TABLE 2

EFFECT OF DAILY ADMINISTRATION OF VARIOUS SUBSTANCES TOGETHER WITH 150 CC OF NEUTRALIZED NORMAL HUMAN GASTRIC JUICE

Substances	Case 96	Case 97	Case 98	Case 99	Case 100
	First Period—10 Days				
			grams daily		
Casein					
"Water soluble vitamin-free" (Harris)*	..	..	50	..	..
Alcohol extracted†	50	50	..	..	..
"Vitamin free—Labco" (Borden)‡	..	..	..	50	50
Accessory Factors§					
Thiamin	0.1	0.1	0.1	0.1	0.1
Riboflavin	0.1	0.025	0.025	0.1	0.1
Niacinamide	0.1	0.1	0.2	0.2	0.2
Pyridoxine hydrochloride	0.1	0.1	0.1	0.1	0.1
d-Calcium pantothenate	..	0.1	0.1	0.1	0.1
p-Aminobenzoic acid	..	..	2.0	2.0	2.0
Choline hydrochloride	..	..	0.3	0.3	0.3
i-Inositol	..	..	0.2	0.2	0.2
Biotin	..	..	..	0.002	0.002
Xanthopterin	..	..	..	0.009	0.005
Folic acid	..	..	..	0.0036	0.0023
Initial R.B.C. (mils./cu.mm.)	1.56	1.79	1.73	1.17	1.50
Reticulocyte peak (per cent.)	1.4	2.2	6.4	1.9	1.0
Interpretation	neg.	neg.	pos.	neg.	neg.
	Second Period—10 Days				
			grams daily		
Beef muscle	..	..	..	200	..
Meat extract ¶	..	..	..	..	35 cc.
Ventriculin N.N.R. (without gastric juice)	30	30	30	..	..
Initial R.B.C. (mils./cu.mm.)	1.66	1.56	1.80	1.54	1.79
Reticulocyte peak (per cent.)	17.2	11.2	7.4	13.6	8.9
Interpretation	pos.	pos.	second pos.	pos.	pos.

* Exhaustively extracted with dilute acids during manufacture.
† "Washed casein" (A. H. Thomas) was extracted with cold 65 per cent. alcohol five times, then once with hot 95 per cent. alcohol.
‡ Repeated isoelectric precipitation in progressively more dilute solutions of sodium chloride during manufacture.
§ Folic acid and folic acid concentrate (Case 100), both prepared by fermentation methods, and xanthopterin were obtained through the courtesy of Dr. Y. SubbaRow, Lederle Laboratories, Pearl River, N. Y.; other accessory factors (except p-aminobenzoic acid) through the courtesy of Dr. D. F. Robertson, Merck and Company, Rahway, N. J.
¶ Courtesy of Mr. Braxton Valentine, Valentine's Meat-Juice Company, Richmond, Va. (35 cc are derived from 600 grams of lean beef).

lowing administration of this type of casein alone (Table 1, Cases 93 and 94). This positive effect is, therefore, probably attributable to the impurity of the casein. The pure vitamin mixtures were inactive when administered in combination with other types of casein (Table 2, Cases 96, 97, 99 and 100).

These observations suggest: (1) that the careful purification required to render crude casein "vitamin free" is also essential for the elimination of the extrinsic factor; (2) that a combination of casein so extracted with the pure accessory factors used and in the dosage indicated did not reconstitute the extrinsic factor activity of the crude casein; and (3) that, nevertheless, it is reasonable to continue to regard the extrinsic factor as a thermostable component of the vitamin B complex as yet unidentified.[8,15]

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[15] R. R. Williams and T. D. Spies, "Vitamin B_1 (Thiamin) and Its Use in Medicine," p. 134. New York: Macmillan Company, 1938.

PHOTOSENSITIVITY AS A CAUSE OF FALSELY POSITIVE CEPHALIN-CHOLESTEROL FLOCCULATION TESTS[1]

THE cephalin-cholesterol flocculation test has been proposed by Hanger[2] as a method of detecting active hepatic disease. Others[3,4,5] have reported that normal individuals showed positive reactions of varying degree and frequency. Our use of this test has also been complicated by the frequent yet irregular occurrence of falsely positive reactions of the 2 and 3 plus grade. A puzzling feature was the fact that sera giving falsely positive reactions in one laboratory consistently gave negative reactions when tested in a

[1] This investigation was conducted under the Commission on Measles and Mumps, Board for the Investigation and Control of Influenza and Other Epidemic Diseases in the Army, Preventive Medicine Service, Office of the Surgeon General, U. S. Army, Washington, D. C. The studies were done at the Medical Clinic, Hospital of the University of Pennsylvania, and at the Biochemical Laboratory, Philadelphia General Hospital.

[2] F. M. Hanger, *Jour. Clin. Investigation*, 18: 261, 1939.

