THE COMPARATIVE BACTERICIDAL ACTION OF NORMAL SERUM, "WHOLE" BLOOD AND SERUM-LEUCOCYTE MIXTURES; WITH FURTHER OBSERVATIONS ON THE BACTERICIDAL MECHANISM OF NORMAL SERUM.

By T. J. MACKIE, M. H. FINKELSTEIN and C. E. VAN ROOYEN.

(*From the Bacteriology Department, Edinburgh University.*)

CONTENTS.

Introduction.

In recent years a considerable amount of attention has been devoted to the bactericidal properties *in vitro* of "whole" blood and serum-leucocyte mixtures, and various methods have been devised for testing these effects (Heist, Solis-Cohen and Solis-Cohen, 1918; Wright, Colebrook and Storer, 1923; Robertson and Sia, 1924[a, b]; and others). Organisms which show little susceptibility to the bactericidal action of serum *per se*, *e.g.* Staphylococcus, Pneumococcus, etc., have been specially studied and it has emerged that "whole" blood or serum-leucocyte mixtures may exert a definite bactericidal effect even when the serum is quite inactive in this respect and the leucocytes have likewise no independent killing properties. Such findings, of course, have merely illustrated by new methods the long-recognised rôle of the leucocytes and the serum-opsonins in antibacterial defence. Some of the data elicited by these tests have suggested a correlation of the bactericidal power of "whole" blood and serum-leucocyte mixtures with actual resistance to infection by particular organisms. Whether or not such *in vitro* reactions exactly reflect the defensive processes which operate *in vivo*, their application has nevertheless contributed valuable additions to our knowledge of the natural and acquired immunity mechanism of the animal body.

https://doi.org/10.1017/S0022172400018210 Published online by Cambridge University Press

When serum *per se* possesses a bactericidal effect for a particular bacterium, it might be expected that the "whole" blood from which the serum is derived would exert an enhanced killing power due to the combined action of the serum-bactericidin and the leucocytes. In the course of a systematic study of the natural bactericidins of serum (Mackie and Finkelstein, 1932) some preliminary comparisons were made between "whole" blood and serum in their respective bactericidal action towards various organisms. The results showed that "whole" blood was frequently not superior to serum in this respect and was in fact often less active—an apparently paradoxical effect. Fiessinger and Cattan (1928) described a similar finding in typhoid infections: thus bactericidal properties were often absent from "whole" blood while well marked in serum, and the addition of leucocytes to the serum frequently annulled its action.

The initial observations referred to above were extended later into a more detailed study of the comparative bactericidal properties of serum, "whole" blood and serum-leucocyte mixtures, and the results of this inquiry are recorded in the following paper.

METHODS.

With certain modifications the system of testing the bactericidal action of serum, "whole" blood and serum-leucocyte mixtures was that previously applied by Mackie and Finkelstein (1931, 1932) for measuring the activity of the serum-bactericidins. This technique has greatly facilitated the carrying out of large numbers of such tests.

Sterile stoppered test-tubes (3 in. by $\frac{1}{2}$ in.), each containing a given quantity of "whole" blood, serum or serum-leucocyte mixture, were inoculated in series with a certain amount of graded bacterial suspensions prepared by successive decimal dilutions from an initial standard suspension ("*S*") of a young culture of the organism to be tested:

$$S/1,\ S/10^1,\ S/10^2,\ \ldots\ S/10^9.$$

Immediately after admixture loop-transfers were made from the contents of each tube to a plate of culture medium (single-stroke inoculations being made on the surface of the plate) and similar transfers were made again after 4 and 24 hours' incubation of the mixtures at 37° C. After incubation of the plates the occurrence of a bactericidal effect could be determined by comparing the end-points of growth from the series of transfers after 4 and 24 hours with the end-point of the immediate transfers. The results obtained by this method and the details of its application to serum have been dealt with in the papers cited above.

The standard density of bacterial suspension was such that the end-point of growth from the transfers made immediately after mixture coincided with the 5th or 6th dilution in the series. The usual standard was a 1/100th dilution of a suspension equivalent to Brown's opacity No. 2, but varied with different organisms according to the results of experience in preparing suitable series of decimal dilutions from particular standards. The bacterial dilutions were made up in gelatin-Locke solution (pH 7·5) as used in the experiments of Robertson and Sia (1924[a]).

