EFFECTS OF MECHANICAL AGITATION AND OF TEMPERATURE UPON COMPLEMENT.*

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In making the fixation tests, the mixtures employed must be frequently shaken, and moderately high temperatures are used. It is of importance, therefore, to know whether these procedures may affect the complement in any way. In order to answer this question the following experiments were undertaken.

THE EFFECTS OF MECHANICAL AGITATION.

The shaking of the serum was made at two different temperatures, at 10° C. and at 37° C. The oscillations were about forty per minute. The serum was sealed in a sterile glass tube without a cork, and the tube containing the serum was placed in a horizontal position in a shaking apparatus. The tube had space enough inside to allow the serum to undergo strong agitation. The titration was made at different periods. At the same time, other portions of the same serum were allowed to stand near the shaken tubes under experiment, and they were examined as controls.

The hemolytic experiments for titration of the complementary activity were made with two different amboceptors, one from an immunized rabbit and the other from an immunized goat. As usual, the amount of corpuscles was 0.1 cubic centimeter of a 10 per cent. suspension of washed human erythrocytes. The total volume was one cubic centimeter.

The foregoing experiments show that the complement of guinea pig serum is considerably injured by continuous shaking at 37° C. Within one hour the reduction was trifling, within three hours the strength of the shaken serum was only one-fourth to one-fifth of

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TABLE I.

Shaking at 37° C.

Varieties of amboceptor.	Guinea pig serum.	1 hour at 37° C. Hemolysis.			3 hours at 37° C. Hemolysis.			6 hours at 37° C. Hemolysis.				
		100 per cent.	50 per cent.	25 per cent.	100 per cent.	50 per cent.	25 per cent.	100 per cent.	50 per cent.	25 per cent.	10 per cent.	0 per cent.
With antihuman amboceptor derived from immune rabbit.	No. 1 Shaken ..	0.0275	0.0125	0.008	0.1	0.04	0.02	—*	—*	0.15	0.005	0.03
	Unshaken	0.02	0.01	0.007				0.05	0.02	0.01	0.003	0.0015
	No. 2 Shaken ..	0.025	0.0125	0.007	0.1	0.04	0.02	—*	—*	0.145	0.05	0.03
	Unshaken	0.02	0.01	0.005				0.05	0.022	0.01	0.003	0.0015
	No. 3 Shaken ..	0.025	0.0125	0.007	0.1	0.04	0.02	—*	—*	—*	0.1	0.05
	Unshaken	0.02	0.01	0.005				0.04	0.02	0.01	0.003	0.0015
With antihuman amboceptor derived from immune goat.	No. 1 Shaken ..	0.015	0.007	0.004	0.06	0.03	0.02	—*	—*	0.15	0.05	0.03
	Unshaken	0.015	0.007	0.003				0.04	0.02	0.007	0.002	0.001
	No. 2 Shaken ..	0.015	0.007	0.004	0.06	0.03	0.02	—*	—*	0.15	0.05	0.03
	Unshaken	0.015	0.007	0.0035				0.03	0.015	0.006	0.002	0.001
	No. 3 Shaken ..	0.02	0.01	0.006	0.06	0.03	0.02	—*	—*	0.175	0.07	0.04
	Unshaken	0.02	0.01	0.005				0.03	0.015	0.007	0.0025	0.0015

*In quantities of .2 c.c., hemolysis did not reach the amount indicated.

TABLE II.

Shaking at 10° C.

Varieties of amboceptor.	Guinea pig serum.	1 hour at 10° C. Hemolysis.			24 hours at 10° C. Hemolysis.		
		100 per cent.	50 per cent.	25 per cent.	100 per cent.	50 per cent.	25 per cent.
With antihuman amboceptor derived from immune rabbit.	No. 1 Shaken.....	0.025	0.0125	0.006	0.05	0.025	0.0125
	Unshaken ...	0.02	0.01	0.005	0.04	0.0175	0.01
	No. 2 Shaken.....	0.022	0.0125	0.006	0.05	0.025	0.0125
	Unshaken ...	0.02	0.01	0.005	0.04	0.0175	0.01
	No. 3 Shaken.....	0.02	0.01	0.006	0.05	0.025	0.0125
	Unshaken ...	0.02	0.01	0.005	0.04	0.0175	0.008
With antihuman amboceptor derived from immune goat.	No. 1 Shaken.....	0.015	0.007		0.05	0.02	0.01
	Unshaken ...	0.015	0.007		0.03	0.01	0.005
	No. 2 Shaken.....	0.015	0.007		0.05	0.0175	0.0075
	Unshaken ...	0.015	0.007		0.03	0.0125	0.006
	No. 3 Shaken.....	0.015	0.007		0.05	0.0175	0.0075
	Unshaken ...	0.015	0.007		0.03	0.013	0.005

