PRESUMPTIVE FALSE POSITIVE SEROLOGIC REACTIONS FOR SYPHILIS IN CENTRAL AMERICA

II. Relation to Serum Ascorbic Acid, Riboflavin, Alkaline Phosphatase, Carotene, and Vitamins A and E in Blood Serum

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THE incidence and distribution of presumptive biologic false positive reactions in serologic tests for syphilis have been discussed in a preceding paper.[1] There is no doubt that reactions of this type are frequent in the population of Central America. No satisfactory explanation has been found for the phenomenon. The incidence in school children does not seem to be influenced by the supplementary feeding of a "snack" rich in protein of good quality.[1] Nevertheless, it was considered possible that variations in the serum level of one or more of the vitamins might show some correlation with serologic reactions of the biologic false positive type.

In the present study, serum levels of ascorbic acid, riboflavin, alkaline phosphatase, carotene, and vitamins A and E, have been studied in relation to this phenomenon and significant correlations established for three of these. Subsequent papers will discuss the relation of biologic false positive reactions to serum protein, albumin and globulin, and to reactions to the cephalin cholesterol (Hanger) flocculation test.

MATERIAL AND METHODS

Only school children within the age range of seven to twelve 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. In general, the blood samples were drawn in the morning but are not necessarily fasting samples. All samples were refrigerated soon after being drawn and

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continuously chilled until the serologic examination could be carried out. The serum samples from Costa Rica, El Salvador, and Honduras were stored in a commercial deep freezer until they could be sent to Guatemala. This procedure did not seem to influence the distribution of biologic false positive reactions in the sera of the different countries.[1]

The vitamin determinations were all done on an ultra microscale according to the following published methods: ascorbic acid,[2,3] riboflavin,[4] alkaline phosphatase,[5] vitamin A[6] and carotene and vitamin E.[7] In the ascorbic acid analyses, 0.6 per cent copper sulphate ($CuSO_4 \cdot 5H_2O$) was substituted for the norite described in the original method.[8] In all cases the samples were analysed before any deterioration in storage occurred.

The battery of tests on which the serologic classifications are based include the Kahn, Mazzini, Kolmer, VDRL, and the Kline cardiolipin.[9] 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 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. The presumptive syphilitic Group III (positive or doubtful with all tests) is not discussed. Sera are 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.[10,11]

RESULTS

The average serum chemistry values for the various serologic reactor groups are given in Table I. The nutritional significance of these values will be discussed elsewhere. No differences are obvious in the serum ascorbic acid, ribo-

TABLE I. AVERAGE SERUM CHEMISTRY VALUES FOR SEROLOGIC REACTOR GROUPS IN CENTRAL AMERICA

REACTOR	GROUP	ASCORBIC ACID (VITAMIN C) mg. %	FREE RIBOFLAVIN mg. %	ALKALINE PHOSPHATASE mM/l/hr.	CAROTENE mg. %	VITAMIN A mg. %	VITAMIN E mg. %
TYPE I	N	53	56	56	56	56	56
	MEAN	1.79	1.62	5.22	87.2	24.7	.64
	σ	1.11	.90	1.52	53.4	9.0	.22
TYPE II	N	20	20	20	20	20	20
	MEAN	1.55	1.72	4.92	71.0	21.9	.64
	σ	.91	1.36	1.31	43.9	8.9	.20
TYPE IV	N	135	141	141	141	130	141
	MEAN	1.70	1.51	5.10	104.2	28.2	.79
	σ	1.02	.85	1.87	71.6	10.8	.10

N = Number of observations
σ = Standard deviation

flavin and alkaline phosphatase levels of the various reactor groups. This is confirmed by Table II, in which no significant difference appears through the use of the tests when the groups are compared for these blood constituents. The calculated probabilities do not suggest any systematic differences among the groups in Table II.

TABLE II. PROBABILITY OF SIGNIFICANT DIFFERENCES IN SERUM VITAMIN E, RIBOFLAVIN AND ALKALINE PHOSPHATASE LEVELS AMONG SEROLOGIC REACTOR GROUPS

COMPARISON		ASCORBIC ACID (VITAMIN C)	FREE RIBOFLAVIN	ALKALINE PHOSPHATASE
GROUP I vs. II	df	73	76	76
	t	.96	.31	.83
	P	.33	.76	.42
GROUP I vs. IV	df	188	197	197
	t	.50	.78	.47
	P	.60	.39	.65
GROUP II vs. IV	df	155	161	161
	t	.68	.68	.54
	P	.50	.50	.60

df = degree of freedom

$$t = \frac{m_1 - m_2}{\sqrt{\frac{6_1^2}{N_1} + \frac{6_2^2}{N_2}}}$$

P = probability

When the values are examined for the fat-soluble vitamins, carotene, vitamin A and vitamin E, the reactor Groups I and II appear to differ from the negative Group IV. This is confirmed by Table III in which differences are tested statistically, although no significant difference appears between reactor Groups I and II in regard to these vitamins. It is clear from the figures of Table I and the highly significant probabilities of Table III that both these groups differ