The method also allowed an estimate to be made of the growth-promoting action of blood or serum after 24 hours' incubation of the mixtures, the end-point in such cases being higher in the scale of dilutions than that after immediate transfers, whereas a bactericidal effect brought about a lowering of the end-point.

The results were denoted by a number representing the difference between the indices of the dilutions which corresponded with the end-points of the immediate and later transfers, the + sign being prefixed to indicate bactericidal action, the − sign to indicate growth-promotion.

In testing "whole" blood both defibrinated and heparinised specimens were used. The amount of heparin was 4–7 mg. to 10 c.c. blood: this was usually effective in preventing coagulation over the period of incubation. Serum from defibrinated blood and the plasma of heparinised blood respectively were examined and compared with the "whole" blood. Leucocytes were obtained from ox or horse blood. It was found that 200 c.c. of citrated blood after centrifugalisation in tubes each containing about 25 c.c. yielded an abundant and easily separable leucocyte layer. This was pipetted off, washed twice in citrate-saline solution and then suspended in 3–5 c.c. gelatin-Locke solution. There was usually a certain admixture of platelets with the leucocytes.

In most of the tests the quantity of blood added to each tube was 0·5 c.c. and the volume of serum taken for comparison with it was that estimated to be present in this test quantity of blood. Thus if a 10 c.c. sample of defibrinated blood on centrifugalisation yielded 6 c.c. of serum, 0·3 c.c. was taken as the test quantity of serum. In some cases in order to equalise the volumes the test quantity of serum was made up with gelatin-Locke solution to that of the blood. In a number of tests also the volumes of blood and serum were equal: here of course the "whole" blood contained a lesser quantity of constituent serum than the separated serum with which it was compared. It may be noted that by all three methods similar results were obtained.

The serum-leucocyte mixtures were prepared by adding to serum one-fifth of its volume of the leucocyte emulsion referred to above. It was found difficult to standardise the leucocyte content of these mixtures, but as prepared they contained usually from 10,000 to 30,000 cells per c.mm.

During incubation the tubes containing the mixtures were shaken in an approximately vertical position by a slowly-moving shaking machine.

The bactericidal effects of serum after short and long periods of incubation.

The comparative tests of blood and serum recorded in this paper included observations of bactericidal effects after 4 hours and of bactericidal or growth-promoting properties also after 24 hours.

In a previous paper (Mackie and Finkelstein, 1932) some reference was made to the fact that the time required for the maximum bactericidal action of serum varied with different organisms. In detailed tests with *Vibrio cholerae* the maximum lysis usually occurred in 3–4 hours at 37° C. and prolonged incubation up to 24 hours did not enhance the effect (see Table 1). Similar results were obtained with *B. dysenteriae* Shiga, *B. influenzae* and an attenuated *B. anthracis*. With other organisms the bactericidal reaction was generally progressive between 4 and 24 hours (*e.g. Streptococcus haemolyticus* and *viridans*, *Diplococcus catarrhalis*, *B. diphtheriae*, *B. welchii*, *B. melitensis*, *B. abortus*) and with certain strains of *B. melitensis* and *B. abortus* killing was only noticeable as a rule after 24 hours' incubation of the mixtures (Table 1). It was found also with some strains which gave almost uniformly negative results with the serum of certain animals after 4 hours that on prolonged incubation of the mixtures a weak or moderate bactericidal effect occurred, *e.g.* certain

strains of *Streptococcus haemolyticus* and *viridans*, and *B. diphtheriae*. In the case of the staphylococci, Enterococcus, Pneumococcus, *B. anthracoides*, *B. pyocyaneus* and certain members of the Pasteurella group, while a bactericidal effect could be demonstrated with certain sera after 4 hours' incubation, generally no such effect was noticeable in the same test after 24 hours, due apparently to the multiplication of the surviving organisms in the mixtures. This is well shown in the illustrative test with *B. avisepticus* (Table 1). Apparently even when such marked killing occurred in 4 hours a certain number of organisms survived though insufficient to be detectable, and later multiplied in the serum.

