THE BACTERICIDAL PROPERTIES OF BLOOD SERUM.

I. THE REACTION-VELOCITY OF THE GERMICIDAL ACTION OF NORMAL RABBIT-SERUM ON *B. COLI COMMUNE* AND THE INFLUENCE OF TEMPERATURE THEREON.

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(With 4 charts.)

The following experiments, which form part of an attempt to investigate the bactericidal properties of serum, are concerned with the process by means of which certain bacteria are killed *in vitro* when immersed in normal sera. Special attention was paid to the temperature relations of this action because it was hoped that some information might be gleaned which might throw light upon the significance of fever as a reaction to infection.

The method of study was in the main similar to that previously employed by me in investigating the germicidal action of other agents (1908 and 1910) and consists in observation of the time relations of the process. As far as I am aware such a method has not hitherto been applied to the study of the bactericidal properties of serum and by its means data have been obtained which are quite new in character. Under these circumstances it does not seem necessary to give any review of the voluminous literature dealing with other aspects of this subject, but such as bears any direct reference to the matter of this paper will be alluded to as occasion occurs.

Materials and method of experiment.

The normal serum used in the following experiments was allowed to remain on the blood clot for about 18 hours, and then separated from any stray red corpuscles by centrifuging. A small quantity, –5 c.c., was measured out into a test tube placed in a thermostat at

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the required temperature of experiment. In order to test the serum for sterility, sterile broth was added to the small deposit left in the centrifuge tube and the whole incubated at 37° C. for two or three days. No difficulty was experienced in keeping the serum sterile.

The bacteria for the experiment were obtained from a 24 hours' culture in broth at 37° C.; this was filtered through paper to get rid of any agglutinated masses and diluted as required (from 50 to 250-fold) with distilled water. A small quantity of the diluted culture, measured by means of special capillary pipettes each delivering a standard drop equal to 0·02 c.c., was added to the serum tube in the thermostat and the time noted. The serum tube was also fitted with a standard capillary pipette by means of which samples could be withdrawn after successive intervals of time and a definite number of drops taken for plate cultures. Duplicate plates were poured at each time of sampling, necessitating the help of at least one assistant. Gelatine plates were usually employed and they were counted after three to four days' incubation at 20° C.

A more detailed account of the method, including the expedients adopted for maintaining a culture of constant resistance, is given in the previous paper already referred to (1908, pp. 96 and 118).

The experiments set forth in Tables I–X (with the exception of Table IV which is concerned with the action of normal goat-serum upon *B. typhosus*) were all made with normal rabbit-serum and *B. coli commune.*

Table I contains the results of three experiments carried out in duplicate at three different temperatures, viz. 20·2° C., 30° C. and 40° C. The general course of events is more readily comprehended by a glance at Fig. 1, where concentration of surviving bacteria is plotted against time.

After the addition of the bacteria to the serum an interesting series of phenomena takes place which, in the case of all three experiments, can be grouped into three distinct phases.

1. *A latent period,* lasting from 1–4½ hours according to the temperature, in which the number of living bacteria remains stationary or may decrease.

2. *A period of multiplication,* the length of which is also inversely related to temperature, during which the number of bacteria is increased until the concentration is about doubled.

The total lag caused by periods 1 and 2 varied from about $2\frac{1}{2}$ hours at 40° C. to about $4\frac{1}{2}$ and 7 hours at 30° C. and 20·2° C. respectively. This may account for the conclusion of Buxton (1905) that normal rabbit-serum was without bactericidal effect on *B. coli*.

3. *A bactericidal period*, during which the bactericidal action of the serum begins to be manifested and the bacteria are submitted to a slow process of disinfection at a rate depending on the temperature.

TABLE I.

Action of normal rabbit-serum upon B. coli commune.

Exp. 30. I. 12	Temperature °C.	Time, hours	Amount of sample taken, no. of drops	Numbers counted on plates		Mean no. of surviving bacteria in 1 drop serum
I	20·2	0·08	1	2220	1478	1850
		0·5	1	1564	1504	1530
		1·5	1	1100	1084	1090
		3·0	1	1158	1284	1220
		4·5	1	1150	1090	1120
		5·9	1	1416	1772	1590
		6·9	1	2368	2372	2370
		13·1	1	1332	1344	1340
		24·2	3	2004		1050
			5	6400*		
II	30	0·08	1	1696	1644	1670
		0·5	1	1240	1388	1310
		1·5	1	1268	1284	1280
		3·0	1	2520	2448	2500
		4·5	1	2684	—	2680
		5·9	1	276	289	282
		6·9	1	59	56	57
		13·0	5	231		46
			1	45		
		24·2	10	20,000*		2000
III	40	0·02	1	2088		2090
		0·05	1	1500		1500
		0·13	1	1296	1362	1330
		0·38	1	1150		1150
		0·87	2	2230		1120
		1·2	2	2576		1290
		1·5	2	4448		2220
		2·5	2	5760		2880
		3·6	3	726		242
		9·6	5	17		3·4
		21·0	10	? 1		0·0

* Approximately.

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In Experiment II, at 30° C., there is also evidence of the existence of a fourth stage. The disinfection occurring in the third stage was not quite complete and the few surviving bacteria began to multiply again. This phenomenon was not noticed in Experiment III at 40° C. and Exp. I at 20·2° C. was not complete enough to reveal it.

