

# THE EFFECT OF INACTIVATION ON COMPLEMENT-FIXING SUBSTANCES IN SYPHILITIC SERUM

## STUDIES ON COMPLEMENT FIXATION. VIII

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It is generally accepted that when syphilitic serum is inactivated for one-half hour at 56 C., it loses some of its complement-binding substances. According to Noguchi,<sup>1</sup> a given serum may lose during the process of inactivation as much as 75% of its antibody content. It is not unlikely that many complement-fixation systems in syphilis which employ raw serum do so not to utilize the easily replaceable complement, but because of the general belief that a large percentage of syphilitic antibody is destroyed during inactivation. In order to overcome this antibody destruction, several workers have, in recent years, recommended a shortening of the inactivation period for the Wassermann test. Among these may be mentioned Simon<sup>2</sup> who employs a 10-minute period of inactivation and Kolmer<sup>3</sup> who recommends in his newly standardized complement-fixation test, a 15-minute period.

Recent studies from this laboratory,<sup>4</sup> however, indicate that the thermolability of syphilitic complement-fixing substances is apparent rather than real, and that in syphilis we are not dealing with true antibody destruction due to inactivation. It was observed that the mode of fixation employed in complement-fixation tests was frequently the determining factor as to whether or not complement-fixing substances disappeared as a result of heating. Thus, if the mode of fixation was 1 hour in the water bath, the effect of heating appeared to be, as a rule, a marked loss of complement-fixing substances. If, on the other hand, fixation was carried out for 4 hours at icebox temperature, the effect of heating resulted either in no loss or some gain in these substances, in most cases. Not being able to interpret this observation, it was felt that technical errors might have entered into it. It seemed worth while, therefore, to extend these studies to a comparatively large number of syphilitic serums in order to establish

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<sup>1</sup> Serum Diagnosis of Syphilis, p. 97.

<sup>2</sup> Jour. Am. Med. Assn., 1919, 72, p. 1535.

<sup>3</sup> Jour. of Syphilis, 1922, 6, p. 82.

<sup>4</sup> Kahn, R. L.; Johnson, S. R., and Boyd, A. G.: Jour. Infect. Dis., 1921, 29, p. 639.

particularly whether the inactivation of serum for one-half hour at 56 C. is capable of bringing about an apparent increase in the number of complement-fixing substances over unheated serum.

It is frequently assumed that the purpose of inactivation is the destruction of native complement and the reduction of the anticomplementary properties of a given serum. Yet, in the precipitation reaction for syphilis recently proposed by one of us,<sup>5</sup> which consists of mixing extract antigen with syphilitic serum and where complement apparently plays no rôle, inactivation nevertheless enhances the reaction. Furthermore, the reduction of anticomplementary properties of a given serum by heat does not apply to the precipitation reaction, as anticomplementary serums exert no special effect on this test. The question arises, therefore, if inactivation is capable of increasing the activity of the precipitation elements of the serum, might it not also be capable of increasing the activity of the complement-fixing substances of the same serum?

#### EXPERIMENTS

Altogether 100 serums were tested. Each serum was examined for its complement-fixing substances in a raw state and after inactivation for one-half hour at 56 C. The loss or gain in antibody content due to inactivation could thus be readily ascertained. The complement-fixation tests were carried out with a sheep cell system and guinea-pig complement, as in the preceding studies of this series. These tests were carried out in duplication, employing a 1-hour fixation period in the water bath at 37.5 C. in one case, and a 4-hour period in the icebox at 8-12 C. in the other. Two antigens were employed; an alcoholic extract of dried beef heart previously freed from ether soluble lipoids and a similar antigen fortified with 0.4% cholesterin.

One of the first observations made in connection with these studies was that strongly positive serums do not seem to be influenced by inactivation. These serums show, in most cases, practically the same number of complement-fixing substances before and after inactivation. Neither are they markedly influenced by the mode of fixation. Whether the fixation period is 1 hour in the water bath or 4 hours in the icebox, the results are practically the same. This, it seems to us, is of some significance. It will be recalled that in the preceding paper<sup>6</sup> it was shown that strongly positive serums are not markedly affected by the

<sup>5</sup> Kahn, R. L.: *Arch. Dermat. & Syph.*, 1922, 5, pp. 570 and 734.

<sup>6</sup> Kahn, R. L., and Johnson, S. R.: *Jour. Infect. Dis.*, 1922.