Table 1. *Bactericidal effects by serum after* 4 *and* 24 *hours.*

	Incubation	Bacterial dilutions								
		$S/1$	$S/10^1$	$S/10^2$	$S/10^3$	$S/10^4$	$S/10^5$	$S/10^6$	$S/10^7$	$S/10^8$
V. cholerae "Bombay" + rabbit serum	Before	C	C	C	+ + +	+	f.c.	–	–	–
	4 hr.	C	+	–	–	–	–	–	–	–
	24 hr.	C	+	–	–	–	–	–	–	–
B. melitensis "Arkwright" + horse serum	Before	C	C	C	+ +	+	f.c.	–	–	–
	4 hr.	C	C	C	+ +	+	f.c.	–	–	–
	24 hr.	C	+ +	f.c.	–	–	–	–	–	–
B. avisepticus + ox serum	Before	C	C	+ +	+	f.c.	–	–	–	–
	4 hr.	–	–	–	–	–	–	–	–	–
	24 hr.	C	C	C	C	C	+ +	+	f.c.	–
B. pyocyaneus + rabbit serum	Before	C	C	C	+ +	+	–	–	–	–
	4 hr.	C	+ +	+	f.c.	–	–	–	–	–
	24 hr.	C	C	C	C	C	+ +	+	–	–
Streptococcus haemolyticus + rabbit serum	Before	C	C	C	+ +	+	f.c.	–	–	–
	4 hr.	C	C	+ +	f.c.	–	–	–	–	–
	24 hr.	f.c.	–	–	–	–	–	–	–	–
Staphylococcus aureus + rabbit serum	Before	C	C	+ +	+	f.c.	–	–	–	–
	4 hr.	C	+ +	f.c.	–	–	–	–	–	–
	24 hr.	C	C	C	C	C	C	C	–	–

Symbols. C = a confluent growth; + + + = abundant growth but showing discrete colonies; + = colonies along whole line of inoculation but well separated; + + = intermediate between + + + and +; f.c. = a few scattered colonies.

Multiplication of surviving organisms in a bactericidal serum, while characteristic of certain bacteria, was apparently dependent on the concentration of serum, and the effect could be reduced or even annulled by increasing the quantity of serum used in the test. Similarly in the case of organisms which showed sustained bactericidal or growth-inhibitory effects over 24 hours, a reduction in the usual concentration of serum tested (by dilution with saline) led to multiplication after the initial bactericidal reaction. The higher concentration of serum either produced more complete killing or inhibited growth of any survivors.

Apparently among some organisms when acted on by serum, individuals resist bactericidal action, may not be inhibited in their growth and multiply in the serum. Experiments have been made with *Staphylococcus aureus* and *B. pyocyaneus* to ascertain whether such survivors give rise to a strain which is more resistant than the parent strain. Strains derived in this way did not,

however, prove more resistant than the original strain when tested comparatively.

With many organisms the comparative results at 4 and 24 hours varied according to the sample of serum tested, the late effect being greater than, equal to, or less than the early result. Such variability was particularly noticeable with *B. anthracis*, *B. typhosus*, *B. paratyphosus* B, *B. proteus*, *B. coli*, and to some extent with *V. cholerae*.

These varying results with different organisms were independent of the animal species from which the serum used in the test was obtained.

Comparison of serum with "whole" blood and serum-leucocyte mixtures.

Samples of blood mainly from the rabbit and ox were used for these tests. These species were selected in view of the fact that ox serum is specially active towards the Gram-negative bacteria and less frequently bactericidal to the Gram-positive types, whereas rabbit serum is more frequently bactericidal to the Gram-positive types and less pronounced in its activity towards the Gram-negative organisms. Both defibrinated and heparinised blood samples were compared with serum and plasma respectively derived from them. Ox leucocytes were used in making serum-leucocyte preparations with ox and rabbit serum; horse leucocytes were also used with rabbit serum. Leucocyte counts of "whole" blood and serum-leucocyte preparations were made and stated as numbers of cells per c.mm. In the initial experiments (1) defibrinated "whole" blood, (2) serum from defibrinated blood, (3) a serum-leucocyte mixture, (4) leucocytes suspended in gelatin-Locke solution (in the same concentration as the serum-leucocyte preparation) were compared as regards their bactericidal power. Heparinised blood and the separated plasma were compared with defibrinated blood and serum from the same animal.