The sequence of the first three stages set out above was found to be a constant characteristic of the action of normal rabbit-serum upon

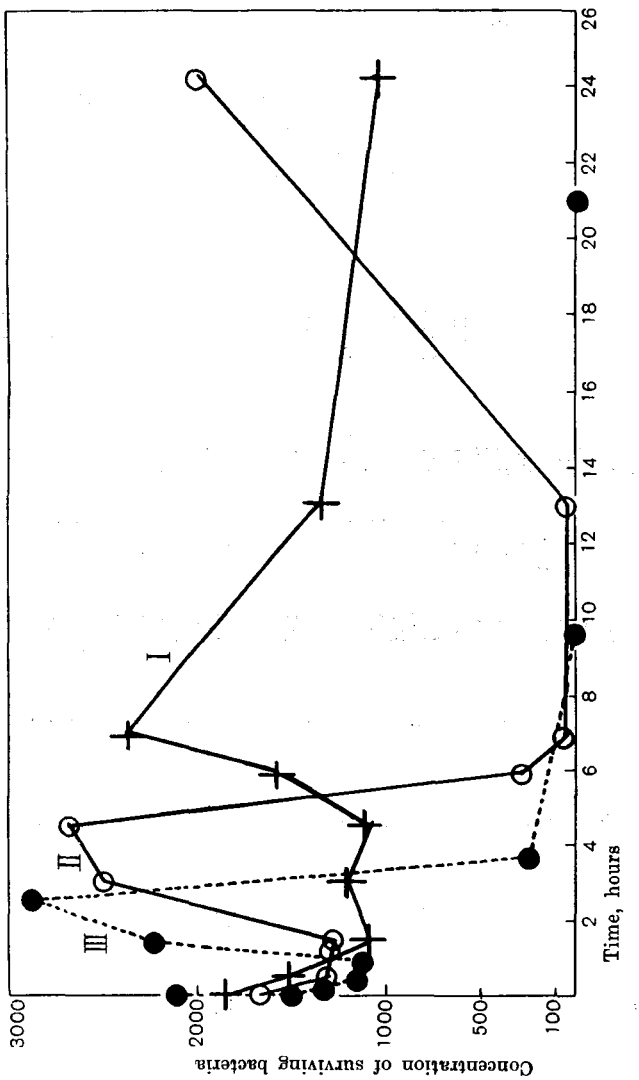

Fig. 1. Action of normal rabbit-serum upon *B. coli commune*, effect of temperature, see Table I, I. at 20·2° C. II. at 30° C. III. at 40° C.

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TABLE II.

Action of normal rabbit-serum (II) *upon* B. coli commune.

Exp. 26. III. 12	Temperature °C.	Time, hours $=t$	Amount of sample taken, no. of drops	Numbers counted on plates		Mean no. of surviving bacteria in 1 drop serum	$\text{Log}_{10} N$*	$K=\frac{1}{t_n-t_0}\log\frac{N_0}{N_n}$	Growth constant $A=\frac{1}{t_n-t_0}\log\frac{N_n}{N_0}$	Generation-time, hours $\frac{\log 2}{A}$
I	20	0·08	1	387	378	382				
		1·1	1	327	285	306				
		2·5	1	287	331	309				
		3·5	1	235	253	244				
		4·5	1	267	269	268				
		5·25	1	267	271	269				
		6·3	1	375	348	361	2·557		($N_0=361$; $t_0=6·3$)	
		7·3	1	450	437	443	2·646		·089	
		8·7	1	555	474	514	2·711	($N_0=514$; $t_0=8·7$)	·064	
		10·7	1	264		267	2·426	·142	·076 = mean	3·96
			2	538						
		12·5	1	171		148	2·170	·142		
			3	420						
		22·0	1	9		15·9	1·201	·114		
			10	166						
		25·0	1	1		9·8	0·991	·105		
			10	107				·126 = mean		
II	30·1	0·08	1	454	417	435				
		0·5	1	325	343	334				
		1·1	1	286	274	280				
		1·65	1	312	317	314				
		2·5	1	384	340	362				
		3·0	1	499	452	475	2·677		($N_0=475$; $t_0=3·0$)	
		3·5	1	525		547	2·738	($N_0=547$; $t_0=3·5$)	·122	2·47
			2	1116						
		4·0	1	379		377	2·576	·32		
			3	1128						
		4·5	1	179		151	2·179	·56		
			3	426						
		5·2	2	170	179	87·2	1·940	·47		
		5·8	2	106	106	53	1·724	·44		

		6·3	2	56		32	1·505	·44		
			5	167						
		7·35	5	81	66	14·7	1·167	·41		
		8·7	5	36	40	7·6	0·881	·36		
		10·7	5	13	9	2·2	0·342	·33		
		12·5	1	1		0		·42 = mean		
			10	0						
		22·0	1	0		0				
			10	0						
		25·0	1	0		0				
			10	0						
III	40·3	0·08	1	325	351	338				
		0·52	1	186	177	181				
		1·1	1	223	255	239				
		1·7	1	346	375	360	2·557		(N_0=360; t_0=1·7)	
		2·4	1	604	—	604	2·781	(N_0=604; t_0=2·4)	·32	0·94
		3·0	1	267	268	267	2·427	—†		
		3·5	1	56		45	1·653	1·03		
			3	124						
		4·0	1	13		12	1·079	1·06		
			5	59						
		4·5	2	9		4·2	0·623	1·03		
			5	20						
		5·2	3	1	4	1·0	0·00	0·99		
		5·8	5	Not more				1·03 = mean		
		6·3	5	than 1 or 2						
		7·3	5	organisms						
		8·7	5	per plate						
		10·7	1	13		14·2	1·152	(N_0=14·2; t_0=10·7)		
			10	143						
		12·4	1	0		0·36	−0·444	0·94		
			10	4						
		22·0	1	0		0				
			10	1						
		25·0	1	4300		4300				
			10	∞						

* Briggs' logarithms are used in calculating the value of K in this and all subsequent tables.
† Value obtained inconsistent with the others.

B. coli commune. In about an equal number of cases the third stage remained final or was succeeded by a period of further bacterial growth. It is not clear upon what circumstances this depends; the matter is further discussed below, p. 421, where some suggested explanations are put forward.

