OPTIMUM CONDITIONS OF FIXATION OF COMPLEMENT IN THE WASSERMANN TEST *

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An investigation of the rate of fixation of complement at different temperatures carried out with protein antigens and specific immune rabbit serums, reported elsewhere,¹ led to the following conclusions:

1. The rate of fixation of complement is approximately the same at icebox (6 to 12 C.), room (18 to 25 C.), and water-bath (37.5 C.) temperatures, the tendency being for somewhat greater fixation at icebox temperature.

2. From 60 to 80 per cent. of complement is "fixed" during the first hour of fixation at either icebox, room, or water-bath temperature, the amount being directly proportional to the number of antibodies in the immune serum, fixation being completed in about four hours at icebox temperature.

The fixation periods at water-bath and room temperatures were not extended in these experiments beyond two hours, in view of the deterioration of complement after prolonged exposure at these temperatures.

The proteins used in these studies were edestin from hempseed and phaseolin from the kidney bean, kindly furnished by Dr. Thomas B. Osborne of the Connecticut Agricultural Experiment Station.

A study of the velocity of fixation of complement at different temperatures in the Wassermann test was next undertaken. A detailed presentation of this work will be given elsewhere. Briefly, this investigation was carried out with six different antigens: (1) a crude alcoholic extract of guinea-pig hearts; (2) a crude alcoholic extract of beef hearts; (3) an alcoholic extract of beef hearts previously freed from ether-soluble lipoids; (4) the same antigen cholesterinized with 0.4 per cent. cholesterol; (5) a Noguchi antigen; and (6) a human heart antigen cholesterinized with 0.4 per cent. cholesterol. The periods of fixation were 0, 5, 15, 30 and 60 minutes, and 2, 3, 4, 5 and 6 hours. The fixation temperatures were, as in the case of the protein antigens, icebox, room and water-bath.

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1. Kahn, R. L.: Proc. Soc. Exper. Biol. & Med. **18**:168, 1921. Complete report to appear shortly in J. Exper. M.

It was found with these antigens, with the exception of the Noguchi antigen, that the velocity of fixation of complement at these temperatures was approximately the same, the tendency being for somewhat greater fixation at icebox temperature. The velocity of fixation with the Noguchi antigen was somewhat greater at water-bath than at icebox temperature. The fixation periods at water-bath and room temperatures were in these experiments, also, not extended beyond two hours.

It was further found that the velocity of fixation of complement was directly proportional to the concentration of the so-called syphilitic antibodies in the syphilitic serums, and that maximum fixation was attained in about four hours at icebox temperature.

The problem that presented itself as a result of the foregoing studies—and which I shall discuss—was to establish whether these experimental findings would be applicable to the routine Wassermann tests carried out in this laboratory, particularly whether icebox fixation could safely be substituted for water-bath fixation in the case of the cholesterinized antigen tests and whether there would be any advantage in it.

EXPERIMENTAL

Until Feb. 9, 1921, we made duplicate Wassermann tests with two antigens and two modes of fixation on all specimens received for examination, employing a period of four hours' fixation in the icebox in the case of the alcoholic antigen tests, and a fixation period of forty-five minutes in the water-bath in the case of the cholesterinized antigen tests. Of about 20,000 Wassermann tests carried out with these two modes of fixation, the tendency has been for weaker reactions with the cholesterinized antigen and water-bath fixation, as compared with the alcoholic-antigen tests. The same specimens which gave a + + + + reaction with the alcoholic antigen after four hours' fixation in the icebox, frequently gave a + + + reaction with the cholesterinized antigen and forty-five minutes' fixation in the water-bath. One which gave a + + + reaction with the alcoholic antigen occasionally gave a + + or even + reaction with the cholesterinized antigen.

Early in February, a series of duplicate Wassermann tests were carried out with the cholesterinized antigens, employing forty-five minutes' fixation in the water-bath in one case and the same fixation period in the icebox in the other. The results of 100 such tests are recorded in the table. The tendency for slightly stronger fixation at icebox temperature is clearly shown.

Accordingly, on Feb. 9, 1921, we eliminated water-bath fixation with the cholesterinized antigens and substituted a fixation period of one hour in the icebox instead. This period was chosen instead of

forty-five minutes because the anticomplementary tendency of cholesterinized antigen observed with water-bath fixation is reduced to a minimum when icebox fixation is resorted to. It was further felt that one hour's fixation would not be likely to give false positive results with this antigen.