Organisms tested.

Staphylococcus aureus (2 strains) and *albus*, *Streptococcus haemolyticus* (2 strains) and *viridans* (2 strains), Enterococcus, Pneumococcus (2 strains—Types I and II), *Diplococcus catarrhalis*, *B. diphtheriae*, *B. anthracis* (2 strains—virulent and attenuated), *B. anthracoides*, *B. coli* (2 strains), *B. typhosus* (2 strains), *B. paratyphosus* A and B, *B. dysenteriae* Shiga and Y types, *B. proteus* X 19, *B. pyocyaneus*, *B. influenzae*, *V. cholerae* and *paracholerae* A, *B. abortus* (2 strains), *B. suisepticus*.

When initial experiments with a particular organism gave varying results a series of tests were carried out in order to ascertain the average result with this organism. In all about 200 tests were made.

Staphylococcus aureus.

Towards this organism ox serum was inactive after 4 hours, and showed considerable growth-promoting action after a longer period of incubation. "Whole" blood, however, and also serum-leucocyte mixtures yielded a definite though weak bactericidal effect after 4 hours; after 24 hours "whole" blood

and "serum-leucocytes" were growth-promoting like serum but to a less degree (Table 2 (*a*)).

Rabbit serum was weakly active as a rule after 4 hours, and "whole" blood and "serum-leucocytes" showed an enhanced effect; after the longer period serum was markedly growth-promoting and blood and "serum-leucocytes" relatively inhibitory (Table 2 (*b*)).

In most cases the leucocyte suspension *per se* exerted an initial bactericidal action but was later strongly growth-promoting. In some tests the activity of the "serum-leucocytes" was greater than the sum of the effects of the serum

Table 2. *Bactericidal effects.*

Staphylococcus aureus.

	B	S	S-L	L
(*a*)	+1/−4	0/−5	+1/−4	0/−4
Ox blood; ox leucocytes	*3100*		*12,400*	
(*b*)	+3/−4	+1/−5	+5/−4	+1/−4
Rabbit blood; ox leucocytes	*1700*		*5200*	
(*c*)	+2/−1	+1/−1	+2/−1	+1/−3
Rabbit blood; ox leucocytes	*3810*		*10,200*	
(*d*)	+1/−1	+2/0	+1/−5	+1/−5
Rabbit blood; ox leucocytes	*2900*		*18,700*	
	B	S	Bh	Ph
(*e*)	+2/0	+1/−3	+2/−1	+1/−3
Rabbit blood	*3200*		*4000*	

Explanation of symbols, etc., in this and subsequent tables:

B = "whole" blood defibrinated; Bh = heparinised "whole" blood; S = serum from defibrinated blood; Ph = plasma from heparinised blood; S-L = serum-leucocyte mixture; L = leucocytes suspended in gelatin-Locke solution.

The first figure (in front of oblique stroke) under B, S, etc., denotes the bactericidal or other effect at 4 hours in accordance with the notation described in the section on Methods; the second figure is the effect after 24 hours.

The leucocyte counts are given in italicised numbers under B and S-L.

Note. Where no strain-designation of an organism is specified the strain used was a standard culture from the laboratory stock.

and leucocytes acting separately but in other instances this was not so (Table 2 (*c*)).

Ox leucocytes acted equally well with rabbit serum as with the homologous (ox) serum; horse leucocytes gave the same effects with rabbit serum as ox leucocytes.

These were the general results obtained, but exceptional effects were also noted. Thus, in certain cases, blood and "serum-leucocytes" were not less growth-promoting than serum after 24 hours (Table 2 (*c*)). In one test the serum was more bactericidal than blood or "serum-leucocytes" (Table 2 (*d*)), though the leucocytes were active *per se* at 4 hours. In this case the serum was moderately active at 4 hours and inhibited growth during the longer period of incubation.

