

# PRESUMPTIVE FALSE POSITIVE SEROLOGIC REACTIONS FOR SYPHILIS IN CENTRAL AMERICA

## IV. RELATION TO POSITIVE REACTIONS FOR CEPHALIN CHOLESTEROL (HANGER) FLOCCULATION TEST

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**R**EACTIONS of the presumptive biologic false positive type in serologic tests for syphilis are unusually frequent in the population of Central America.<sup>1</sup> No satisfactory explanation has been found for this phenomenon. Studies of the relation of serum ascorbic acid, riboflavin, alkaline phosphatase,<sup>2</sup> and of serum protein, albumin and globulin,<sup>3</sup> have failed to reveal significant correlations of these components with reactions of the false positive type. However, serum carotene and vitamins A and E were significantly lower in reactor groups.<sup>2</sup> The reason for these lower values is unknown.

In the course of field studies of the Institute of Nutrition of Central America and Panama, it had been noted that the incidence of positive reactions to the cephalin cholesterol (Hanger) flocculation tests were unusually high in the same population groups in which a similar percentage of presumptive false positive reactions had been encountered. This indicated that an attempt should be made to correlate the reactions to the two tests. The important role of the liver in antibody production and the close relation between liver function and the storage of fat soluble vitamins were further indications for the study reported below.

### MATERIAL AND METHODS

Only school children within the age range of 7 to 12 years in Guatemala have been included in this study. Most of these children were examined on two or

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more occasions during 1950 and again during the first half of 1951. The cephalin cholesterol flocculation determinations were done by the standard method<sup>4</sup> as were the determinations of Icterus Index, Van Den Bergh, and quantitative bilirubin.<sup>5</sup> The flocculation reactions were considered negative if the results were negative at 24 hours and negative or doubtful at 48 hours. Doubtful results consisted of doubtful or 1+ at 24 hours and 1+ at 48 hours. Weak positives consisted of doubtful or 1+ at 24 hours and 2+ at 48 hours. All reactions 2+ at 24 hours or greater than 2+ after 48 hours were considered strong positives for purposes of this classification.

The battery of tests on which the serologic classifications were based includes the Kahn, the Mazzini, the Kolmer, the VDRL, and the Kline cardiolipin.<sup>6</sup> Group I contained 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 both the Kahn or Mazzini or a positive reaction with one of these tests and a doubtful in the other. In both Groups I and II, the Kolmer, VDRL, and Kline tests were negative. Positive or doubtful reactions on all tests are classified in Group III, that of presumptive syphilis or true positives. No patient is considered negative unless he is completely negative in all five tests. The basis of this classification has already been discussed.<sup>1</sup>

## RESULTS

A comparison of the distribution of negative, doubtful, weak and strong cephalin cholesterol flocculation reactions among the serologic reaction Groups I, II, and IV are shown in Table I. Reaction Group III, the serologic positives, are omitted from this table because of the small numbers involved. In Table I, distribution of cephalin cholesterol reactions in each serologic group is compared with the distribution in the total group by means of the Chi square test. A high "Chi square" and resulting significant probability would indicate that the distribution was not uniform in the three serologic groups. Since all of the "Chi squares" are low it is concluded that serologic classification has no effect on the distribution of cephaline cholesterol results.

TABLE I. RELATION OF CEPHALIN CHOLESTEROL (HANGER) FLOCCULATION TEST RESULTS TO SEROLOGIC REACTOR GROUPS

CEPHALIN CHOLESTEROL GROUPS	TOTAL OBSERVATIONS	NEGATIVE		DOUBTFUL		WEAK POSITIVE		STRONG POSITIVE	
		N	X <sup>2</sup>	N	X <sup>2</sup>	N	X <sup>2</sup>	N	X <sup>2</sup>
Reactor Group I	102	55	0.34	24	0.19	12	0.88	11	.01
Reactor Group II	49	26	0.23	10	0.03	4	.04	9	2.50
Reactor Group IV	160	101	0.59	33	0.05	12	.40	14	.68
Total	311	182	1.16	67	0.28	28	1.32	34	3.19

N = Number of observations; X<sup>2</sup> = Chi square based on difference from total frequency. No significant frequency differences appear in this table.