TABLE 1  
DIFFERENCE IN COMPLEMENT-FIXATION POWER OF RAW AND INACTIVATED SYPHILITIC  
SERUM. TESTS WITH ALCOHOLIC ANTIGEN OF BEEF HEART

Serum		Mode of Fixa- tion	Serum, C c								Total Plus Signs	Effect of Inactivation	
No.	Kind		0.01	0.007	0.004	0.003	0.002	0.001	0.0005	0.0001		Antibody	
												Loss %	Gain %
1	Raw Heated	Water bath	4 3	4 1	1 —	1 —	— —	— —	— —	— —	10 4	60	
	Raw Heated	Ice- box	4 4	4 4	2 1	1 1	— —	— —	— —	— —	11 10		
2	Raw Heated	Water bath	4 4	4 1	4 —	— —	— —	— —	— —	— —	12 5	59	
	Raw Heated	Ice- box	4 4	4 4	4 4	1 3	1 2	— —	— —	— —	14 15		
3	Raw Heated	Water bath	4 4	4 3	4 1	1 1	1 —	— —	— —	— —	14 9	36	
	Raw Heated	Ice- box	4 4	4 4	4 3	1 2	— 1	— —	— —	— —	15 14		
4	Raw Heated	Water bath	3 2	3 2	2 1	1 1	1 —	1 —	— —	— —	11 6	46	
	Raw Heated	Ice- box	4 4	4 4	2 3	2 3	1 2	— 2	— —	— —	13 18		
5	Raw Heated	Water bath	4 —	4 —	3 —	2 —	1 —	1 —	— —	— —	15 0	100	
	Raw Heated	Ice- box	3 4	2 4	1 4	1 3	1 2	— 1	— —	— —	8 18		
6	Raw Heated	Water bath	4 4	4 4	4 2	4 1	3 —	1 —	— —	— —	20 11	44	
	Raw Heated	Ice- box	4 4	4 4	4 4	4 2	2 1	1 —	— —	— —	19 15		
7	Raw Heated	Water bath	— 1	— —	— —	— —	— —	— —	— —	— —	0 1		
	Raw Heated	Ice- box	— 4	— 4	— 1	— 1	— —	— —	— —	— —	0 10		
8	Raw Heated	Water bath	4 4	4 4	4 4	4 1	2 1	— —	— —	— —	18 14	22	
	Raw Heated	Ice- box	4 4	4 4	4 4	4 4	1 1	— —	— —	— —	16 17		
9	Raw Heated	Water bath	4 1	3 —	1 —	— —	— —	— —	— —	— —	8 1	87	
	Raw Heated	Ice- box	4 4	4 4	1 1	— —	— —	— —	— —	— —	9 9		
10	Raw Heated	Water bath	4 4	4 4	2 1	— —	— —	— —	— —	— —	10 9	10	
	Raw Heated	Ice- box	4 4	4 4	4 4	2 3	— 1	— —	— —	— —	12 16		

The period of water bath fixation was 1 hour; of icebox fixation, 4 hours.  
4 = +++++, 3 = ++++, 2 = ++, 1 = +, and — = negative.

TABLE 2  
DIFFERENCE IN COMPLEMENT-FIXATION POWER OF RAW AND INACTIVATED SYPHILITIC  
SERUM. TESTS WITH CHOLESTERINIZED ANTIGEN OF BEEF HEART

