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INFLUENCE OF TEMPERATURE UPON THE VELOCITY OF THE COMPLEMENT FIXATION REACTION IN SYPHILIS.

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It has become customary to carry out immunity reactions *in vitro* at the temperature of 37°C., because most of the reactions between specific antibodies and antigens take place only at 37°C. or near that point. The reactions of bacteriolysis, cytolysis, hemolysis, agglutination, precipitation, phagocytosis, opsonization, etc., manifest their maximum activities at 37°C. Certain biological reactions, however, do not necessarily follow this general rule. For example, hemolysis produced by saponin, bile salts, cobra lecithid, or sodium oleate is complete within a very short time whether at 4° or 37°C.[1, 2] The velocity of the reaction in these instances is such that time and temperature play but a slight part.

The mechanism of the lipotropic complement fixation of syphilitic serum or spinal fluid is not understood, but it is certain that the lipoids are an important factor. The question in regard to the velocity of this reaction has not received as much attention as it deserves. The prevailing idea is that it is one of the immunity reactions which manifest their maximum activity at 37°C. Whether or not the reaction can take place at lower temperatures has not been carefully studied. We have been interested in this phase of the problem and have carried out numerous experiments to determine the relation between time, temperature, and reaction. The determination

[1] Madsen, T., and Walbum, L., Toxines et antitoxines. L'influence de la température sur la vitesse de réaction. I, *Overs. k. Danske Vidensk. Selsk. Forh.*, 1904, No. 6, 425.

[2] Madsen, T., and Noguchi, H., Toxines et antitoxines. L'influence de la température sur la vitesse de réaction. II, *Overs. k. Danske Vidensk. Selsk. Forh.*, 1904, No. 6, 447.

of these points is of practical importance at the present time, since, should the reaction prove to take place satisfactorily at a temperature which can be obtained without the aid of a special incubator, the performance of the test becomes much more widely adaptable. That this is the case is shown in the following experiments.

Detection of Varying Known Quantities of the Fixing Substance ("Syphilitic Antibody") at Different Temperatures.

It was the purpose in this series of experiments to study whether or not a given quantity of the fixing substance can be detected, not only at the usual incubation temperature of 37°C., but also at 30° or 23°. Graduated doses of a strongly positive syphilitic serum were chosen, so that a series would represent two and one fixing units and three-fifths and one-fifth of a unit. For the first experiment a serum was selected which produced complete fixation in a dose of 0.05 cc. within 30 minutes at 37°C. (water bath). Of this 0.1, 0.05, 0.03, and 0.01 cc. were measured into different tubes and tested for their fixing capacity at different temperatures, 37°, 30°, and 23°C.

In the first series human complement, 0.1 cc., and in the second guinea pig complement, 0.04 cc. (or 0.1 cc. of 40 per cent guinea pig serum dilution), were used. The quantities of the other elements were as usual, antihuman amboceptor, 0.1 cc., containing one unit for the human and two units for the guinea pig complement, 10 per cent human corpuscle suspension, 0.1 cc., and antigen, 0.1 cc. The total volume in each tube was made 1.5 cc.

Four groups of tubes, representing 0.1, 0.05, 0.03, and 0.01 cc. of the serum, were prepared for studying the influence of temperature upon the velocity of the reaction (Table I). Each group had seven duplicate sets for each dose of serum, as it was necessary to add amboceptor and corpuscle suspension at seven different intervals after the first incubation period began. The addition of the amboceptor and corpuscle suspension was made simultaneously 10 minutes, 20 minutes, 30 minutes, 60 minutes, 2 hours, and 4 hours after the contents were mixed.

For the temperature of 37°C. a water bath was used, for 30°C. a special thermostat room, and for 23°C. the laboratory room. Not

only the first incubation, but also the second or hemolytic phase of the reaction, was carried out at the temperatures indicated; that is, the results recorded under the heading of 23°C. were obtained at that temperature throughout, and those at 30° and 37°C. also.

