PRESUMPTIVE FALSE POSITIVE SEROLOGIC REACTIONS FOR SYPHILIS IN CENTRAL AMERICA

III. Relation to Serum Protein, Albumin, and Globulin.

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THE incidence and distribution of presumptive biologic false positive reactions in serologic tests for syphilis and their relation to serum levels of ascorbic acid, riboflavin, alkaline phosphatase, carotene and vitamins A and E, have been discussed in preceding papers.[1,2] The latter report by us indicates these reactions are associated with low serum levels of vitamins A and E and carotene. No satisfactory explanation has been found for the unusual frequency of biologic false positive reactions in Central America.

In both syphilitic and biologic false positive human sera, the reactive antibodies are associated with the serum globulin[3]; therefore, it seemed desirable to investigate the relation of serum total protein, albumin, and globulin to the occurrence of false positives in Central America. These studies appeared particularly worthwhile because serum protein levels tend to be normal or higher than normal in nutrition surveys conducted in the countries of Central America despite the generally unsatisfactory character of the diets upon which these groups subsist.[4] The parallel between the high incidence of biologic false positive reactivity and the apparent elevation of serum protein values, led to the hope that some causative relationship or common factor might be found.

The fourth paper in this series will discuss serologic reactivity in relation to the cephalin cholesterol (Hanger) flocculation tests.

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METHODS

Only school children within the age range of 7 to 12 years in the Central American countries of Costa Rica, El Salvador, Guatemala, and Honduras have been included in this study. Most of these children were examined on two or more occasions during 1950 and again during the first half of 1951. Although the blood samples were drawn in the morning, they are not necessarily fasting samples. The serum protein determinations reported for all of Central America, were done by the gradient method[5] the same day that the sample was drawn. The potassium sulphate standards were supplied at intervals by the central laboratory in Guatemala to the branch laboratories in each country. The separation of protein fractions was carried out only on samples from Guatemala, using the technique of Kibrick and Blonstein.[6] The relative final concentrations as well as the serum total protein values in this work were determined by the biuret method.[7]

The battery of tests on which the serologic classification is based include the Kahn, Mazzini, Kolmer, VDRL, and the Kline cardiolipin.[8] Group I contains doubtful reactions to the Kahn and/or Mazzini tests or positive reactions to one of them. Group II, the presumptive false positives, gave positive reactions to to both Kahn and Mazzini or a positive reaction with one of these tests and doubtful with the other. In both Groups I and II, the Kolmer, VDRL, and Kline tests were negative. Positive or doubtful in all tests are classed in Group III, that of presumptive syphilis. A patient is considered negative when negative reactions are obtained on all five tests. The basis of this classification has already been discussed.[1]

The standard deviation, t values and probabilities were determined by standard statistical methods.[9,10]

RESULTS

The average serum protein values as determined for Central American children by the gradient method in the four reaction Groups are given in Table I.

TABLE I. SERUM PROTEIN VALUES IN SEROLOGIC REACTOR GROUPS IN CENTRAL AMERICA

GROUP I	N	134	GROUP III	N	27
	Mean	6.98		Mean	6.82
	σ	.64		σ	.49
GROUP II	N	60	GROUP IV	N	284
	Mean	7.10		Mean	7.09
	σ	.55		σ	.48

GROUP I vs. II	t	1.33	P –	.19
GROUP I vs. IV	t	1.77	P –	.08
GROUP II vs. IV	t	.12	P –	.92

N = Number of observations

σ = Standard Deviation

$$t = \frac{m_1 - m_2}{\sqrt{\frac{\sigma_1^2}{N_1} + \frac{\sigma_2^2}{N_2}}}$$

P = Probability

Gross differences in average values do not appear and no significant differences are noted when the doubtful and false positive Groups I and II are statistically compared with each other and with the negative Group IV.

In Table II are presented average serum protein values determined by the biuret method for a group of Guatemalan children. The average percentage of albumin and globulin is given together with the albumin/globulin ratio. In each case the standard deviation appears. No gross differences are apparent in the average values; and the standard deviations are similar for all three groups with the exception of the total protein values for Group II. No explanation can be offered for the smaller standard deviation and hence less variation within this group.

