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Presence of Typhus Antibodies in Commercial Frozen and Dried Complement.

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Frozen and dried complement from commercial sources has been used by this laboratory for the past two years in the performance of the virus and rickettsial[1,2] complement fixation tests. With the exception of an occasional ampule giving a low titer due to a poor vacuum or inadequate drying, at no time did we experience any serious difficulties with this material. However, in a recent series of tests, we have had results which can be best explained on the basis of the presence of specific antibodies in the particular product being used.

The initial observations were as follows: In a test containing a large number of human sera being titrated against both the epidemic and murine antigens, it was noted that in all tubes containing the epidemic antigen there was complete fixation regardless of the dilution of the test sera even when known negative sera were being tested. As there was no parallel abnormal complement fixation in the presence of the murine antigen (the positive and negative controls gave expected results) the immediate suspicion was that the epidemic antigen had for some reason become anticomplementary. Another epidemic antigen was therefore used on the following day but the complement was unchanged. Again all tubes containing epidemic antigen gave complete fixation even with known negative sera while the series with murine antigen behaved as ex pected. It was obvious that we were not dealing with a chance phenomenon. The first thought, that the antigen had become anticomplementary, was improbable as our epidemic antigens had been recently shown to be free from anticomplementary action in the dilutions used. It seemed unreasonable to assume a sudden change in 2 antigens particularly as other similar antigens (murine) stored in the identical manner were unaltered. It was therefore suspected that a specific fixation was occurring between the complement and the epidemic antigen as opposed to the murine antigen. Furthermore, this lot of complement was employed in psittacosis and lymphocytic choriomeningitis complement fixation tests without any evidence of abnormal fixation. This complement is hereafter referred to as complement lot No. 1.

Complement lot No. 1 was therefore tested as an unknown serum to see whether the presence of antibodies could be detected. It was inactivated at 56°C for 30 minutes and tested in the usual manner against epidemic antigen in the presence of a completely different complement (complement lot No. 2). At the same time this new complement, lot No. 2, was similarly tested as a control. Routine specific control sera were included in the same test. (Table I.)

It was noted that complement lot No. 1 was capable of fixing part of the complement with epidemic antigen at a serum dilution of 1/80 while complement lot No. 2 gave no fixation.

The epidemic antigen (E-26) that was employed in the preliminary test was also titrated at the same time in the presence of the heat inactivated complement lot No. 1 as well as known positive and negative sera. The results in Table II were obtained.

It is obvious from the above tests that the antigen (E-26) was not anticomplementary at 1/10 in saline nor did it give fixation at 1/10 in 10% normal serum. On the other hand when tested against control Epidemic Human No. 7 it gave a 3+ fixation at 1/160 dilution, the same titer for this antigen as observed in previous titrations. In the presence of complement lot No. 1 diluted 1/30 this antigen titered 1/120 indicating again that there was a considerable degree of react-

[1] Plotz, H., and Wertman, K., *Science*, 1942, **95**, 441.

[2] Plotz, H., *Science*, 1943, **97**, 20.

TABLE I.
Serum Titrations with Epidemic Antigen E-26.*

Sera	Dilutions: 1/10	1/20	1/40	1/80	1 160	1/320	1/640	SC†
Complement No. 1 inactivated	4	4	4	2+	0	0	0	0
Complement No. 2 inactivated	0	0	0	0	0	0	0	0
Epidemic human serum No. 7 (control)	4	4	4	4	4	4	3—	0

Complement No. 2 titration	0.1 cc	0.15	0.2	0.25	0.3	0.4	0.5
1/42 dil. of complement and 0.25 cc E-26 1/80	4	4	2	±	0	0	0

* 0.5 cc of a 1/42 dilution of complement No. 2 was used in the test.
† Serum control 1/10 dilution with no antigen.

TABLE II.
Epidemic Antigen Titration.

Antigen dilution	Complement lot No. 1 inact. 1/30 dil.	Control Ep. H No. 7 1/80 dil.	Control normal 1/10 dil.	Saline control
1/10	4	4	0	0
1/15	4	4	0	0
1/20	4	4	0	0
1/30	4	4	0	0
1/40	4	4	0	0
1/60	4	4	0	0
1/80	4	4	0	0
1/120	3	4	0	0
1/160	1	3	0	0

*Complement lot No. 2 titration	0.1 cc	0.15	0.2	0.25	0.3	0.4	0.5
1/42 dil. (saline)	4	4	1	0	0	0	0

* 0.5 cc of 1/42 dilution of complement lot No. 2 was used in the test.

ing substances in the heat inactivated complement.

To obtain further evidence that complement lot No. 1 contained specific antibodies it was subjected to a mouse neutralization test. Accordingly, mouse toxicity tests were set up using an epidemic and murine toxic substance. It was shown that no antibodies were present in the complement lot No. 1 which were capable of neutralizing the murine toxic substance. The epidemic toxic substance, however, was completely neutralized by a dilution of 1/32 and partially neutralized by a dilution of 1/64 of complement lot No. 1. This indicates clearly that specific epidemic neutralizing antibodies were present in complement lot No. 1.