The effects were not dependent on the relative numbers of leucocytes in

https://doi.org/10.1017/S0022172400018210 Published online by Cambridge University Press

the blood or serum-leucocyte preparations. Even with a leucocyte count of 1700 "whole" blood was more active than serum (Table 2 (*b*)) and in one instance a serum-leucocyte mixture containing 18,700 cells per c.mm. was less active than serum (Table 2 (*d*)). "Serum-leucocytes" with a count of 24,000 showed no greater enhancement of bactericidal action (as compared with serum) than a mixture containing 5200 leucocytes.

When defibrinated and heparinised blood from the same animal were compared quantitatively the effects were practically equal. The same applied to the serum and plasma respectively separated from the blood samples (Table 2 (*e*)).

Twelve tests were carried out with this organism, and the general result was that after a short period of incubation "whole" blood and "serum-leucocytes" were more actively bactericidal than plasma or serum, and though growth-promoting after 24 hours were less so than serum. Serum was initially inactive, weakly or only moderately bactericidal (0 to +2). In one instance blood and "serum-leucocytes" were less active than serum, and it seemed as if the leucocytes interfered with the action of the serum bactericidin.

As compared with serum, "whole" blood and "serum-leucocytes" generally acted similarly, and in the majority of cases the effect of "serum-leucocytes" was quantitatively equal to that of "whole" blood though the number of leucocytes present was greater in the former.

The bactericidal action of the leucocytes *per se* was specially noteworthy, and in some cases the enhanced action of the "serum-leucocytes" was no greater than the sum of the effects of serum and leucocytes. In other tests, however, there was apparently a definite reaction which might be interpreted as opsonic in nature, the "serum-leucocytes" showing a greater activity than the sum of the effects produced by serum and leucocytes individually.

The same general results were obtained with another strain of *Staphylococcus aureus* and with *S. albus*.

Streptococcus haemolyticus and *viridans*.

Towards the strains used (including a recently isolated virulent *S. haemolyticus*) serum was either initially inactive or weakly bactericidal, but after 24 hours appeared to be more active, sometimes to a marked degree. The comparative results with "whole" blood and "serum-leucocytes" were specially interesting in that the effects were greater than that of serum after the short period of incubation but definitely weaker at 24 hours.

This type of comparative result was obtained in a series of tests with great uniformity. Thus when serum was inactive or weak, blood and "serum-leucocytes" showed an enhanced effect; on the other hand when (as at 24 hours) the serum reacted strongly the presence of leucocytes seemed to reduce the effect (Table 3).

The relative numbers of leucocytes did not influence the results: in test (*c*)

of Table 3 a serum-leucocyte mixture containing 30,400 cells per c.mm. was less active than serum after 24 hours.

Leucocytes alone frequently exhibited an initial bactericidal effect, and in some cases this apparently accounted for the enhanced activity of the serum-leucocyte preparation. Leucocytes were always growth-promoting during the longer period of incubation.

Heparinised blood was more active than defibrinated blood and similarly the plasma was more active than serum. The heparin *per se* was, however, slightly bactericidal at 24 hours (Table 3 (*d*)).

These results with streptococci show how under certain conditions "whole" blood and serum-leucocyte preparations may be less actively bactericidal than plasma or serum, and it might appear that organisms may actually be protected by leucocytes from the bactericidal action of the plasma or serum.

Table 3. *Bactericidal effects.*

Streptococcus haemolyticus.

	B	S	S-L	L
(*a*)	+2/0	0/+5	+2/+2	0/−2
Rabbit blood; ox leucocytes	*9200*		*25,000*	
(*b*)	+1/0	0/+4	+2/−3	3/−3
Rabbit blood; ox leucocytes	*3800*		*10,200*	

Streptococcus viridans.

	B	S	S-L	L
(*c*)	+2/−1	0/+6	+1/+2	0/−2
Rabbit blood; ox leucocytes	*9400*		*30,400*	

	B	S	Bh	Ph	h
(*d*)	+2/−1	0/+2	+3/+1	0/+4	0/+1
Ox blood; ox leucocytes					

Enterococcus.

	B	S	S-L	L
(*e*)	+1/−2	0/−3	+1/−2	0/−3
Ox blood; ox leucocytes	*6750*		*13,500*	

Enterococcus.

