THE INACTIVATION OF BACTERICIDAL AND HAEMOLYTIC COMPLEMENT ON STANDING.

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Hecht (1923) states that the haemolytic activity of serum complement disappears on standing, but that the activity can be restored by the addition of heated fresh serum. It has also been shown (Gordon and Wormall, 1928; Gordon, 1930; Gordon and Carter, 1932) that the bactericidal action of serum involves the activity of a complement similar to haemolytic complement and that inactivation of the latter by dilute ammonia and congo red causes, at the same time, inactivation of the bactericidal complement. The experiments now to be described show that the similarity of the two complements is exhibited once again in the effect of long standing on serum.

Serum was taken under sterile conditions from the hearts of several guinea-pigs, and the pooled serum was allowed to stand at room temperature for a month. The maintenance of sterility was verified by subculturing from time to time. The following tests for haemolytic complement activity were then made:

I. 0·1 c.c. serum, after standing 1 month, *plus* 0·5 c.c. 4 per cent. sensitised ox cells. No haemolysis.

II. 0·1 c.c. serum as above, *plus* 0·1 c.c. fresh serum heated 30 min. at 55° C., *plus* 0·5 c.c. suspension of red cells as above. Complete haemolysis.

III. 0·1 c.c. serum, after standing 1 month, *plus* 0·1 c.c. serum after standing 1 month but then heated for 30 min. at 55° C., *plus* 0·5 c.c. cell suspension as above. No haemolysis.

IV. 0·1 c.c. serum, fresh, but heated for 30 min. at 55° C., *plus* 0·5 c.c. cell suspension. No haemolysis.

The tubes were kept in the incubator at 37° C. for 2 hours and the readings then taken.

It will be seen that Hecht's statement was fully confirmed. The next step was to investigate the effect of long standing on bactericidal activity. The tests made were as follows:

Five series of tubes were set up.

Series I. 0·05 c.c. of bacterial suspension in broth, of a strength of about 100 millions per c.c., was added to 0·5 c.c. of sterile guinea-pig serum which had become inactivated by standing at room temperature for 1 month.

Series II. 0·05 c.c. of bacterial suspension was added to 0·5 c.c. of sterile guinea-pig serum inactivated by standing as above, together with 0·5 c.c. of fresh serum inactivated by heating for 30 min. at 55° C.

Series III. 0·05 c.c. of bacterial suspension was added to 0·5 c.c. of the serum inactivated by standing, together with 0·5 c.c. of serum also inactivated by standing but afterwards heated for 30 min. at 55° C.

Series IV. 0·05 c.c. of bacterial suspension was added to 0·5 c.c. of fresh guinea-pig serum.

Series V. 0·05 c.c. of bacterial suspension was added to 0·5 c.c. of fresh guinea-pig serum inactivated by heating for 30 min. at 55° C.

Series IV and V were set up as controls.

The organisms used were: (1) *V. cholerae*, (2) *B. dysenteriae* (Flexner), (3) *B. typhosus*, (4) *B. dysenteriae* (Shiga), and (5) *B. enteritidis* (Gaertner).

Table I.

+ + = heavy growth; + = good growth; +/2 = slight growth; 0 = no growth.
The numbers refer to the number of colonies where these could be counted.

Contents of each tube	Organism	Growth			
		At once	4 hr.	7 hr.	11 hr.
Series I:					
0·5 c.c. guinea-pig serum inactivated by standing for 1 month, together with 0·05 c.c. of bacterial suspension	(1) *V. cholerae*	+ +	+ +	+ +	+ +
	(2) *B. dysenteriae* (Flexner)	+	+	+	+ +
	(3) *B. typhosus*	+ +	+ +	+ +	+ +
	(4) *B. dysenteriae* (Shiga)	+ +	+ +	+ +	+ +
	(5) *B. enteritidis* (Gaertner)	+ +	+ +	+ +	+ +
Series II:					
0·5 c.c. guinea-pig serum inactivated as above, *plus* 0·5 c.c. of heat-inactivated fresh serum, together with 0·05 c.c. of bacterial suspension as above	(1) *V. cholerae*	+ +	0	0	0
	(2) *B. dysenteriae* (Flexner)	+	0	0	0
	(3) *B. typhosus*	+ +	+/2	0	0
	(4) *B. dysenteriae* (Shiga)	+ +	82	0	0
	(5) *B. enteritidis* (Gaertner)	+ +	+/2	32	0
Series III:					
0·5 c.c. guinea-pig serum inactivated by standing, *plus* 0·5 c.c. serum also inactivated by standing but afterwards heated 30 min. at 55° C., together with 0·05 c.c. bacterial suspension as above	(1) *V. cholerae*	+ +	+ +	+ +	+ +
	(2) *B. dysenteriae* (Flexner)	+	+ +	+ +	+ +
	(3) *B. typhosus*	+ +	+ +	+ +	+ +
	(4) *B. dysenteriae* (Shiga)	+ +	+ +	+ +	+ +
	(5) *B. enteritidis* (Gaertner)	+ +	+ +	+ +	+ +
Series IV:					
0·5 c.c. fresh guinea-pig serum together with 0·05 c.c. bacterial suspension	(1) *V. cholerae*	+ +	0	0	0
	(2) *B. dysenteriae* (Flexner)	+ +	0	0	0
	(3) *B. typhosus*	+ +	45	0	0
	(4) *B. dysenteriae*	+ +	10	0	0
	(5) *B. enteritidis* (Gaertner)	+ +	+/2	0	0
Series V:					
0·5 c.c. fresh guinea-pig serum heated 30 min. at 55° C., together with 0·05 c.c. bacterial suspension	(1) *V. cholerae*	+ +	+ +	+ +	+ +
	(2) *B. dysenteriae* (Flexner)	+ +	+ +	+ +	+ +
	(3) *B. typhosus*	+ +	+ +	+ +	+ +
	(4) *B. dysenteriae* (Shiga)	+ +	+ +	+ +	+ +
	(5) *B. enteritidis* (Gaertner)	+ +	+ +	+ +	+ +

The tubes were incubated at 37° C., loopfulls being withdrawn immediately and after intervals of 4, 7 and 11 hours, and inoculated to plates of 10 per cent. heated blood agar. The amount of growth shown after 20 hours' incubation at 37° C. was recorded.

The experimental results are given in Table I.

These results show that the serum which has lost its haemolytic complement on standing has lost its bactericidal power, that the addition of fresh heated serum restores both the haemolytic and bactericidal power, but that the addition of serum that has been inactivated by standing and then heated does not reactivate either the bactericidal or haemolytic power. Controls show that the heated fresh serum alone has no bactericidal power on these organisms, and that fresh unheated serum possesses marked bactericidal power.

Thus the similarity of bactericidal and haemolytic complements is brought out clearly in the further respect of the effect of long standing on serum.

The inactivation described in this paper is clearly a destruction of a heat-stable factor in serum complement. Two heat-stable factors are known, namely third and fourth components, but it would be rash to attribute inactivation on standing to the loss or destruction of either of these, since it cannot be assumed that they are the only heat-stable factors present in complement.

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