[3] F. J. Pohle and J. K. Stewart, *Jour. Clin. Investigation*, 20: 241, 1941.

[4] J. G. Mateer, J. I. Baltz, P. F. Marion and J. M. MacMillan, *Jour. Am. Med. Asn.*, 121: 723, 1943, Postscript.

[5] J. W. Oliphant, A. G. Gilliam and C. L. Larson, *Pub. Health Rep.*, 58: 1233, 1943.

different laboratory. This discrepancy occurred even when the same technician, using the same reagents and glassware, performed the tests in the two laboratories. During the winter months, the differences were not great, but with the advent of spring and summer, they became progressively more accentuated. Attempts to relate this to improper cleansing of glassware, impurities in reagents, differences in sensitivity of the antigen and details of manipulation failed to provide more than a partial explanation. It was then noted that the two laboratories differed markedly with respect to illumination and that the falsely positive reactions always occurred in the one which was well lighted by several large windows. The other laboratory, in which satisfactory results were usually obtained, was poorly lighted.

In order to study the effect of light on this reaction, a number of experiments of the following type were carried out: Fresh serum was obtained from normal subjects who had no detectable evidence of liver disease and also from subjects with known liver disease. Duplicate preparations of each serum with saline and antigen were then made according to the technique of Hanger.[2] The Difco cephalin-cholesterol antigen was used. One set was placed before a window so that the preparations would be exposed to daylight. The duplicate set was placed in a dark cabinet. Readings were made after 24 and 48 hours. This procedure was carried out at both laboratories previously described, and also at a third laboratory in a different city.

The results in all three laboratories were nearly identical. Table 1 shows representative results of such experiments. When the serum-saline-antigen mixtures were protected from light, none of the 13 normal sera produced flocculation. The results with the duplicate set, which had not been protected from light, were strikingly different. In these, positive reactions, 1 to 4 plus, were obtained in 9 of the 13 after 24 hours, and in all after 48 hours. A similar effect was obtained with exposure to the bright light of an ordinary incandescent filament lamp. None of the men in this group presented evidence of liver disease detectable by physical examination or by application of a group of liver function tests. Thus, the positive reactions obtained in the exposed tubes were false.

The tests on sera from the patients with known liver disease prove that protection from light does not prevent the truly positive reactions. All showed positive reactions of varying degree in the tubes protected from light, and in 2 the flocculation was maximal (Table 1). However, the degree of flocculation given by 4 of the 6 pathological sera was greater when the serum-antigen mixtures were exposed to light. The other 2 showed maximal flocculation under both conditions of illumination.

TABLE 1

EFFECT OF ILLUMINATION ON RESPONSE OF THE CEPHALIN-CHOLESTEROL FLOCCULATION TEST

Case No. or initial	Protected from light		Exposed to light		Remarks
	24 hrs.	48 hrs.	24 hrs.	48 hrs.	
12	0	0	0	1+	Normal
"	0		3+		3 days later
"	0	0	0	1+	12 days later
16	0	0	1+	1+	Normal
"	0	0	0	2+	15 days later
15	0	0	3+	3+	Normal
"	0	0	4+	4+	6 days later
"	0	0	3+	3+	15 days later
9	0	0	2+	3+	Normal
11	0	0	2+		Normal
14	0	0	3+	3+	Normal
6	0	0	0	1+	Normal
13	0	0	3+	3+	Normal
8	?1+	1+	2+	3+	Subsiding hepatitis
5	0	1+	3+	4+	Subsiding hepatitis
T.G.	?1+	?1+	4+	4+	? liver abscess
W.	1+	2+	4+	4+	Chronic extra-hepatic obstruction
G.	4+	4+	4+	4+	Hepatitis and pneumonia
S.	4+	4+	4+	4+	Biliary cirrhosis

It was also noted that the results varied with the location of the test preparations in any one laboratory, the number of falsely positive tests being directly related to the intensity of illumination. The reactions in subdued light and total darkness were usually the same.

The evidence that the reaction is photosensitive seems unquestionable.[6] It has been previously observed that the sensitivity of the cephalin-cholesterol antigen is influenced by the extent to which it is exposed to light in the course of "ripening."[3] Presumably due to this difference in sensitivity of various antigens, which even seems to involve vials with the same lot number purchased at the same time, some discrepancies in results have been obtained. However, the influence of light on the reaction itself appears to be a more frequent source of variable results, and this factor apparently has not been previously recognized.