The quantitative data of the experiments in Table I were not complete enough for satisfactory analysis, such as might throw light upon the nature of the bactericidal action or the effect of temperature thereon. Accordingly a similar set of experiments was undertaken in which determinations of surviving bacteria were made with much greater frequency. The results are set forth in Table II and present a scheme similar to that deduced from the results of Table I, notwithstanding the fact that the serum was obtained from a different rabbit. The first three stages described above are present in all three experiments. In Exp. I at 20° C. the third or bactericidal stage was not completed by the 25th hour, and in Exp. II at 30·1° C. there is no evidence that this phase is other than final. It must be remembered, however, that the march of events in Exp. II is very slow, compared for example with Exp. III at 40·3° C., and it is possible for the final result to remain undiscovered if, as in this instance, the experiment is not continued beyond 24 hours.

In Exp. III, the third or bactericidal stage is succeeded by further multiplication[1] at the tenth hour. Towards the end of the experiment, *from the 10th to the 25th hour, this period of growth is followed by* a second phase of bacterial death which itself gives place to another period of multiplication. The latter stages in the action of serum upon bacteria need further investigation and for this purpose it will be necessary to arrange experiments of even longer duration than those described in the present communication.

The first three stages of the process were, however, studied with some care in the experiments in Table II and the results obtained may be summarised as follows:

The first or latent period lasted for about 0·5, 1·0 and 5 hours at 20°, 30·1° and 40·3° C. respectively. This stage seems to be merely an instance of the phenomenon which is usually observed when living bacteria are transferred to fresh media and for which no entirely satisfactory explanation has been advanced. For a variable length of time, depending on the conditions of experiment, including the previous

[1] A similar phenomenon was shown by Trommsdorf (1901) in the action of rabbit- and dog-serum upon *B. typhosus*.

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history of the seed material and the composition and temperature of the new medium, the number of living organisms remains almost stationary or may even decrease slightly [see Müller (1895); Rahn (1900); Hehewerth (1901); Barber (1908); Lane-Claypon (1909)]. In the present case the initial reduction in numbers, when present, is usually much too small to be explained by any hypothesis of agglutination.

The determinations made during the second or growth period were too infrequent for study and this stage was studied separately later on.

During the third or bactericidal period, however, frequent observations were made in order that the process might be investigated in greater detail. It was found that the action proceeded logarithmically, in other words death-rate of the surviving bacteria remained proportional to the concentration of surviving bacteria thus:

$$-\frac{dN}{dt} = KN,$$

where K is a constant and N is equal to the number of bacteria surviving in unit volume. In the seventh column of Table II are given the values of the velocity constant

$$K = \frac{1}{t_n - t_0} \log \frac{N_0}{N_n},$$

where N_0 and N_n are equal to the number of bacteria surviving in one standard drop of serum at times t_0 and t_n respectively. It will be seen that very fair constancy is maintained in the value of K. The same relationship is expressed graphically in Fig. 2 where logarithms of concentration of surviving bacteria as ordinates are plotted against times as abscissae and the experimental points are found to lie upon approximately straight lines.

The fact that the germicidal action proceeds as a "uni-molecular" reaction would suggest that the bactericidal agent was not being exhausted, but was remaining approximately constant in concentration as long as the above relation was maintained. A departure was, however, frequently noticed towards the end of the bactericidal period. At the same time the concentration of living bacteria became so small that it was very difficult to make an accurate evaluation by means of plate cultures. It is possible, however, that a decrease in the value of K such as occurs, for example, in Exp. I might be due to a falling off in concentration of the bactericidal substance or that a few specially resistant forms were being bred which are able to withstand its influence (see

Trommsdorf, 1901). The time occupied for even the most rapid of the experiments was long enough for such a phenomenon to take place and the suggestion receives some support from the fact that a growth period frequently succeeded that of bacterial death.

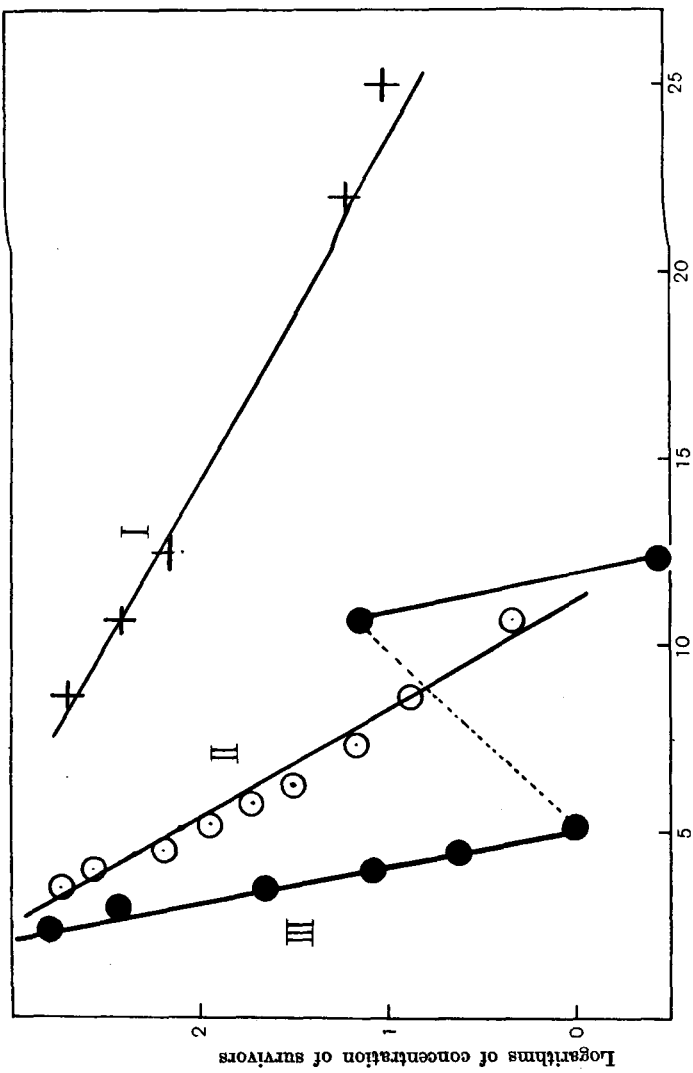

Fig. 2. Influence of temperature upon the germicidal action of normal rabbit-serum on *B. coli commune*, see Table II.
I. at 20° C. II. at 30·1° C. III. at 40·3° C.