Subsequently, we have observed this same phenomenon in a lot of complement from a different commercial establishment. In this latter instance, identical qualitative results were obtained. Not only did the inactivated complement fix in the presence of 3 of our epidemic typhus antigens, but also with 2 commerical epidemic typhus antigens. No fixation occurred in the presence of murine antigen. Furthermore, this same complement completely neutralized epidemic toxic substance at a 1/16 dilution. However, in all these tests the titers were approximately one-half those observed with complement lot No. 1.

During 3 years of experience with the complement fixation test in typhus fever, we have never encountered normal guinea pig sera which gave positive tests. The demonstration therefore of a heat stable substance capable of fixing complement in the presence of epidemic rickettsial antigen but not in the presence of murine rickettsial antigen, psittacosis or lymphocytic choriomeningitis antigens is highly indicative that these particular lots of complement were derived from guinea pigs possessing epidemic typhus antibodies. This opinion is further substantiated by the demonstration of specific neutralizing antibodies for epidemic toxic substance.

The possibility that the complement in question originated from guinea pigs that were used in testing epidemic typhus vaccine is admitted by one of the commercial concerns

who furnished the complement. They likewise corroborated our results in finding specific antibodies in the specimen of complement examined. Measures have been taken by the commercial concern to enforce a previous order to destroy all used animals and further to test all lots of frozen and dried complement for the presence of typhus antibodies.

It is obvious from the above that serious errors in complement fixation can be obtained if complement from guinea pigs of unknown origin is used. It is therefore advisable when complement is obtained from unknown origin to test it for the presence of specific antibodies before use.

The neutralization tests were performed by Captain Byron L. Bennett, Sn.C., and Captain Howard L. Hamilton, Sn.C.; the psittacosis and lymphocytic choriomeningitis virus complement fixation tests were performed by First Lt. Joel Warren, Sn.C., of the Division of Virus and Rickettsial Diseases, Army Medical School.

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Lipoxidase, a Dehydrogenase.

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The action of lipoxidase on unsaturated fats has recently been shown[1] to be of a complex nature; coupled with the oxidation of the fat is a loss of associated carotene which first directed attention to the existence of this enzyme. It appears to catalyze autoxidative rancidity, which has long been known to bleach carotene and hasten the oxidative destruction of fat-soluble vitamins.[2] Since autoxidation can be delayed by phenolic inhibitors and by tocopherol, application of these antioxidants offered a possible means of further studying the manner of action of lipoxidase, whether it is merely a catalyst or whether it modifies the course of autoxidation.

The substrate was the ethyl esters of lard fatty acids,[3] 75 mg properly emulsified in 75 cc of a solution comprising 60 cc water, 9 cc of 95% alcohol, 3 cc of acetone, and 3 cc of 0.1 M phosphate buffer at pH 6.5.

Oxygen absorption was measured at 38° in a macro-modification of the Warburg respirometer; when equilibrium conditions were attained, 3 cc of enzyme solution* were added by upsetting a small container within the flask. Iodimetric determinations of peroxide oxygen[4] were made on parallel samples under identical conditions.

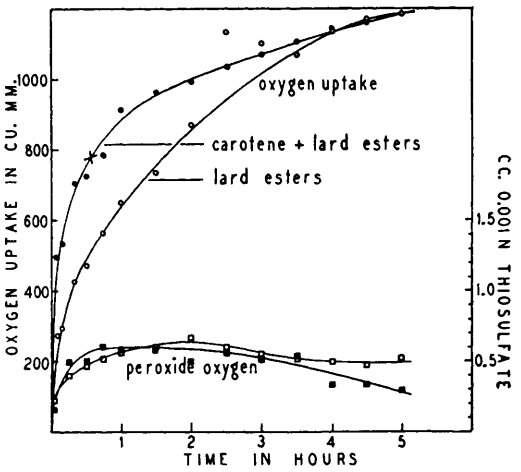

FIG. 1.

Oxygen uptake of esters with ● and without ○ carotene; peroxide oxygen with ■ and without □ carotene. X marks decolorization of carotene.

As seen in Fig. 1, the typical enzymatic

[1] Balls, A. K., Axelrod, B., and Kies, M. W., *J. Biol. Chem.*, 1943, **149**, 491.

[2] Mattill, H. A., *J. Am. Med. Assn.*, 1927, **89**, 1505.

[3] Olcott, H. S., and Mattill, H. A., *J. Am. Chem. Soc.*, 1936, **58**, 2204.

* This crude extract was prepared by suspending 5 g of powdered soybean protein (Glidden flakes, courtesy of Dr. P. L. Julian) in 100 cc of 2% sodium chloride solution. After standing for 30 minutes at room temperature the solution was centrifuged.

[4] French, R. B., Olcott, H. S., and Mattill, H. A., *Ind. Eng. Chem.*, 1935, **27**, 724.