In addition to the effect of light on the serum-saline-antigen mixtures, preliminary studies have suggested that the following other factors may influence the cephalin flocculation procedure: (1) serum diluted and allowed to stand for 5 hours, before mixing with the antigen, resulted in falsely positive reactions whether exposed to light or kept in the dark. The number of falsely positive tests and the degree of flocculation was increased, however, if the diluted serum, alone, was exposed to light before mixing with the antigen. (2) Flocculation generally occurred more rapidly and completely at 37.5° C. than at room temperature. Positive results frequently have been ob-

[6] The possibility that light may influence other flocculation procedures deserves investigation.

tained at 37.5° C. when the response was negative at room temperature.

The need for standardization of the conditions under which the cephalin flocculation procedure is carried out is obvious. This is especially important in connection with the use of this test in various geographical locations, in which widely differing conditions, in respect to light and temperature, may be encountered. The most satisfactory results have been obtained when the reagents and sera were protected from prolonged exposure to bright light, and when the antigen was added soon after the serum was diluted with saline. It is not yet possible to define the ideal temperature conditions for this reaction, but it appears that more reliable results are obtained at 20 to 25° C. than at 37.5 degrees. Variable results due to differences in sensitivity of the antigen can be partially eliminated by the frequent inclusion of normal control samples. Work is continuing in an attempt to define more exactly the conditions that will yield the most dependable results. However, the procedure is even now capable of providing useful information when performed under the conditions described above.

SUMMARY

(1) Flocculation of cephalin-cholesterol emulsions by blood serum is markedly influenced by the amount of light to which the serum-saline-antigen suspensions are exposed. Protection from bright light, natural and artificial, has eliminated many falsely positive reactions. (2) Other factors that appear to influence the cephalin flocculation procedure have been briefly mentioned. Misses Dorothy Feinberg, Arvilla Howley and Mary Lanning contributed helpful technical assistance.

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A RHODOTORULA DEFICIENT FOR PARA-AMINO-BENZOIC ACID

IN November, 1943, Mr. Manfred Wahl of Philadelphia furnished us with a pink yeast which he had isolated and cultivated from a development in an old culture of beer yeast which Mr. Wahl had attempted to rejuvenate with other dormant cultures. Through the courtesy of Dr. Lynferd J. Wickerham, associate zymologist, Fermentation Division, Northern Regional Research Laboratory, Peoria, Ill., it was identified as a strain of *Rhodotorula aurantiaca* (Saito) Lodder. Preliminary experiments showed that this yeast grew well at 25° C on a basal medium composed of KH_2PO_4, $MgSO_4$, asparagine and dextrose solidified with purified agar and supplemented with thiamine and peptone. When peptone was omitted the growth in the first transfer was scanty, and subcultures on the same medium failed completely. It appeared probable that this strain of *R. aurantiaca* suffered from growth-substance deficiencies which were not corrected by the addition of thiamine to the basal medium but were satisfied by the substances supplied by peptone.

Yeasts with complete or partial deficiencies for thiamine, biotin, *i*-inositol, pyridoxine and pantothenic acid have been described. However, we were unable to induce the growth of *R. aurantiaca* by the addition of a mixture of these 5 substances to our basal medium. Excellent growth was obtained when the basal agar medium was supplemented with a mixture of para-amino-benzoic acid, calcium pantothenate, guanine, hypoxanthine, *i*-inositol, nicotinamide, pyridoxine, pimelic acid, riboflavin, thiamine, biotin methyl-ester and 2-methyl-1, 4-napthohydroquinone diacetate. In fact, the growth was more vigorous and the color a deeper red on the medium supplemented with the pure growth substances than on the peptone medium (containing 8 mg of neopeptone per tube). Good growth was obtained also on a medium prepared by adding 5 per cent. of desiccated malt extract to 1.5 per cent. Difco agar, but the color was a deep dull red instead of the bright red observed on the medium supplemented with the mixture of growth substances.

In order to determine the effective substances the basal agar medium was supplemented with 11 of the growth substances mentioned above, one being omitted. Growth was scant when thiamine or para-amino-benzoic acid was omitted; it was unaffected by the omission of any one of the other ten. It appeared, therefore, that *R. aurantiaca* suffered from a complete deficiency for these two growth substances. Further experiments confirmed this finding. The yeast grew well on the basal medium prepared with purified agar supplemented with thiamine and para-amino-benzoic acid. It did not grow on the same medium to which thiamine alone or PAB alone was added. It required molecular thiamine, as no growth was obtained when thiamine was replaced by the thiazole or pyrimidine intermediates of thiamine singly or together. The intensity of the pink color was related to the supply of PAB. In media with less than an optimum quantity of PAB the color was paler and tended toward orange as compared to the deeper pink which developed when more PAB was supplied.

The yeast was grown at 25° in test-tubes containing 5 ml of the basal solution and 10 mμ moles of thiamine per tube plus various amounts of PAB. Turbidity measurements were made after 48, 72, 96 and 149 hours incubation. Under these conditions a positive effect of 0.001 mμ mole of PAB (0.000137 μg) was observed after 72 hours. Growth increased with