The bactericidal action of normal serum thus falls into line with other types of disinfection. In all the cases which have been investigated

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the process has been shown to take place in accordance with the "unimolecular law," so that the reaction velocity at any time is proportional to the concentration of surviving bacteria. The cases studied include disinfection both of vegetative forms and spores by metallic salts, phenol and other coal-tar derivatives (Chick, 1908); hot water (Madsen and Nyman, 1907; Chick, 1910); sunlight (Clark and Gage, 1903 and Chick, 1910, p. 280); drying (Paul, 1909); the radium emanation (Chambers and Russ, 1912).

Influence of temperature.

The influence of temperature upon the bactericidal action was estimated by a comparison of velocity constants in the different experiments. These are collected in Table III, and it is seen that the effect of temperature is fairly consistent and a logarithmic relationship with reaction velocity is approximately maintained between the temperatures of 20° and 40·3° C., with a temperature coefficient of about 2·8 per rise of 10° C.

TABLE III.

Temperature coefficient of the bactericidal action of normal rabbit-serum (II) *upon* B. coli commune (*from Table II*).

	Temperature °C.	Value of the velocity constant, K	Log_{10} ($K\times10$)	Log_{10} (temperature coefficient per 1° C.)	Temperature coefficient per 1° C.	Temperature coefficient per 10° C.
Exp. I	20	·126	·100	·0518	1·13	3·30
Exp. II	30·1	·42	·623	·0379	1·09	2·39
Exp. III	40·3	1·03*	1·01			2·84 = mean

* Not including 1st value of K (see Table II).

In Table IV are given the results of a set of experiments made with normal goat-serum and *B. typhosus*. The general character of the action is rather different from that observed with *B. coli commune* and rabbit-serum, the bactericidal action in this case being altogether more powerful. The logarithmic nature of the action is, however, again confirmed, and the effect of temperature is seen to be of the same order, the mean velocity constant being increased 1·93-fold for a rise in temperature of 10° C. between the temperatures of 20·2° and 40·45° C.

The value of this temperature coefficient is lower than that found for most cases of germicidal action by other agents. With phenol, the velocity of disinfection was found, for anthrax spores, to be increased

TABLE IV.

Action of normal goat-serum upon B. typhosus; *effect of temperature.*

Exp. 23. III. 12	Temperature °C.	Time, hours	Number of surviving bacteria in 1 standard drop serum = N	$\log_{10} N$	$K = \frac{1}{t_n - t_0} \log \frac{N_0}{N_n}$	$\log_{10}(K \times 10)$	$\log_{10}$ (temperature coefficient per 1° C.)	Temperature coefficient per 1° C.	Temperature coefficient per 10° C.
I	20·2	0·05	526	2·721	($N_0 = 526$; $t_0 = 0·05$)				
		1·3	226	2·354	·29				
		2·08	97	1·987	·36				
		2·7	51	1·708	·38				
		3·8	31	1·491	·33				
		5·0	9	0·954	·36				
		5·8	10	1·000	·39				
		7·8	5	0·699	——				
		9·2	2	0·301	·34 = mean	·531	·0291	1·069	1·95
II	30·1	0·05	577	2·761	($N_0 = 577$; $t_0 = 0·05$)				
		1·3	108	2·033	·58				
		2·05	30	1·477	·64				
		2·6	7	0·845	·75				
		3·7	0	—	——				
					·66 = mean	·819	·0282	1·067	1·91
III	40·45	0·05	339	2·530	($N_0 = 339$; $t_0 = 0·05$)				
		0·65	40	1·602	1·55				
		1·3	17	1·230	1·04				
					——			1·068 = mean	1·93 = mean
					1·29 = mean	1·111			

5·5-fold per 10° C. rise in temperature and as much as 15-fold for *B. paratyphosus*, under certain conditions. Disinfection of the latter organism by higher tar-acids gave a figure of 7–8 per rise of 10° C. (Chick, 1908). In the case of disinfection by hot water the effect of temperature is even more enhanced, the coefficient reaching the high value of 136 per 10° C. rise in temperature for *B. typhosus* (Chick, 1910). Disinfection by means of drying, on the other hand, has a temperature coefficient of only about 2 to 3 per 10° C. (Paul Birstein and Reuss, 1910), and a similar value has been obtained for the germicidal action of metallic salts upon both spores and vegetative organisms (Madsen and Nyman, 1907; Chick, 1908). In the latter case, where there is some

evidence of direct chemical union between the salt and the bacterial protein, it is significant that the effect of temperature should be of the same order as that corresponding to most chemical reactions.

The high temperature coefficient of disinfection by hot water recalls a similar effect of temperature upon other reactions where bodies of a protein nature are involved. Such are the destruction by hot water of certain antigens (Famulener and Madsen, 1908) and agglutinins (Madsen and Streng, 1909) and the "heat coagulation" of proteins (Chick and Martin, 1910). In these cases the suggestion has been made that rise of temperature might be accompanied by changes in the nature of the colloidal solution resulting in a greatly increased surface

TABLE V.

Action of normal rabbit-serum (II) *upon* B. coli commune *at* 30° *C.; effect of the magnitude of the sowing.*

Exp. I. 8 standard drops (·16 c.c.) of a diluted (1 in 250) paper-filtered culture, added to 2 c.c. serum.

Exp. II. 1 standard drop (·02 c.c.) of a diluted (1 in 250) paper-filtered culture, added to 5 c.c. serum.