Two strains were examined. Serum was practically inactive and growth-promoting on continued incubation of the test mixtures. The comparative results with blood and "serum-leucocytes" were similar to those with the staphylococci (Table 3 (*e*)).

Pneumococcus.

A moderately virulent type I strain was used for most of the tests. The results were variable: either like those with *Staphylococcus aureus*, or resembling the effects with streptococci, depending on the relative activity of the serum at 4 and 24 hours. Serum was either inactive or weakly bactericidal (0 to +1) at 4 hours, and blood and "serum-leucocytes" showed distinctly enhanced effects (Table 4). In some cases serum showed a considerable degree of bactericidal action at 24 hours, and then blood and "serum-leucocytes"

were less active and even growth-promoting (Table 4 (*a*)). When, however, the serum was weakly active at 24 hours, the blood and "serum-leucocytes" exerted a greater killing effect; when serum was growth-promoting, blood and "serum-leucocytes" were inhibitory. Leucocytes were on occasions initially bactericidal *per se*, but were usually growth-promoting on continued incubation with the organisms. Heparinised blood was more active than defibrinated specimens and the same difference was noted between the plasma and serum, but heparin *per se* was somewhat bactericidal at 24 hours.

Such variable findings clearly showed that the comparative effects of blood and serum depended on the degree of activity of the serum.

Table 4. *Bactericidal effects.*

	Pneumococcus, type I.			
	B	S	S-L	L
(*a*)	+3/0	0/+3	+3/−1	0/−2
Ox blood; ox leucocytes	*6750*		*13,500*	
(*b*)	+2/+1	+1/0	+3/+1	+1/0
Rabbit blood; horse leucocytes	*4000*		*24,000*	
	B. diphtheriae.			
	B	S	S-L	L
(*c*)	+2/0	0/+3	+1/0	0/0
Rabbit blood; horse leucocytes	*4000*		*26,000*	

Bacillus diphtheriae.

In most cases this organism behaved like the streptococci (Table 4 (*c*)). Leucocytes alone were occasionally bactericidal at 4 hours. No difference was elicited between heparinised and defibrinated blood samples.

Bacillus anthracis.

As shown in a previous paper (Mackie and Finkelstein, 1932) the effects of ox and rabbit serum on a virulent strain of this organism differed considerably. The former was inactive and growth-promoting; the latter was often strongly bactericidal and the effect was frequently progressive during 24 hours.

The comparative results with blood and serum varied in type and, as with other organisms, depended on the quantitative reaction of the serum in each case (Table 5). When the serum was initially inactive, weak or moderately active (0 to +2) "whole" blood and "serum-leucocytes" showed enhanced effects; when on the other hand serum was strongly bactericidal (+3 to +5) "whole" blood was weaker. After the longer interval when serum was growth-promoting (*e.g.* ox serum with a virulent strain) blood and "serum-leucocytes" were inhibitory; when, however, serum was strongly bactericidal blood was definitely weaker. In some instances where serum was moderately active (+2) blood and "serum-leucocytes" were quantitatively equal to it in bactericidal action. Leucocytes were occasionally bactericidal *per se* at 4 hours.

Bacillus anthracoides.

Serum was initially inactive (ox) or only weakly active (rabbit) and after a longer period, growth-promoting or weakly active, the comparative results generally resembling those with the staphylococci (see Table 5 (*d*)).

Table 5. *Bactericidal effects.*

B. anthracis—virulent strain.

	B	S	S-L	L
(*a*)	+2/0	0/−1	+1/0	0/−4
Ox blood ox leucocytes	*5500*		*9600*	
(*b*)	+4/+4	+5/+6	+4/+2	+1/−4
Rabbit blood; ox leucocytes	*4200*		*24,000*	

B. anthracis—attenuated strain.

	B	S	S-L	L
(*c*)	+2/−2	+3/+3	+1/−2	0/−4
Ox blood; ox leucocytes	*5950*		*26,200*	

B. anthracoides.

	B	S	S-L	L
(*d*)	+2/−1	0/−2	+1/−1	0/−4
Ox blood; ox leucocytes	*10,000*		*11,500*	

Bacillus dysenteriae.

