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Rôle of Leucocytes and Serum in Streptococcidal Activity of Blood.

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In testing the defibrinated blood and serum of patients and normal individuals for killing effect against streptococci we found that, while the majority of samples of blood exhibited bactericidal power, the serum alone rarely caused reduction in the number of bacteria. This finding supported the idea that the effective bactericidal agent in whole blood acting against streptococci resides in the cellular elements and confirms the earlier observations of several workers, notably Mackie, Finklestein and Van Rooyden[1] with regard to gram positive cocci in general. Most of the previous work on gram positive bacteria, however, has been done with pneumococci[2] and staphylococci[3] and we considered it worth while to check and extend the work with streptococci.

Leucocyte counts were made at the time of withdrawal of samples of blood and repeated after defibrination was completed. The defibrination was accomplished by rotating the blood with glass beads in an Erlenmeyer flask under sterile precautions. In 6 samples of freshly drawn blood, leucocyte counts of 6930 to 9550 per cu. mm. were obtained and in the same samples after defibrination the leucocyte number was between 5200 and 7160, an average reduction

TABLE I.
Effect of Aging and Chilling on Bactericidal Test.

Case No.		Colony Count-Bc Test				
		Start	4 hrs.		8 hrs.	Leucocytes
				%		%
1	Fresh blood	256	6	(—97)	320	6150
	Chilled "	200	960	(+380)	8000±	4850 (--21)
2	Fresh blood	256	31	(—88)	2080	5300
	Chilled "	245	8000±	(+3200)	8000±	2435 (—54)
3	Fresh blood	448	8000±	(+1700)	8000±	5940
	Chilled "	320	8000±	(+2400)	8000±	2825 (—52)
4	Fresh blood	140	5	(—97)	15	6400
	Chilled "	110	800	(+630)	8000±	2600 (--59)
5	Fresh blood	175	0	(—100)	50	6600
	Chilled "	160	3	(—98)	576	5350 (—19)

[1] Mackie, J., Finklestein, M. H., and Van Rooyden, C. E., *J. Hyg.*, 1932, **32**, 494.

[2] Robertson, O. H., and Sia, R. H. P., *J. Exp. Med.*, 1924, **39**, 219.

[3] Thalhimer, W., and Colwell, C., *J. Lab. and Clin. Med.*, 1929, **24**, 441.

of 23%. This reduction was not sufficient to produce an appreciable alteration of the bactericidal power of the blood as proved by previous experiments. After rotatory agitation for periods of 4 hours without the addition of bacteria, however, the reduction in number of intact leucocytes was from 70% to 90%.

The effect of standing and refrigeration is illustrated in Table I, which gives the comparative colony counts from bactericidal tests made on fresh samples and samples allowed to stand in the refrigerator overnight. These were incubated with the test culture for periods of 4 and 8 hours in the agitating machine. In each blood sample that exhibited definite killing action when fresh, there was marked reduction of bactericidal power after refrigeration. In these samples the degree of reduction in number of leucocytes varied between 19% and 59%. It is obvious that the numerical reduction in leucocytes is not the most important factor. The phagocytic activity of the intact leucocytes must have been seriously impaired by such treatment.

Next a small series of tests was made to determine whether or not some of the inhibitory effect of the blood would remain after complete removal of the leucocytes. Four samples of defibrinated blood from different patients were divided into 2 portions and one part centrifuged at low speed to throw down the formed elements. The buffy coat was removed as completely as possible with sterile pipettes and parallel tests were run on these samples and on the complete defibrinated blood as controls. The results (Table II) indicate

TABLE II.
Effect of Removal of Leucocytes.

Case	Colony Count (Control)			Colony Count (Leucocytes removed)		
No.	Start	4 hrs.	8 hrs.	Start	4 hrs.	8 hrs.
1	2880	8000±	8000±	2180	8000±	8000±
2	2240	37	86	2200	8000±	8000±
3	190	0	0	210	8000±	8000±
4	200	1	160	215	8000±	8000±

that after the more or less complete removal of leucocytes, blood no longer inhibits the growth of streptococci. In case 1, however, the blood failed to show any inhibitory effect even in the control sample.

The apparent exhaustion of bactericidal power after about 4 hours of incubation and the demonstration of marked reduction of leucocytes in mixtures that had been incubated for a few hours with rotatory agitation led us to suspect that the loss of killing power was due merely to exhaustion and disintegration of the leucocytes. If true, an addition of fresh leucocytes should restore the exhausted

factor. This was found to be the case in each of 5 samples from different patients where the blood was fortified by the addition of leucocytes at the end of 4 hours of incubation. The volume of leucocyte suspension added to each tube was equal approximately to that at the beginning of the test. (Table III.)

TABLE III.
Bactericidal Power after Fortification with Leucocytes.

Case	Control			Fortified at 4 hours		
No.	Start	4 hrs.	8 hrs.	Start	4 hrs.	8 hrs.
1	520	8000±	8000±	560	8000±	1216
2	80	160	4000±	90	175	115
3	2880	8000±	8000±	2900	8000±	1940
4	240	19	160	250	28	15
5	152	4	200	160	7	30

In a similar group of cases the blood samples were each divided into 2 equal portions. One was run as usual as the control and the other was subjected to rotatory agitation in the incubator for 4 hours before the suspension of bacteria was added. The killing effect of the blood was completely destroyed in 3 samples and seriously impaired in the other 2 as a result of the preliminary incubation (Table IV).