In Table II, the distribution of serologic groups among the cephalin cholesterol flocculation groups is tested. In the first three columns the frequency of flocculation reactions in each of the three serologic groups does not differ significantly from the total distribution of these groups in the present study. Neither the individual nor the total "Chi squares" are high enough to yield a probability even approaching significance.

TABLE II. RELATION OF SEROLOGIC REACTOR GROUPS TO RESULTS OF THE CEPHALIN CHOLESTEROL (HANGER) FLOCCULATION TESTS

CEPHALIN CHOLESTEROL	TOTAL OBSERVATIONS	REACTOR GROUP I		REACTOR GROUP II		REACTOR GROUP IV		REACTOR GROUP I AND II*	
		N	X <sup>2</sup>	N	X <sup>2</sup>	N	X <sup>2</sup>	N	X <sup>2</sup>
Negative	182	55	0.37	26	0.25	101	0.59	81 127	1.77
Doubtful	67	24	0.21	10	0.03	33	.06	34 43	0.53
Weak and Strong Positive	62	23	0.36	13	1.08	26	1.07	31 39	1.07
Group X <sup>2</sup>		102	.94	49	1.35	160	1.72	142	3.37
df, P		2	.65	2	.54	2	0.40	2	.20

df = degrees of freedom

P = Probability

\*Compared against distribution of flocculation results in the negative Serologic Group IV instead of with the overall distribution as is done for the other comparisons.

In the last column of Table II, serologic Groups I and II have been combined. This is done in order to yield larger numbers for the statistical treatment and is justified by the failure of the present series of studies to reveal differences between these groups for the substances tested. The frequency of flocculation results in these combined groups has been compared with the frequency in the negative serologic group. Again, no significant difference is found. It must be concluded that despite the high incidence of positive results on both tests in the population studied, there was no detectable tendency for positive results to coincide in the same individual more often than would occur by chance.

Actually, a complete battery of liver function tests were run which included the Icterus Index, the serum bilirubin, and the Van Den Bergh reactions. However, the frequency of positive responses to these tests was so far below the frequency of false positives, that statistical comparisons were not worthwhile.

#### DISCUSSION

In the previous paper of this series it was reported that the distribution of high serum protein seemed to parallel the high incidence of false positive serology, yet when correlation between the two findings was sought, none was found. The same conclusion applies to the attempt to correlate the incidence of false positive serologic tests with the high incidence of doubtful or positive reactors with the cephalin flocculation test in the same populations. It is unusual for

three abnormal serum findings (high serum protein,<sup>2</sup> false positive serologic tests<sup>7</sup> and positive cephalin cholesterol flocculation results) in the same population to have no etiologic relationship. Yet this would appear to be the case. In view of the primary role of the liver in antibody formation and the occurrence of biologic false positives in certain liver diseases, the above data are disappointing. No satisfactory explanations are at present available for any of the three serum abnormalities mentioned. The search for the causative factors must be continued.

#### SUMMARY

Three hundred and eleven Guatemalan school children were examined for their reactions to the cephalin cholesterol (Hanger) flocculation test as well as a battery of serologic tests for syphilis. A high incidence of doubtful and presumptive false positive serologic reactions with the tests for syphilis and frequent doubtful or positive reactions to the cephalin flocculation test are found in this population. However, there is no detectable tendency for positive results on both tests to be found in the same individual more often than would occur by chance. Icterus Index, Van Den Bergh, and quantitative bilirubin were also determined but there are very few abnormal findings on these tests in comparison to the number of doubtful and presumptive false positive reactors in the tests for syphilis.

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