Strains of the Shiga and Y types respectively were tested. The results with these organisms were entirely uniform and constitute an interesting category among the comparative bactericidal reactions of "whole" blood and serum. In all cases serum produced marked bactericidal reactions both at 4 and 24 hours, and invariably "whole" blood and "serum-leucocytes" were less active than blood (Table 6 (*a*)). The effects of blood and "serum-leucocytes"

Table 6. *Bactericidal effects.*

B. dysenteriae Shiga.

	B	S	S-L	L
(*a*)	+4/+3	>+5/>+5	+4/+3	0/−3
Rabbit blood; ox leucocytes	*4400*		*10,000*	

	B	S	Bh	Ph	h
(*b*)	+2/+1	+3/+4	+2/+2	>+4/>+4	0/−2
Ox blood					

B. proteus X 19.

	B	S	S-L	L
(*c*)	+4/+3	>+5/>+5	+5/+3	0/−4
Rabbit blood; ox leucocytes				

B. influenzae.

	B	S	S-L	L
(*d*)	+4/+3	>+5/>+5	+5/+4	0/−3
Rabbit blood; ox leucocytes			*15,000*	
(*e*)	+3/+3	+4/+4	+3/+3	+1/−2
Ox blood; ox leucocytes				

B. suisepticus.

	B	S	S-L	L
(*f*)	+3/0	>+6/+4	+2/+1	0/−3
Rabbit blood; horse leucocytes			*32,000*	

https://doi.org/10.1017/S0022172400018210 Published online by Cambridge University Press

also showed a close quantitative similarity irrespective of the difference in leucocyte content. Leucocytes *per se* were inactive and growth-promoting. Heparinised blood gave stronger effects than defibrinated specimens, and the same applied to the plasma and serum respectively.

These results correspond to those with *B. anthracis* and rabbit blood and serum; they show further that when serum is strongly bactericidal, "whole" blood and "serum-leucocytes" are of lesser activity.

Bacillus proteus X 19, *Bacillus influenzae* and *Bacillus suisepticus.*

The results with these organisms were almost uniformly like those with *B. dysenteriae*, the serum reacting strongly both initially and after 24 hours (Table 6 (*c*), (*d*), (*e*) and (*f*)). In some instances leucocytes were weakly bactericidal at 4 hours.

Vibrio cholerae and *paracholerae.*

These organisms were strongly lysed by serum, the effects being usually marked both at 4 and 24 hours. As in the case of *B. dysenteriae*, blood and "serum-leucocytes" showed bactericidal reactions which were generally weaker than those with serum (Table 7 (*a*)). In one instance with *V. cholerae* and also with *V. paracholerae* the serum was less bactericidal at 24 hours than at 4 hours and in these cases though blood and "serum-leucocytes" were initially less active than serum, they became more active after the longer period (Table 7 (*b*)). These results illustrate further the quantitative relationship between the action of serum and blood: when serum reacted strongly blood was weaker, when the former gave a weak reaction blood was stronger. In no case were leucocytes bactericidal *per se.*

Table 7. *Bactericidal effects.*

	V. cholerae "Bombay."			
	B	S	S-L	L
(*a*)	+4/+5	>+5/>+5	+4/+5	0/−3
Rabbit blood; ox leucocytes				
(*b*)	+2/>+4	+4/+2	+2/+3	0/−3
Rabbit blood; ox leucocytes	*4100*		*18,000*	

Bacillus typhosus and *Bacillus coli.*

A considerable number of tests were carried out with two strains of each of these organisms. In the majority of tests serum proved more bactericidal than blood or "serum-leucocytes," the serum reacting strongly (Table 8 (*a*), (*e*)). In some cases, however, the initial action of the serum was weak or moderate (+1 to +3) though the effect was later progressive. Under such conditions the blood and "serum-leucocytes" were either more active than serum or quantitatively equal to it at 4 hours though less bactericidal at 24 hours (Table 8 (*b*), (*c*), (*f*), (*g*)). A test was made in which leucocytes were added to "whole" blood, the result being to depress further the action of the latter as compared with serum (Table 8 (*a*)). Heparinised samples in some cases

gave stronger effects than defibrinated blood (Table 8 (*a*)): in others the effects were quantitatively equal. Leucocytes alone were very rarely bactericidal, and always growth-promoting during 24 hours.