COMPARATIVE STUDY OF SYPHILITIC SERUMS TESTED WITH CHOLESTERINIZED ANTIGEN AT WATER-BATH AND ICEBOX TEMPERATURES

Serum Number	Fixation for 45 Minutes in the		Serum Number	Fixation for 45 Minutes in the	
	Water-bath	Icebox		Water-bath	Icebox
1	±	±	51	—	—
2	—	—	52	—	—
3	—	—	53	—	—
4	—	—	54	—	—
5	—	—	55	++	+
6	++	±	56	—	—
7	—	—	57	—	—
8	—	—	58	—	—
9	—	—	59	—	—
10	—	—	60	++	+
11	—	—	61	—	—
12	++++	++++	62	—	—
13	—	—	63	—	—
14	—	—	64	++	±
15	+++	++++	65	—	—
16	—	—	66	—	—
17	—	—	67	—	—
18	+	—	68	++++	++++
19	—	—	69	—	—
20	—	—	70	—	—
21	—	—	71	—	—
22	—	—	72	—	—
23	—	±	73	—	—
24	—	—	74	—	—
25	+	+	75	+	+
26	—	—	76	—	—
27	±	±	77	—	—
28	—	—	78	—	—
29	—	—	79	—	—
30	—	—	80	—	—
31	+	++	81	—	—
32	—	—	82	—	—
33	++	++	83	—	—
34	±	±	84	++++	++++
35	+++	+++	85	++++	++++
36	—	—	86	—	—
37	—	—	87	—	—
38	±	±	88	—	—
39	—	—	89	—	—
40	—	—	90	—	—
41	+	+	91	—	—
42	—	—	92	—	—
43	++++	++++	93	—	—
44	—	—	94	±	±
45	—	—	95	—	—
46	—	—	96	—	—
47	+++	+++	97	+++	+++
48	—	—	98	—	—
49	—	—	99	—	—
50	—	—	100	—	—

Of 6,000 Wassermann tests performed with the cholesterinized antigen and one hour's fixation in the icebox, the results approximate the alcoholic antigen tests with four hours' fixation more closely than when the cholesterinized tests were carried out with water-bath fixation. A small percentage of specimens show a tendency for slightly stronger fixation with the cholesterinized antigen; then again, an equally small number show a tendency to slightly stronger fixation with the alcoholic antigen.

Regarding our Wassermann procedure, it might be stated that a sheep cell system is employed. The quantities are one tenth of the original Wassermann technic. All reagents entering into the test are measured in 0.1 c.c. quantities, except the patient's serum, which is employed in quantities of 0.01 c.c. and 0.02 c.c. Two units of complement and 2 units of amboceptor are employed and from 8 to 10 antigenic units in the case of the alcoholic extract antigen and from 3 to 4 antigenic units in the case of the cholesterinized antigen. For further details the reader is referred to other papers from this laboratory.²

DISCUSSION

The fact that the velocity of fixation of complement in the Wassermann test is about the same at icebox or water-bath temperature, and that fixation of complement is completed in about four hours suggests the possibility that those who employ a one-half or one hour period of fixation in the water-bath do not obtain the maximum amount of fixation. It is true that strongly positive syphilitic serums show marked fixation of complement after from five to fifteen minutes. In the case of weaker positives, however, no more than 50 per cent. of fixation is frequently obtained after one hour as compared with four hours' fixation.

The question of the period of fixation necessary to obtain the maximum sensitiveness of the cholesterinized antigen, without picking up false positives, is a problem in itself. We are inclined to agree with Smith and MacNeal,³ Ottenberg⁴ and others⁵ that prolonged periods of fixation with cholesterinized antigen will occasionally pick up a false positive result and, therefore, limit the fixation period with this antigen to one hour. We are not, however, in agreement with those workers as to the temperature of fixation, preferring the icebox to the water-bath.

The main advantage of icebox over water-bath fixation in the case of the cholesterinized antigen lies in the fact that the former procedure renders the antigen less anticomplementary as compared with the latter. The tendency for anticomplementary properties, which is inherent with the cholesterinized antigen, is reduced to a minimum when a one hour icebox fixation period is employed. A probable explanation for this is that a small amount of complement deteriorates during water-bath fixation, while the complement is practically preserved in the icebox.

2. Kahn, R. L.: *J. Lab. & Clin. M.* **6**:153 and 218, 1920 and 1921, and forthcoming issue. *Am. J. Pub. Health* **11**:410, 1921.

3. Smith, J. W., and MacNeal, W. J.: *J. Infect. Dis.* **21**:232, 1917.

4. Ottenberg, R.: *Arch. Int. Med.* **19**:457, 1917.

5. Owen, R. G., and Martin, F. A.: *J. Lab. & Clin. M.* **5**:232, 1920.

Our studies indicate further that the velocity of fixation with cholesterinized antigens is somewhat greater than with alcoholic antigens. This undoubtedly explains why our results with the cholesterinized antigens and one hour fixation approximate the results of the alcoholic antigen tests after four hours' fixation.

Kolmer and co-workers,⁶ who have made a careful study of the effects of temperature and time on fixation of complement, conclude that "The (fixation) method of choice narrows down to a selection of three or four hours at 8 to 10 degrees C. plus one hour in the water-bath at 38 degrees C. or eighteen hours at 8 to 10 degrees C. The latter is preferred on the basis of somewhat more sensitive reactions, but the differences are not marked."

Judging from our experience with the technic employed in this laboratory, the slight increase in the amount of complement "fixed" after 5, 7 and 9 hours' fixation, as compared with four hours, is largely nonspecific, due, most likely, to the deterioration of complement. We do not doubt that eighteen hours' fixation will result in a more sensitive reaction than four hours. But this increase in sensitiveness, judged from our studies is largely nonspecific, and a fixation period of twenty-four hours would, in our opinion, for the same reason result in a more sensitive reaction than eighteen hours.

CONCLUSIONS

1. The optimum conditions of fixation of complement in the Wassermann test with alcoholic-extract and Noguchi antigens appear to be four hours at ordinary icebox temperature (from 6 to 12 C.).

2. With cholesterinized antigens, a one-hour fixation period at icebox temperature is recommended. Icebox temperature renders the reactions somewhat sharper than water-bath temperature, and a one hour period precludes the possibility of picking up false positive reactions with these antigens.

6. Kolmer, J. A.: Matsunami, T., and Trist, M. E.: *J. Syph.* **5**:63, 1921.