TABLE III. PROBABILITY OF SIGNIFICANT DIFFERENCES IN SERUM CAROTENE VITAMIN E AND VITAMIN A LEVELS AMONG SEROLOGIC REACTOR GROUPS

COMPARISON		CAROTENE	VITAMIN E	VITAMIN A
GROUP I vs. II	df	76	76	76
	t	1.3	0	1.2
	P	.20	1.0	.23
GROUP I vs. IV	df	197	197	186
	t	1.83	3.5	2.3
	P	.068b	.0005	.02
GROUP II vs. IV	df	161	161	150
	t	2.9	3.75	2.2
	P	.004	.0002	.025

For abbreviations see Table II.

markedly in vitamin A and vitamin E from the negative Group IV. In the case of carotene, the difference between Groups II and IV is also statistically significant. The difference between Groups I and IV however, while strongly suggestive, is not within the 5 per cent level of significance (probability less than .05).

Since no differences appear between Groups I and II, these might be combined and tested against Group IV. This procedure would serve only to emphasize the already high statistical validity of the relation of lower serum levels of vitamin A and E to reactor Groups I and II. When the carotene values for Groups I and II are combined for comparison against Group IV, the difference is definitely significant (probability—.007).

DISCUSSION

When the reaction types were originally set up, it was not known whether Type I should be considered a weak biologic, false positive group or a doubtful group so heterogeneous in composition as to be of little value for providing information regarding the relation of false positives to other observations. The above results suggest that Group I is in fact quite different from the negative group in respect to serum vitamin A and E, two vitamins in which the Group II reactors, the presumptive false positives, also differ. Furthermore, the difference between Group I and IV in serum carotene, while not statistically significant, shows the same trend as that between Group II and Group IV. In contrast, Groups I and II do not differ significantly in respect to vitamin A, vitamin E or carotene. It would appear from these data, at least, that as a group even the doubtful reactions and the positive reactions appearing on only one test, are frequently significant manifestations of the false positive phenomenon.

The meaning of these lower carotene, vitamin A and vitamin E levels in the presumptive false positive groups is not apparent at the present time. In view of the failure of biologic false positive tests to decrease with feeding,[1] or show any relation to the other serum constituents tested, it is unlikely that these findings can be taken to indicate a primary relationship. Although the nutritional significance of these data will be discussed elsewhere, there is no indication that the blood levels of these substances in Groups I and II are below normal averages.* It also seems improbable that the ingestion of carotene, vitamin E or A as such has any relation to the phenomenon under discussion.

It is more likely that some common factor is responsible for the parallel observations.[12]

Vitamin A, vitamin E and carotene are fat soluble factors and must be carried in the blood in relation to the serum lipids or lipo-proteins. Volkin[13] has reported that extraction with fat solvents tends to reduce the activity of serum fractions that inhibit the development of biologic false positive reactions. It has also been reported that human serum lecithin fractions are more effective than whole serum fractions in inhibitory activity toward euglobulin fractions of biologic false positive origin.[14] Neurath and his associates[15] suggest that possibly serologic activity will vary with the specificity and activity of the inhibitor in whole serum.[15]

*The carotene levels would be considered low by some authorities.

It is not impossible that serum vitamin A and E, because of their relation to the lipids of the plasma, reflect in some way the amount of inhibitor present.

The diets of these children are also known to be low in fat.[16] Under these circumstances the absorption of fat from the gastrointestinal tract and its metabolism in the body may not be normal. Whether this has any relation to the phenomenon described is unknown. That some relation between the fat soluble constituents measured, however indirect, must exist is apparent from the data.

SUMMARY

Serum levels for ascorbic acid (vitamin C), riboflavin, alkaline phosphatase, carotene, vitamin A and vitamin E are given for negative, doubtful, and presumptive biologic false positive reactor groups in Central American school children 7 to 12 years of age. A minimum of 130 in the negative group, 53 in the doubtful, and 20 in the presumptive false positive are tabulated. The classification has been based on the reactions to the standard Kahn, Mazzini, Kolmer, VDRL, and Kline test for syphilis.

No significant differences appear among the various groups in relation to serum ascorbic acid, riboflavin, and alkaline phosphatase. The presumptive false positive group shows a statistically significant difference from the negative serologic group in carotene and vitamins A and E. The difference was also significant for vitamins A and E in the case of the doubtful compared with the negative group. No significant difference was found between the average carotene, vitamin A and vitamin E levels of the doubtful and presumptive false positive groups. A primary nutritional relationship to vitamins A and E is not postulated, but certain other possibilities are discussed.

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