TABLE II. SERUM PROTEIN, PERCENT ALBUMEN AND GLOBULIN, ALBUMIN/GLOBULIN RATIO IN SEROLOGIC REACTOR GROUPS IN GUATEMALA

	N		PROTEIN GM. %	ALBUMIN %	GLOBULIN %	A/G RATIO
GROUP I	54	Mean	7.47	55.4	44.6	1.26
		σ	.50	3.6	3.6	.17
GROUP II	26	Mean	7.53	56.4	43.6	1.32
		σ	.17	4.4	4.4	.26
GROUP IV	115	Mean	7.52	56.4	43.6	1.31
		σ	.51	3.2	3.3	.14

N = Number of observations
σ = Standard Deviation

In Table III, Groups I and II (the doubtfuls and the presumptive false positives) are again compared with each other and with the negative Group IV for total protein, as well as the percentage of albumin and globulin and the albumin globulin ratio. None of the statistical comparisons show a significant difference. The distribution of alpha, beta, and gamma globulin in the total globulin fraction was studied in two patients in Group I, one in Group II and twenty-three in Group IV. Combining Groups I and II the average values were *1.09*, (.14), *.86*, (.03) and *1.29*, (.17) for alpha, beta, and gamma globulin

TABLE III. SIGNIFICANCE OF DIFFERENCE IN SERUM PROTEIN, ALBUMIN, GLOBULIN AND ALBUMIN GLOBULIN RATIO IN SEROLOGIC REACTOR GROUPS IN GUATEMALA

		TOTAL PROTEIN	PERCENTAGE ALBUMIN	PERCENTAGE GLOBULIN	ALB/GLOB RATIO
GROUP I vs. II	t	.75	.99	1.01	1.07
	P	.45	.32	.31	.26
GROUP I vs. IV	t	.62	1.69	1.69	1.67
	P	.55	.09	.09	.09
GROUP II vs. IV	t	.08	.02	.02	.18
	P	.95	.97	.97	.85

For abbreviations see Table I.

respectively compared with *1.11*, (.17), *0.92*, (.33) and *1.35*, (.20) for the negative Group IV. Standard deviations are given in parenthesis. These differences are not statistically, significant, although the small number of cases greatly decreases the chance of detecting a significant difference if it occurs.

DISCUSSION

Despite the occurrence of anomalous serum protein values among the same population groups found to have a high incidence of false positive serology, the direct comparison has not led to significant findings. Neither the serum total protein nor the albumin and globulin fractions appeared to be different in negative and false positive groups. This is consistent with the report that electrophoresis, ultracentrifugation, and purification of antibody failed to show significant differences for false positive sera.[11] Others have reported no unique components and no significant mobility differences among serum protein constituents of syphilitic, biologic false positive or negative sera.[12,13]

Davis[14] considers the impression unwarranted that false positive reactions are often caused by conditions which give rise to marked hyperglobulinemia. In the three cases studied the gamma globulin was within normal limits in doubtfuls and false positives. Since it has been reported[3] that the reactive antibodies are associated with the serum gamma globulin in both syphilitic and biologic false positive human sera, differences in the serum gamma globulin would not necessarily be found.

The reversal to negativity of nonspecific serologic reactions reported by Barnes[15] following the change to a meat free, milk free, abundant liquid diet is different from the findings in Central America. The diets of the individuals in the present studies were low in animal protein[4,16] and yet false positive reactions occurred with great frequency. Breazeale is quoted as finding no relation between nutritional habits and unsatisfactory serologic findings in southwestern Indian groups.[17]

Although it seems probable that serum proteins are involved in the biologic false positive phenomenon, it is apparent that satisfactory methods for detecting this are not available or have not been applied to the problem. Certainly studies of serum protein fractions to date, including this one, have failed to demonstrate consistent differences between negative and false positive reactors.

SUMMARY

Serum protein values are reported for school children 7 to 12 years of age in Costa Rica, El Salvador, Guatemala, and Honduras, divided into four serologic reactor groups for syphilis. The classification is based on reactions to the standard Kahn, Mazzini, Kolmer, VDRL, and Kline cardiolipin tests. One hundred thirty-four children are listed in a doubtful reactor group, 60 as presumptive false positive, 27 as presumptive syphilitics and 284 were negative on all tests. The differences in serum protein among the groups are not statistically significant.

In Guatemala 54 children in the doubtful category, 26 presumptive false positives, and 115 negatives are included in a study of serum total protein, albumin

and globulin. No statistically significant differences appeared among any of these three groups for these blood components or the albumin globulin ratio. Three children showing doubtful or presumptive false positive serology did not differ from 23 normals in alpha, beta, or gamma globulin as separated by sodium sulfate precipitation.

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