Serum		Mode of Fixa- tion	Serum, C c								Total Plus Signs	Effect of Inactivation	
No.	Kind		0.01	0.007	0.004	0.003	0.002	0.001	0.0005	0.0001		Antibody	
												Loss %	Gain %
1	Raw Heated	Water bath	4 4	4 4	4 1	2 1	1 —	— —	— —	— —	15 10	33	
	Raw Heated	Ice- box	4 4	4 4	4 4	3 4	— 2	— 1	— —	— —	15 19		
2	Raw Heated	Water bath	4 4	4 3	2 1	1 —	— —	— —	— —	— —	11 8	28	
	Raw Heated	Ice- box	4 4	4 4	2 3	2 2	— 1	— —	— —	— —	12 14		
3	Raw Heated	Water bath	4 3	4 2	1 —	— —	— —	— —	— —	— —	9 5	45	
	Raw Heated	Ice- box	4 4	4 4	1 —	— —	— —	— —	— —	— —	9 8		
4	Raw Heated	Water bath	4 3	4 1	2 —	— —	— —	— —	— —	— —	10 4	60	
	Raw Heated	Ice- box	4 4	4 4	4 4	1 4	— —	— —	— —	— —	13 16		
5	Raw Heated	Water bath	4 4	4 4	4 1	2 1	1 —	— —	— —	— —	15 10	33	
	Raw Heated	Ice- box	4 4	4 4	3 4	1 2	— 1	— —	— —	— —	12 15		
6	Raw Heated	Water bath	4 3	4 1	1 —	1 —	1 —	— —	— —	— —	11 4	64	
	Raw Heated	Ice- box	4 4	4 4	1 1	1 1	— —	— —	— —	— —	10 10		
7	Raw Heated	Water bath	4 3	4 2	1 —	— —	— —	— —	— —	— —	9 5	45	
	Raw Heated	Ice- box	4 4	3 3	1 1	— —	— —	— —	— —	— —	8 8		
8	Raw Heated	Water bath	2 2	2 1	— —	— —	— —	— —	— —	— —	4 3	25	
	Raw Heated	Ice- box	2 4	1 4	— 1	— —	— —	— —	— —	— —	3 9		
9	Raw Heated	Water bath	4 1	4 —	1 —	— —	— —	— —	— —	— —	9 1	89	
	Raw Heated	Ice- box	4 4	4 3	1 1	1 —	— —	— —	— —	— —	10 8		
10	Raw Heated	Water bath	4 4	4 4	4 3	4 2	3 —	1 —	— —	— —	20 13	35	
	Raw Heated	Ice- box	4 4	4 4	4 4	4 2	2 —	— —	— —	— —	18 14		

amount of antigen employed in the test. It thus appears that these serums belong to a class by themselves and are not representative of the behavior of moderately and weakly reacting serums.

Turning to the effect of inactivation on serums of weak and moderate potency we noted again the phenomenon already alluded to in our previous communication, namely, that the mode of fixation plays a marked rôle in the final results. Thus, after a fixation period of 1 hour in the water bath there was, in most cases, what appeared to be a marked antibody destruction due to inactivation. After a fixation period of 4 hours in the icebox, however, there was usually found, either some gain or a comparatively small loss in antibody content. Tables 1 and 2 illustrate this point.

The experiments in table 1 were carried out with an alcoholic antigen of beef heart and those in table 2 with a cholesterinized antigen. Due to the large amount of tabular material, the effect of inactivation on 10 serums only are recorded in each case. It will be seen that antibody loss is occasionally observed after inactivation even when fixation is carried out in the cold. This loss, however, is comparatively small, while the gain in antibody content is, as a whole, quite pronounced.

#### DISCUSSION

When employing an alcoholic and cholesterinized beef heart antigen, it was observed that inactivation of serum for one-half hour at 56 C., showed a tendency for stronger complement-fixation reactions compared with raw serums in many cases. This finding is not entirely in disagreement with those of the numerous workers who have shown that inactivation of syphilitic serum has a marked destructive effect on complement-fixing substances. The experiments which led these workers to this conclusion are based on complement-fixation tests carried out with a comparatively short period of fixation. To our knowledge, this period extended from one half to 1 hour in the water bath, in every case. Our findings with a 1-hour period of fixation in the water bath also show marked antibody destruction following inactivation. Our disagreement with these workers is limited to their interpretation of the results. They appear to have assumed that their findings with short fixation periods in the water bath is applicable to prolonged fixation periods in the icebox. This, our studies indicate, is not entirely true. A 4-hour fixation period in the icebox not only shows little antibody destruction due to inactivation, but shows a considerable gain in antibody content in many cases.

Whether the gain in antibody content is due to the colder temperature of fixation or the prolonged period (4 hours) has been considered in our previous paper.<sup>4</sup> The results indicated that the gain was largely due to the prolonged period rather than to the temperature of fixation.

It would appear that in syphilis we are dealing not with antibody destruction following inactivation, but probably with molecular rearrangement. This rearrangement has a tendency to decrease the velocity of fixation. After a 1-hour period of fixation, the reaction is quite weak; after sufficient time, however, such as 4 hours, the complement-fixation power of the serum is brought forth again. This does not explain why, in many cases, there is an antibody gain following inactivation. Probably the same factors which enhance the precipitation reaction because of inactivation play a rôle in increasing the complement-fixation power.

#### CONCLUSIONS

The inactivation of syphilitic serum for one-half hour at 56 C. was found to enhance the complement-fixation reaction in many cases, providing the period of fixation was no less than 4 hours at icebox temperature. A small loss in antibody content following inactivation was observed in some cases. The proportional antibody gain, however, due to inactivation, was greater than the antibody loss.