that of the fresh serum, and after six hours it was only one-fifteenth of that of the fresh serum. During the same periods, the unshaken serum also showed deterioration, and had after six hours only two-fifths of its original strength.

The effect of shaking was, however, much less injurious at 10°

C. Even after constant shaking at this temperature for twenty-four hours, the destruction of complement was not marked. The strength was reduced to a little less than half of what it was originally. The control had also deteriorated to half of its original power.

From this it appears that mechanical agitation at a low temperature has only a slightly destructive effect upon guinea pig complement, while at a higher temperature the complement is destroyed very rapidly, especially after the lapse of a few hours. In this last respect the destruction of complement differs markedly from that of certain ferments described by Meltzer and Shaklee.[1]

THE EFFECTS OF TEMPERATURE.

The effect of another physical force, heat, upon guinea pig complement has been studied by numerous investigators since the time of Buchner, and it is generally accepted that complement kept at 55° C. is destroyed in about thirty minutes. Ehrlich and Morgenroth showed, however, that goat complement resists this temperature, while one of us met with complements in cold-blooded animals which were destroyed, to a considerable extent, at 45° C. Zeissler found that the complement in human serum may still be active after heating the serum to 60° C. for one hour or longer.

Our study was made with six different samples of guinea pig serum. Each sample was divided into four parts, giving us four series. One of these was kept at room temperature as a control. The other three were heated for thirty minutes in a water bath at 45°, 50°, and 55° C. respectively.

Our reason for employing two different amboceptors was that we thought in this way we might be able to discover two different complements specific for each amboceptor, and that one might be destroyed while the other remained comparatively intact. But, as may be seen from the protocol, upon heating the sera at gradually increasing temperatures, the complementary activity for both amboceptors decreased progressively and equally. It was also quite unexpected to find that the complementary property was not completely lost at 55° C. in thirty minutes. It was, however, so weak-

[1] *Proc. Soc. Exper. Biol. and Med.*, 1908–9, vi, 103.

TABLE III.

Varieties of amboceptor.	Guinea pig serum.	Fresh serum. Hemolysis. 50 per cent.	25 per cent.	15 per cent.	0 per cent.	45° C.—30 minutes. Hemolysis. 50 per cent.	25 per cent.	0 per cent.	50° C.—30 minutes. Hemolysis. 50 per cent.	25 per cent.	15 per cent.	0 per cent.	55° C.—30 minutes. Hemolysis. 50 per cent.	25 per cent.	15 per cent.	0 per cent.
Amboceptor (rabbit)	No. 7	0.02	0.01	0.007	0.0025	0.04		0.02			0.1	0.05			0.25	0.075
	No. 8	0.02	0.01	0.007	0.002	0.025	0.015	0.01			0.1	0.05			0.2	0.075
	No. 9	0.02	0.01	0.007	0.002	0.025		0.0075			0.1	0.05			0.3	0.075
	No. 10	0.02	0.01	0.007	0.002	0.0275		0.01			0.1	0.035			0.15	0.075
	No. 11	0.015	0.007	0.004	0.002	0.02	0.015	0.01			0.1	0.04			0.15	0.075
	No. 12	0.0175	0.008	0.004	0.002	0.02	0.01	0.005			0.1	0.05			0.2	0.075
Amboceptor (goat)	No. 7	0.0125	0.005	0.0035	0.002	0.0125	0.007		0.15		0.075	0.02			0.25	0.05
	No. 8	0.015	0.0075	0.004	0.0015	0.015	0.0075			0.1		0.04			0.15	0.05
	No. 9	0.015	0.0075	0.004	0.0015	0.105	0.0075				0.1	0.05			0.15	0.04
	No. 10	0.01	0.005	0.003	0.0015	0.01	0.005				0.1	0.03			0.15	0.03
	No. 11	0.015	0.0075	0.004	0.0015	0.015	0.0075				0.1	0.04			0.15	0.05
	No. 12	0.015	0.0075	0.004	0.0015	0.015	0.0075				0.1	0.03			0.15	0.04