Exp. 13. III. 12	Time, hours $=t$	Amount of sample taken, no. of drops	Numbers counted on plates		Mean no. of bacteria surviving in 1 standard drop of serum $=N$	$\text{Log}_{10} N$	$K=\frac{1}{t_n-t_0}\log\frac{N_0}{N_n}$
I	0·08	1	1890		1890		($N_0=2230$; $t_0=4·8$)
	0·66	1	1600		1600		
	2·0	1	1960		1960		
	2·85	1	3310		3310		
	4·8	1	2250	2210	2230	3·348	
	5·8	1	341		341	2·533	·81
	6·5	1	147		147	2·167	·69
	7·0	1	94		94	1·973	·62
	8·05	1	49		49	1·690	·51
	24·0	5	0		0		·66 = mean
II	0·08	8	719		90·9		($N_0=104·5$; $t_0=4·7$)
	0·66	5	430		86		
	2·0	5	682		136		
	2·85	5	1142		228		
	4·7	3	281		104·5	2·019	
		5	555				
	5·8	5	56		11·2	1·049	·88
	6·5	3	17		5·4	0·732	·71
	7·0	5	2		0·4		·79 = mean
	8·1	1	0		0·2		
		3	1				
	24·0	10	0		0		

for action. In disinfection by hot water some such hypothesis might also explain the enhanced effect of temperature. The comparatively small influence of temperature in the present instance indicates that the mechanism involved in the germicidal action of serum must be of a different and probably simpler character. At the same time it is necessary to point out that a fallacy may be involved when attempts are made to elucidate the nature of a process by such indirect means as the study of the temperature effect. The value of the temperature coefficient need not necessarily refer to the essential part of the process but only to some other factor limiting the velocity of the action without necessarily being closely involved in it.

Effect of magnitude of the sowing.

The results of Tables I–IV received further confirmation in the case of experiments specially undertaken to investigate other points.

For example, Exps. I and II in Table V were made in order to ascertain what influence the initial number of bacteria added might have upon the bactericidal action of the serum. The data are somewhat scanty, but show approximately the logarithmic nature of the process. In the case of the heavier sowing, a concentration of about 1900 bacteria per drop, disinfection appears to be distinctly retarded, but the effect is not very marked when the mean value of the velocity constant, 0·66, is compared with that for an initial concentration of bacteria of only 90 per drop, viz. 0·79.

Influence of previously heating the serum.

A comparison of Experiments A and B in Table VI show the effect upon bactericidal action of previously heating the serum to 55–57° C. for about 20 minutes. As would be expected, the bacteria experienced no germicidal action in Exp. B with heated serum and, after a latent period which lasted about one hour, underwent steady multiplication.

The rate of multiplication was measured by calculating the mean generation time. If T = the mean generation time, and N_n and N_0 = the number of bacteria present in unit volume after times t_n and t_0 respectively, then, assuming cell-division to be steadily and universally maintained, the number of successive cell-divisions taking place in the interval of time $t_n - t_0$ will be equal to

$$\frac{t_n - t_0}{T}$$

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and $N_n = N_0 \times 2^{\left(\frac{t_n - t_0}{T}\right)}$

or $\log N_n - \log N_0 = \frac{t_n - t_0}{T} \log 2$

and $T = \frac{\log 2}{\frac{\log N_n - \log N_0}{t_n - t_0}}$,

the denominator of which fraction has been represented by A and called the "growth constant."

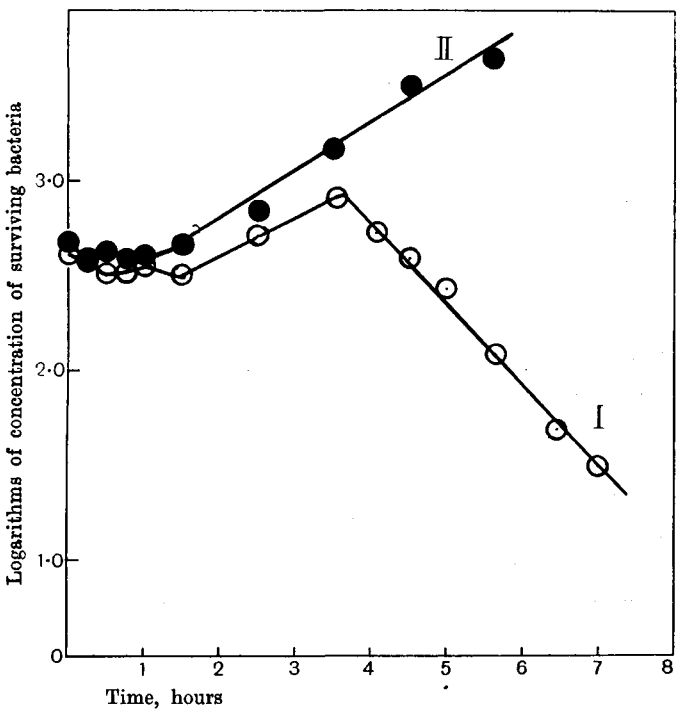

Fig. 3. Action of normal rabbit-serum upon *B. coli commune* (see Table VI).
I. Serum not heated.
II. Serum previously heated to 55–57° C. for 20 minutes.

The values of T were calculated up to 5·6 hours in Exp. B and show a fair constancy considering the great difficulty experienced in counting the very crowded plates with any accuracy. The mean generation was 1·4 hours or 84 minutes.

In Exp. A, made with unheated serum to form an exact control for B, the initial latent-period also lasted about one hour. In the period of growth which followed it is interesting to notice that the calculated

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value of the generation-time is not very dissimilar to that obtained in Exp. B. In fact when allowance is made for the difficulty of setting boundaries to the various stages in Exp. A, each of which is of short duration, the agreement in the two experiments is surprising. For the first three and a half hours the course of events runs almost parallel in the two cases.

