of a recording for a particular test indicates that non-reactive results were obtained with all listed fractions in the particular test or tests.

plus the mixed beta-gamma globulin fractions as measured by the VDRL slide test; reactivity was also found in the fast alpha-2 globulin. Reactivity with the Kolmer cardiolipin complement-fixation test was confined to two of the gamma globulin areas. Antibody as detected by the FTA 1/5 was found in the area covering the gamma-3, -2, -1, and beta-gamma globulin zone.

### Discussion

The results obtained in these limited studies suggest that the fractionation procedure used was effective. The similarity of the paper electrophoretic mobilities of the isolated fractions with those obtained with unfractionated serum suggested that the technique employed did not significantly alter the proteins. This was a factor of considerable importance with respect to the serological analyses, since alterations in the serum proteins might be expected to effect serological reactivity. The generally high concentrations of serum fractions used in the serological tests add to the validity of these determinations. These concentrations were considerably higher in all instances than those present in the unfractionated sera.

The finding of reagin over a broad area covering beta and gamma globulins and the confinement of TPI antibody to the gamma globulin zone is in agreement with previous reports (Laurell, 1955; Laurell and Hederstedt, 1958).

Although the FTA-200 is the recommended method for performing the fluorescent treponemal antibody (FTA) test, it seemed advisable to perform this test as well at a 1 : 5 dilution in order to obtain greater sensitivity. Reactivity with the FTA 1/5 was observed over a greater number of fractions than was noted with the FTA 1/200. No attempt was made to differentiate the virulent *T. pallidum* component of these reactions from the Reiter component (Deacon and Hunter, 1962).

The observation of reactivity in the VDRL slide test with the fast alpha-2 globulin of the biologic false positive serum and one of the two normal sera was anomalous; this bears further investigation, particularly in the continuing search for the differentiation of the biologic false positive reactors.

The findings contained in this report are of a preliminary nature and await substantiation by the performance of similar analyses on additional cases.

### Summary

Continuous flow paper electrophoresis was employed to fractionate five human sera, obtained from two patients with early syphilis, two persons presumed non-syphilitic, and one biologic-false positive reactor.

Serological analyses, including treponemal and non-treponemal tests, were performed on these fractions.

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### Études de la fractionation des anticorps découverts au cours des réactions sérologiques de la syphilis

#### RÉSUMÉ

L'électrophorèse sur papier par effusion continue fut utilisée pour fractionner 5 sérums humains (obtenus de deux cas de syphilis précoce, deux individus non atteints de syphilis, et un individu ayant une réaction positive fautive).

Ces fractions furent donc examinées par les tests sérologiques tréponémaux et non-tréponémaux.

**STI**

# Fractionation Studies of Antibodies detected in Serological Tests for Syphilis: A PRELIMINARY REPORT

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