In the experiments recorded in Tables I and II the same quantities of fixing substance ("syphilitic antibody") were employed, but the source and amount of complement used were different. It will be noted that the reaction was somewhat stronger when 0.04 cc. of guinea

TABLE I.

Fixation of Human Complement at Various Temperatures.

Incubation period.	37°C.				30°C.				23°C.			
	Serum.											
	0.1 cc.	0.05 cc.	0.03 cc.	0.01 cc.	0.1 cc.	0.05 cc.	0.03 cc.	0.01 cc.	0.1 cc.	0.05 cc.	0.03 cc.	0.01 cc.
Immediately.	−	−	−	−	−	−	−	−	−	−	−	−
10 min.	++++	++	−	−	+++	−	−	−	+++	−	−	−
20 "	++++	+++	+	−	++++	++	−	−	++++	+	−	−
30 "		++++	++	±	++++	+++	+	−	++++	++	±	−
60 "		++++	++	±		++++	++	±	++++	+++	+	−
2 hrs.						++++	++	±	++++	++++	++	±
4 "						++++	++	±	++++	++++	++	±

Human complement, 0.1 cc.; diluted antihuman amboceptor, 0.1 cc. (one unit); 10 per cent human corpuscle suspension, 0.1 cc.; antigen, 0.1 cc. Total volume, 1.5 cc.

The second incubation also was carried out at the temperatures indicated.

pig complement was used instead of 0.1 cc. of human complement. The difference seems to be due to the fact[3] that the more active the complement the easier it is fixed, since guinea pig serum contains the requisite complement in a much smaller volume.

Some striking facts are brought out in these experiments. When the amount of fixing substance exceeds two units (0.1 cc.), complete

[3] Noguchi, H., and Bronfenbrenner, J., The interference of inactive serum and egg-white in the phenomenon of complement fixation, *J. Exp. Med.*, 1911, xiii, 92.

fixation takes place within 20 minutes at 23° or 30°C., while at 37°C. the fixation is complete within 10 minutes. When one unit of fixing substance (0.05 cc.) is used, the fixation is complete within 30 minutes at 37°C., 60 minutes at 30°C., and 2 hours at 23°C. With three-fifths (0.03 cc.) and one-fifth (0.01 cc.) of the fixing unit the minimum time required for completion of the fixation is 30 minutes for 37°C., 60 minutes for 30°C., and 2 hours for 23°C.

TABLE II.

Fixation of Guinea Pig Complement at Various Temperatures.

Incubation period.	37°C.				30°C.				23°C.			
	Serum.											
	0.1 cc.	0.05 cc.	0.03 cc.	0.01 cc.	0.1 cc.	0.05 cc.	0.03 cc.	0.01 cc.	0.1 cc.	0.05 cc.	0.03 cc.	0.01 cc.
Immediately.	−	−	−	−	−	−	−	−	−	−	−	−
10 min.	++++	+++	+	−	++++	+++	−	−	+++	++	−	−
20 "	++++	++++	++	−	++++	+++	+	−	++++	+++	±	−
30 "	++++	++++	++	±	++++	++++	++	−	++++	+++	+	−
60 "	++++	++++	++	±	++++	++++	++	±	++++	++++	++	−
2 hrs.					++++	++++	++	±	++++	++++	++	±
4 "					++++	++++	++	±	++++	++++	++	±

40 per cent guinea pig complement, 0.1 cc.; diluted antihuman amboceptor, 0.1 cc. (representing two units for this system); 10 per cent human corpuscle suspension, 0.1 cc.; antigen, 0.1 cc. Total volume, 1.5 cc.

The second incubation also was carried out at the temperatures indicated.

The velocity of the reaction of complement fixation and subsequent hemolysis is much greater at 37°C. than at 30° or 23°C. A quantity of complement which will completely hemolyze the mixture in 20 minutes at 37°C. will require 35 minutes at 30°C. and 45 minutes at 23°C. for complete hemolysis. It is therefore understood that the test of syphilitic serum at 30° or 23°C. requires a period of nearly 3 hours, while at 37°C. the reaction is complete at the end of 1 hour.