This is well shown in Fig. 3, where logarithms of concentration of living bacteria are plotted against time. The same curve shows the logarithmic nature of the subsequent disinfection in Exp. A to be well maintained for a period of about four hours after which there appears to be some discrepancy. The values of the velocity constant, given in the last column of Table VI, also show a falling off towards the end of the experiment. It should be noted, however, that the concentration of survivors have been reduced so low that the enumerations are not very trustworthy.

The results of a second experiment are set out in Table VII. It was less complete than that of Table VI, and the numbers obtained on the plates were too great for accurate counting. The calculations must be regarded as approximate only, but serve as useful confirmation of the previous results. In this case in Exp. A, between the sixth and tenth hour, after the majority of the bacteria had been killed, the residue began to multiply. The rate of growth was similar to that obtaining in the short period of growth (second to fourth hour) previous to the disinfection, and also similar to that steadily maintained throughout in Exp. B with the heated serum.

Influence of temperature upon growth in serum.

The temperature coefficient of bacterial growth in serum was investigated in material previously heated to 55–57° C. for about 20 minutes. Three similar experiments at different temperatures are given in Table VIII and graphically shown in Fig. 4. They all show a preliminary latent period followed by regular multiplication which was maintained as long as the observations could be made. In Table IX are given the results of two experiments made at the same time at 30·1° C. with nutrient broth and normal saline solution respectively for comparison with Exp. II in Table VIII. By comparing the calculated values of the generation-time 0·54 hours (or 32 minutes) in broth and 0·69 hours (or 41 minutes) in serum, it will be seen that multiplication took place rather more slowly in the latter medium. The values of the

TABLE VI.

Action of normal rabbit-serum (I) *upon* B. coli commune *at* 30·3° *C.*

A. Serum unheated.

B. Serum previously heated for 20 minutes at 55–57° C.

Two standard drops (0·04 c.c.) of a 24 hours' culture of *B. coli commune* in broth, diluted 120-fold in distilled water, added to 3 c.c. serum.

Exp. 28. II. 12	Time, hours $=t$	Amount of sample taken, no. of drops	Numbers counted on plates		Mean no. of surviving bacteria in 1 drop serum $=N$	$\text{Log}_{10} N$	Growth constant $A = \frac{1}{t_n - t_0} \log \frac{N_n}{N_0}$	Generation time, hours $= \frac{\log 2}{A}$	Disinfection constant $K = \frac{1}{t_n - t_0} \log \frac{N_0}{N_n}$
A	0·03	1	409	402	405	2·607			
	0·25	1	391	359	375	2·574			
	0·5	1	332	313	322	2·508			
	0·75	1	322	330	326	2·513			
	1·0	1	350	366	358	2·554	($N_0 = 327$;		
	1·5	1	332	322	327	2·514	$t_0 = 1·5$)		
	2·5	1	502	546	524	2·719	·205	1·47	($N_0 = 813$;
	3·55	1	816	810	813	2·910	·193	1·56	$t_0 = 3·55$)
	4·07	1	532	538	535	2·728			·35
	4·5	1	396	392	394	2·595		1·51 = mean	·33
	5·0	1	263	276	269·5	2·431			·33
	5·66	1	117	123	120	2·079			·39
	6·45	1	57	42	49·5	1·695			·42
	7·0	1	29	33	31·5	1·498			·41
	8·25	1	30	20	25	1·398			·32
	9·1	1	16	18	17	1·230			·30
	10·25	1	17		11·8	1·072			·27
		5	54						
	11·4	1	6		5·3	0·724			·28
		5	26						
	13·6	1	2	1	1·0	·00			·29
		5	4						
	18·0	1	0	(?) 1	0				·34 =
		5	0						mean
	24·0	1	0		0				
		4	0						
	31·0	1	0		0				
		4	0						
B	0·03	1	468	476	472	2·674			
	0·25	1	377	389	383	2·583			
	0·5	1	430	416	423	2·626			
	0·75	1	386	387	386	2·587			
	1·0	1	412	377	395	2·597	($N_0 = 444$;		
	1·5	1	453	435	444	2·647	$t_0 = 1·5$)		
	2·5	1	690	718	704	2·848	·201	1·50	
	3·5	1	1572	1368	1470	3·167	·260	1·16	
	4·5	1	3152	3240	3200	3·505	·286	1·05	
	5·6	1	4052	4928	4490	3·652	·245	1·23	
								1·26 = mean	

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TABLE VII.

Action of normal rabbit-serum (I) *upon* B. coli commune *at* 30·3° *C.*

A. Serum unheated.

B. Serum previously heated for about 25 minutes at 55–56° C.

Two standard drops (0·04 c.c.) of a 24 hours' culture of *B. coli commune* in broth, diluted 60-fold in distilled water, added to 3 c.c. and 2·7 c.c. serum in Experiments A and B respectively.

Exp. 20. II. 12	Time, hours $=t$	Amount of sample taken, no. of drops	Numbers counted on plates		Mean no. of surviving bacteria in 1 drop serum $=N$	$\text{Log}_{10} N$	Growth constant $A = \frac{1}{t_n - t_0} \log \frac{N_n}{N_0}$	Generation time, hours $= \frac{\log 2}{A}$	Disinfection constant $K = \frac{1}{t_n - t_0} \log \frac{N_0}{N_n}$
A	0·05	1	1336	1412	1370		($N_0 = 1460$;		
	1·0	1	1388	1532	1460	3·164	$t_0 = 1·0$)		
	2·0	1	2120	2412	2270	3·356	·192	1·57	
	3·0	1	6888	6064	6480	3·812	·324	0·93	($N_0 = 7810$;
	4·33	1	7808		7810	3·893	·219	1·37	$t_0 = 4·33$)
	6·4	1	1396	1408	1400	3·146	($N_0 = 1400$; $t_0 = 10·6$)		·36
	10·6	1	16770	24350	20560	4·313	·278	1·08	
	12·1	1	∞					1·21 = mean	
B	0·05	1	1460	1512	1490				
	0·86	1	1660	1680	1670		($N_0 = 1970$;		
	1·9	1	1840	2092	1970	3·294	$t_0 = 1·9$)		
	3·0	1	3640	4152	3900	3·591	·270	1·11	
	4·25	1	8064	7712	7890	3·897	·257	1·17	
	6·33	1	18944	19584	19260	4·285	·224	1·34	
	10·6	1	∞					1·21 = mean	

TABLE VIII.