TABLE IV.
Reduction of Bactericidal Power after Preliminary Incubation.

Case	Control			4 hrs. Preliminary Incubation		
No.	Start	4 hrs.	8 hrs.	Start	4 hrs.	8 hrs.
1	288	0	58	300	728	8000±
2	432	0	0	400	368	124
3	800	13	62	780	8000±	8000±
4	820	125	548	800	8000±	8000±
5	592	11	0	610	8000±	8000±

The mode of destruction of the bacteria and the rate at which they were removed were determined by the examination of stained films made from the mixtures at intervals during the tests. For this experiment suspensions containing about 200 million bacteria per cc. were used. Blood films were made in the usual manner from a drop removed by sterile capillary pipette and stained with Wright's blood stain. The relative numbers of polymorphonuclear cells containing bacteria and those showing no bacteria in their cytoplasm at the different intervals are recorded in Table V. Toward the end of the 4-hour period the leucocytes were so few that only 10 or 15 leucocytes were found by systematic examination of the entire film. In each case the majority of the leucocytes contained phagocytosed

TABLE V.
Rate of Phagocytosis in Bactericidal Tests.

Case No.		Start	½	1	1½	2	3	4	8 hrs.
1	Pmn with bac.	3	6	12	13	25	15	10	
	No intrac. bac.	22	19	13	2	0	0	0	
	Col. in Bc test	100						20	64
2	Pmn with bac.	5	8	18	25	25	18	15	
	No intrac. bac.	20	17	7	0	0	0	0	
	Col. in Bc test	80						160	6000±
3	Pmn with bac	10	23	25	25	25	20	10	
	No intrac. bac.	15	2	0	0	0	0	0	
	Col. in Bc test	150						1	1
4	Pmn with bac.	4	11	15	24	25	15	15	
	No intrac. bac.	21	14	10	1	0	0	0	
	Col. in Bc test	190						160	640
5	Pmn with bac.	12	40	44	46	50	50	50	
	No intrac. bac.	38	10	6	4	0	0	0	
	Col. in Bc test	200						1	1

cocci by the end of one or one and one-half hour and in 2 hours all of the cells contained bacteria in various stages of disintegration. No significant differences in rate of phagocytosis were observed in those blood samples exhibiting marked inhibitory power in the bactericidal tests and those showing less power.

In preliminary experiments it was found that while the presence of serum was necessary for the obtaining of bactericidal effects, dilutions up to 1-25 were apparently as effective as undiluted serum. The agglutinating titer had been found to bear no constant relationship to the bactericidal power of whole blood, defibrinated blood or serum. The effect of varying complement concentration remained to be determined. In 10 cases the complement titer and bactericidal power of the defibrinated blood were determined on the

TABLE VI.
Bactericidal Power and Complement Titer.

Case No.	Serum Dilution	Beginning Lysis cc.	Complete Lysis cc.	Colony Count in Bc Test		
				Start	4 hrs.	8 hrs.
1	1-50	.2	0.5	160	2	368
2	1-50	.1	0.2	340	1420	8000±
3	1-50	.2	1.0	256	31	2080
4	1-25	.5	0.8	240	0	0
5	1-25		0.3	288	23	380
6	1-50	.5	1.0	240	8000±	8000±
7	1-50	.5	1.0	240	3	450
8	1-50	.9	1.0	152	4	200
9	1-25		0.5	250	10	50
10	1-50	.7	1.0	192	0	0

same sample (Table VI). No correlation was observed. One of the two samples of blood showing the least bactericidal power, contained the highest concentration of complement and the 2 samples having the greatest killing power yielded very low complement titer.

Finally the thermostability of the active factor was tested by removing the serum as completely as possible from samples of defibrinated blood after centrifugation and heating it at 55°C. for 30 minutes. The inactivated serum was then restored to the cells from which it had been removed and bactericidal tests run. For controls we used a part of the same sample, subjected to the same treatment with the exception that the serum was not heated. In a few cases a part of the serum was heated to 68°, returned to the cells and then tested for killing effect. Similar tests were made with serum alone after heating at 55° or 68° for 30 minutes and with unheated controls. In none of these cases was there any evidence that the bactericidal property was impaired by the heating of the serum.

Conclusions. The power of defibrinated blood to inhibit the growth of or destroy streptococci is dependent upon the presence of surviving leucocytes and is roughly proportional to the number of leucocytes, other factors being equal. A minimal number of leucocytes is necessary for the demonstration of the bactericidal effect. The bacteria are removed by phagocytosis and finally destroyed by intracellular lysis. Complement is not necessary for the bactericidal action of defibrinated blood or serum. The bactericidal element in defibrinated blood which is effective against streptococci is not destroyed by heating the separated serum at 55°C. for 30 minutes. The results of a small number of tests suggest that the heating of the serum even at 68°C. does not impair the bactericidal effect of the restored defibrinated blood.

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Development of Gastric Ulcers and Decrease in Reducing Power of Adrenals Following Injection of Bile Salts.

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Sellards[1] reported the development of acute gastric ulcers in guinea pigs following intraperitoneal bile salt injection. His ob-