ened that to produce the same hemolytic effect as the original, at least thirty to forty times as large a quantity was required. The serum heated for thirty minutes at 50° C. had about one-fifteenth of the original strength, and at 45° C. it decreased to about one-half to one-third of the original. The reduction of the activity of the serum heated to 45° C. was scarcely detectable with goat amboceptor. But the destruction of complement for this and for rabbit amboceptor was equally marked when the serum was exposed to 50° and 55° C.

In the following experiments we determined the rate of destruction of this complement after various lengths of time, at the temperature of 50° C.

The protocol given above is of some interest. It will be noticed that the rate of destruction at 50° C. is not proportional to the length of time of exposure. During the first five minutes it was reduced to one-half of its original strength, but in the second five minute period the reduction was far less rapid. In the third and fourth five-minute periods, the reduction was again quite marked, while between the fourth and sixth five minute periods, the velocity of reduction was decreased. The periodic acceleration of physical force is by no means unusual. Certain ferments undergo a periodic destruction at higher temperatures.

TABLE IV.

	Guinea pig serum.	With rabbit amboceptor. Hemolysis.					With goat amboceptor. Hemolysis.				
		100 per cent.	50 per cent.	25 per cent.	10 per cent.	0 per cent.	100 per cent.	50 per cent.	25 per cent.	10 per cent.	0 per cent.
Unheated serum (original titers)	No. 1	0.022	0.01	0.006			0.0275	0.015	0.0075	0.0045	
	No. 2	0.02	0.01	0.007			0.0275	0.015	0.0075	0.0045	
Serum heated. 5 minutes at 50°C.	No. 1	0.0375	0.02	0.0125	0.006	0.0035	0.075	0.0225	0.015		0.005
	No. 2	0.0375	0.0175	0.0125	0.007	0.005	0.05	0.0225	0.015		0.005
10 minutes at 50°C.	No. 1	0.05	0.025	0.015	0.01	0.007	0.1	0.04	0.025	0.015	0.01
	No. 2	0.04	0.022	0.015	0.012	0.01	0.075	0.035	0.025	0.015	0.01
15 minutes at 50°C.	No. 1	0.1	0.05	0.03	0.025	0.015	0.15	0.075	0.04	0.022	0.015
	No. 2	0.08	0.04	0.025	0.015	0.01	0.15	0.075	0.035	0.022	0.015
20 minutes at 50°C.	No. 1	0.22	0.1	0.05	0.035	0.025		0.175	0.1	0.05	0.03
	No. 2	0.18	0.075	0.04	0.03	0.02		0.15	0.075	0.045	0.02
30 minutes at 50°C.	No. 1		0.22	0.1	0.05	0.025			0.125	0.075	0.045
	No. 2		0.2	0.1	0.04	0.025			0.125	0.075	0.05

CONCLUSIONS.

1. Under certain conditions, mechanical agitation destroys the complementary activity of guinea pig serum. It is most injurious when carried out constantly at a temperature of 37° C., but it is extremely insignificant at 10° C. After the first few hours at 37° C., the destruction of complement proceeded much more rapidly, and after six hours it was almost complete. On the other hand, within one hour shaking had almost no destructive effect on complement, even at 37° C. From this we may conclude that the several shakings which are necessary for fixation experiments during incubation do not modify perceptibly the outcome of the reactions.

2. The rate of destruction of the complement of guinea pig serum at temperatures above 45° C. is progressively greater as it approaches 55° C., at which temperature the activity is reduced in thirty minutes to one-thirtieth to one-fortieth of the original strength of the unheated serum; but it is not completely destroyed, as is commonly assumed.

The velocity of destruction of guinea pig complement when exposed to 55° C. for various lengths of time is found to be quite irregular, and not proportional to the length of time. This irregularity, however, presents a certain rhythm, a period of greater destruction alternating with one of less destruction.