Effect of temperature upon growth of B. coli commune *in normal rabbit-serum* (I) *previously heated to* 55–57° *C. for* 20 *minutes.*

Exp. 6. III. 12	Tempera-ture, °C.	Time, hours $=t$	Amount of sample taken, no. of drops	Numbers counted on plates		Mean no. of living bacteria in 1 standard drop serum $=N$	$\text{Log}_{10} N$	Growth constant $A = \frac{1}{t_n - t_0} \log \frac{N_n}{N_0}$	Generation time, hours $= \frac{\log 2}{A}$
I	19·8	0·08	5	139	111	25	1·398	($N_0 = 48·3$;	
		2·3	5	120	105	22·5	1·352	$t_0 = 6·4$)	
		4·3	4	119	110	28·6	1·456		
		6·4	2 + 4	90 + 200		48·3	1·684		
		8·4	2	247	269	129	2·111	·213	1·41
		10·0	1 + 3	550 + 848		349	2·543	·239	1·26
		11·7	1	619	647	633	2·801	·211	1·43
									1·37 = mean
II	30·1	0·08	5	117	103	22	1·342	($N_0 = 56·4$;	
		2·3	5	293	271	56·4	1·751	$t_0 = 2·3$)	
		4·3	4	2136	1748	485	2·686	·467	·645
		6·4	2	*9376	8160	4380	3·641	·461	·653
		8·4	2	∞					·649 = mean
III	34·9	0·08	5	90	112	20·2	1·305		
		1·0	5	119	113	23·2	1·365		
		2·3	2 + 3	174 + 324		99·6	1·998	($N_0 = 99·6$; $t_0 = 2·3$)	
		4·3	2	3792	3776	1890	3·276	·639	·471
		6·3	1	*38620	34620	36620	4·564	·641	·470
									·470 = mean

* Numbers must be regarded as approximate.

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generation-time are calculated on the assumption that multiplication is universal among the bacteria and maintained without interruption. But it may well be that in serum the longer generation-time is to be

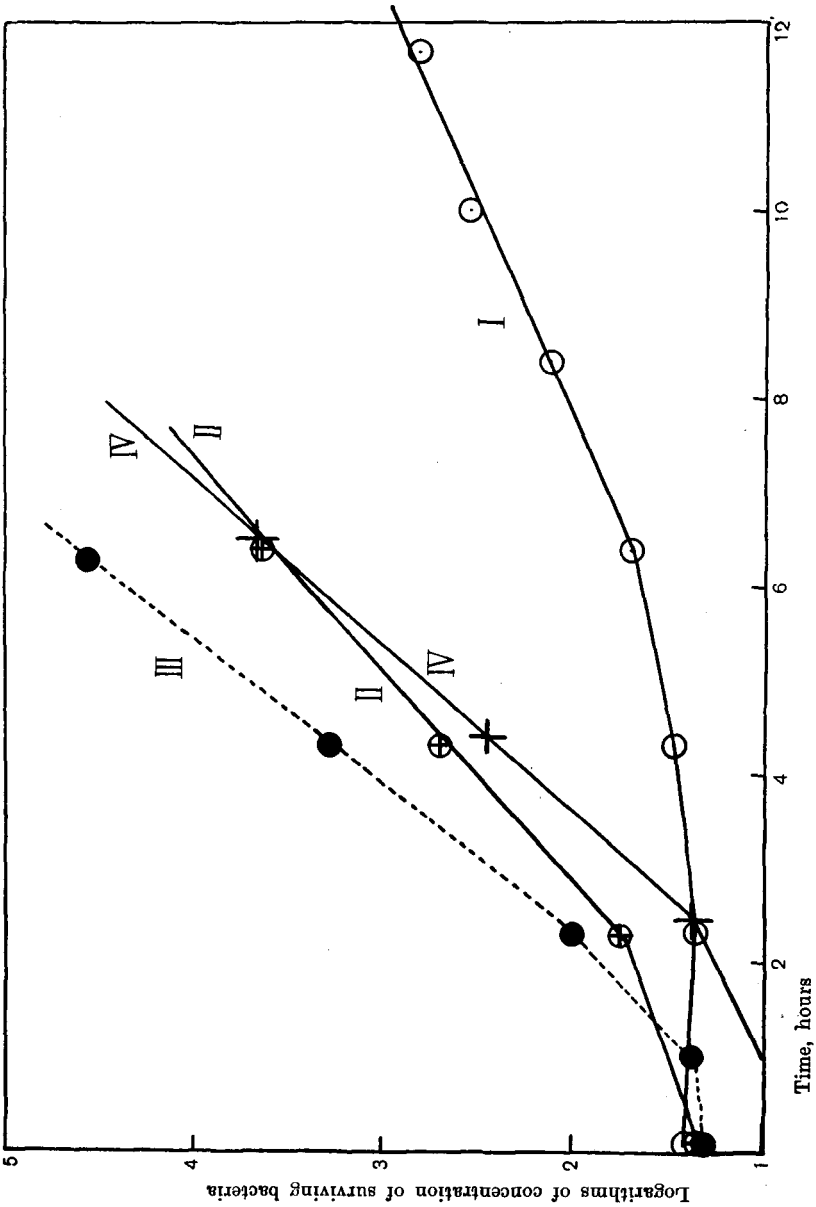

Fig. 4. Influence of temperature upon the growth of *B. coli commune* in normal rabbit-serum, heated to 55–57° C. for 20 minutes (see Table VIII).