TABLE III.

Comparative Study of Specimens Tested at Different Temperatures.

Specimen.	Amount.	37°C. Incubation 30 min.	30°C. Incubation 2 hrs.	23°C. Incubation 2 hrs.
	cc.			
Serum 1	0.2	−	−	−
" 2	0.2	++++	++++	++++
" 3	0.2	−	−	−
" 4	0.2	−	−	−
" 5	0.2	−	−	
" 6	0.2	++++	++++	++++
" 7	0.2	++++	++++	++++
" 8	0.2	−	−	−
" 9	0.2	+++	+++	+++
" 10	0.2	−	−	−
" 11	0.2	++	++	++
" 12	0.2	−	−	−
" 13	0.2	++++	++++	++++
" 14	0.2	+	+	+
" 15	0.2	−	−	−
" 16	0.2	−	−	−
" 17	0.2	−	−	−
" 18	0.2	±	±	±
" 19	0.2	−	−	−
" 20	0.2	−	−	−
Cerebrospinal fluid 1	0.5	++++	++++	++++
Cerebrospinal fluid 2	0.5	++++	++++	++++
Cerebrospinal fluid 3	0.5	−	−	−
Cerebrospinal fluid 4	0.5	−	−	−
Cerebrospinal fluid 5	0.5	+++	+++	+++

Human complement was employed. Diluted antihuman amboceptor, 0.1 cc. (one unit); 10 per cent human corpuscle suspension, 0.1 cc.; antigen, 0.1 cc. Total volume, 1.5 cc.

The first and second incubations were carried out at the temperatures indicated.

Examination of Specimens of Serum and Cerebrospinal Fluid at Different Temperatures.

With a view to the possible practical application of the facts just mentioned, a series of tests was undertaken with specimens of serum and cerobrospinal fluid at 37°, 30°, and 23°C. The serum was used in the active state, and the cerebrospinal fluids were tested by adding human complement, 0.1 cc., to each specimen. Table III gives the results.

There was no disagreement in the results obtained at the three different temperatures. Apparently the examination of syphilitic sera and cerebrospinal fluids can be conducted in a warm laboratory without recourse to an incubator in which a temperature of 37°C. is maintained. It is advisable, however, to make use of the incubator whenever it is available, and, when not accessible, to make the room as warm as possible, in order to accelerate the reaction. 1 hour of incubation is sufficient when the temperature of the air is 32–37°C.

CONCLUSIONS.

Examination of syphilitic serum or cerebrospinal fluid can be made at any temperature between 23° and 37°C. The velocity of the fixation reaction, including the fixation of complement and subsequent hemolysis, is greater at a higher temperature, the optimum point being 37°C. The maximum reaction is also reached, however, when the mixture of lipoids, syphilitic serum, and complement is allowed to stand for a long enough period at a lower temperature, the minimum thermal point being near 23°C. For the optimum temperature (37°C.) an incubation of 30 minutes is sufficient, while for the minimum temperature (23°C.) 2 hours are necessary. At the temperature of 30°C. the reaction proceeds with moderate velocity and is complete within 60 minutes.

Guinea pig complement gave a sharper reaction with the sera which contained less than one unit of the fixing substance. Fixation is complete, however, at any of the three temperatures within 20 minutes when there are more than two units present. A serum containing one unit of fixing substance will complete reaction within 30 minutes at

37°C., 60 minutes at 30°C., and 2 hours at 23°C., irrespective of whether human or guinea pig complement is used.

For many reasons a properly adjusted thermostat for 37°C. is recommended for conducting the serum diagnosis of syphilis when possible, but it should not be overlooked that at a temperature near 30°C. an entirely reliable result can be obtained without a special incubator. Even at a temperature as low as 23°C. the test can be carried out if sufficient length of time is allowed.

The foregoing conclusions refer only to the systems in which the acetone-insoluble fraction of tissue lipoids[4] is used as antigen.

[4] Noguchi, H., Serum diagnosis of syphilis and butyric acid test for syphilis, Philadelphia, 3rd edition, 1912.