I. at 19·8° C. II. at 30·1° C. III. at 34·9° C. IV. in broth at 30·1° C.

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explained by the fact that some individuals do not multiply at all, or that some are dying while others are growing. The point could be settled by a determination of the generation-time by direct observations under the microscope, using a dark ground and side illumination, and it is hoped subsequently to publish the results of a comparable set of experiments to the present in which the microscopic method is substituted for that of plate cultures.

TABLE IX.

Growth of B. coli commune *in* (*a*) *nutrient broth, and* (*b*) "*normal*" *saline solution at* 30·1° *C., for comparison with Exp. II, Table VIII.*

Exp. 6. III. 12	Time, hours $=t$	Amount of sample taken, no. of drops	Numbers counted on plates		Mean no. of living bacteria in 1 standard drop $=N$	$\text{Log}_{10} N$	Growth constant $A = \frac{1}{t_n - t_0} \log \frac{N_n}{N_0}$	Generation time, hours $= \frac{\log 2}{A}$
(*a*)	0·05	5	43	29	7·2		($N_0 = 23{\cdot}4$;	
	2·45	5	123	111	23·4	1·369	$t_0 = 2{\cdot}45$)	
	4·4	2	462	648	277	2·443	·551	·546
	6·5	2	8430	10210	4660	3·668	·568	·530
	23·1	·002 (by dilution)	*12580	13250	6457000	6·810		·538 = mean
(*b*)	0·05	5	28	52	8		($N_0 = 28{\cdot}4$;	
	1·3	5	57	56	11·3		$t_0 = 6{\cdot}5$)	
	2·45	5	60		14·1			
		10	152					
	4·43	5	64	66	13			
	6·5	5	154	130	28·4	1·453		
	8·5	5	260	256	51·6	1·713	·130	
	10·03	5	401		79·7	1·901	·127	
		10	794					
	11·8	10	976		108·1	2·034	·110	
		17	1940					
	22·8	10	6180	6020	610	2·785		

* Number must be regarded as approximate.

The figure for generation-time in broth at 30·1° C., viz. 32 minutes, is in fair agreement with that obtained for *B. coli commune* by Lane-Claypon (1909) using a similar method to the above, viz. 31·8 minutes at 31° C. The fact that the value is also consistent with that obtained by direct observation of the bacterial cells (Barber, 1908, Table II, p. 389), viz. 30·7 minutes at 30° C., goes to prove that during the early hours of a broth culture multiplication is universal. Whether multiplication in serum is really slower or is only less general can only be settled by further experiment.

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The effect of temperature was measured by comparison of "growth constants" at the various temperatures of experiment. These are set out in Table X and the influence of temperature is seen to be fairly consistent and in accordance with a logarithmic law. It is not possible to decide whether the velocity of growth is influenced by rise of temperature in accordance with the law of Arrhenius or in a simple logarithmic manner, for over a small range of temperature the two expressions become almost identical. The same is true of the influence of temperature upon the disinfectant action of serum.

TABLE X.

Temperature coefficient of growth of B. coli commune *in normal rabbit-serum* (I) *heated* (*see Table VIII*).

Exp. 6. III. 12	Temperature °C.	Mean value of the growth constant A	Log_{10} $(A \times 10)$	Log_{10} (temperature coefficient per 1° C.)	Temperature coefficient per 1° C.	Temperature coefficient per 10° C.
I	19·8	·221	·344			
				·0313	1·074	2·06
II	30·1	·463	·666			
				·0292	1·070	1·96
III	34·9	·640	·806			
					1·072 = mean	2·01 = mean

The rate of growth in serum is influenced by temperature to a degree comparable with that shown in the case of the death-rate (see above), being increased 1·07-fold and 2·01-fold for a rise of temperature of 1° C. and 10° C. respectively, between the temperatures of 20° C. and 35° C. That the effect remains consistent at least as high as 40° C. may be gleaned from the somewhat scanty data of the growth phase in Exps. I, II and III, Table II.

These values are in close accord with those obtained for growth of *B. coli* in broth by Lane-Claypon and Barber respectively. The former worker found a temperature coefficient of 2·13 per 10° C. between the temperatures of 20° C. and 34° C. (calculated from data given in Table I, *see* Lane-Claypon, 1909, p. 246) and the latter obtained 2·36 per 10° C. between 20° C. and 35° C. (from curve, *see* Barber, 1908, p. 696).

SUMMARY.

1. The action *in vitro* of normal rabbit-serum upon *B. coli commune* consists usually of three or more phases, the duration of which is inversely related to temperature.

2. The latent period which occurs on first mixing bacteria and serum is followed by a period of bacterial growth, which in its turn

yields to a period of bactericidal action. This may remain final or may be succeeded by a second period of growth.

3. The germicidal action, both in case of normal rabbit-serum upon *B. coli commune* and of normal goat-serum upon *B. typhosus*, takes place in accordance with a logarithmic law so that death-rate remains proportional to the concentration of surviving bacteria.

The bactericidal action of serum thus falls into line with all other cases of disinfection, hitherto investigated.

4. The temperature coefficient of the bactericidal action of serum is low in comparison with that found for other germicides, being 2·84 per 10° C. rise in temperature for normal rabbit-serum and *B. coli commune* and 1·93 in the case of normal goat-serum and *B. typhosus*.

5. Growth of *B. coli commune* in rabbit-serum takes place logarithmically so that the concentration of living bacteria after equal intervals of time form terms of a geometrical series.

The calculated value of the generation-time is slightly greater than that obtaining in broth at the same temperature.

6. The influence of temperature upon rate of bacterial growth in serum is comparable with the effect upon growth in other media. The generation-time is decreased 2·01-fold for a rise in temperature of 10° C.

7. The separate processes of bacterial growth and death in serum being similarly influenced by temperature, there is no support for the view that the significance of fever might be concerned with a tendency to encourage either process relative to the other.

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