Table 8. *Bactericidal effects.*

B. typhosus.

	B	S	S-L	L	B-L*
(*a*)	+4/+5	>+6/>+6	+4/+5	0/−4	+3/+3
Ox blood; ox leucocytes	*6200*		*11,050*		*17,000*
(*b*)	+3/+3	+3/>+5	+3/+1	0/−3	
Rabbit blood; ox leucocytes	*2600*		*10,200*		
(*c*)	+4/+5	+3/>+6	+4/+4	0/−4	
Rabbit blood; horse leucocytes			*30,400*		
	B	S	Bh	Ph	h
(*d*)	+3/+3	+4/>+4	+4/>+4	+5/>+5	0/−4
Rabbit blood	*3200*		*4000*		

B. coli.

	B	S	S-L	L
(*e*)	+3/+4	+5/+5	+3/+4	0/−4
Rabbit blood; ox leucocytes	*7300*		*16,400*	
(*f*)	+2/+2	+2/>+5	+2/+2	0/−4
Rabbit blood; ox leucocytes	*2400*		*12,000*	
(*g*)	+2/+2	+1/>+5	+2/+2	0/−2
Rabbit blood; horse leucocytes	*4000*		*32,000*	

* B-L = "whole" blood with leucocytes added.

Bacillus abortus.

With the strain of this organism used in most of the tests, serum gave weak initial reactions, but the effect was progressive on longer incubation and the comparative results resembled those with the streptococci (Table 9 (*a*)) but even when the serum at 4 hours reacted fairly strongly (*e.g.* +4) the presence

Table 9. *Bactericidal effects.*

B. abortus "Bang."

	B	S	S-L	L
(*a*)	+5/+5	+2/>+6	+4/+5	0/−2
Ox blood; ox leucocytes				
(*b*)	+5/+4	+4/>+6	+5/+6	0/−2
Ox blood; ox leucocytes				

D. catarrhalis.

	B	S	S-L	L
(*c*)	+3/+4	+2/+6	+4/+5	0/−3
Rabbit blood; ox leucocytes			*15,000*	

B. pyocyaneus.

	B	S	S-L	L
(*d*)	+2/0	+1/−1	+2/0	0/−4
Ox blood; ox leucocytes	*6400*		*12,500*	

B. paratyphosus B.

	B	S	S-L	L
(*e*)	+2/+1	+1/−1	+4/+1	0/−4
Rabbit blood; horse leucocytes			*22,000*	

https://doi.org/10.1017/S0022172400018210 Published online by Cambridge University Press

of leucocytes still enhanced the bactericidal effect (Table 9 (*b*)). This was exceptional as compared with results obtained with all other organisms.

Diplococcus catarrhalis.

The serum effects and the comparative results with this organism resembled those with the streptococci (Table 9 (*c*)).

Bacillus pyocyaneus.

This organism corresponded in its reactions with serum and blood to the staphylococci (Table 9 (*d*)).

Bacillus paratyphosus B.

Both ox and rabbit serum were only weakly active towards the strain of this organism tested, and sometimes inactive and later growth-promoting. Thus, following the rule with other organisms "whole" blood and "serum-leucocytes" showed enhanced effects (Table 9 (*e*)). In certain instances, however, when serum was unusually active (+3 or over) "whole" blood and "serum-leucocytes" were weaker than serum. Leucocytes were invariably inactive *per se.*

Bacillus paratyphosus A.

With the strain used serum was strongly active (+5 to +6) and the comparative results were like those with *B. dysenteriae*, blood being weaker than serum.

GENERAL RESULTS.

From these findings with various organisms it was evident that only under certain conditions were "whole" blood and serum-leucocyte mixtures superior in bactericidal power to serum. When serum was inactive or growth-promoting, blood and "serum-leucocytes" were bactericidal or growth-inhibitory respectively, but these effects were nevertheless comparatively weak as compared with the pronounced bactericidal action which serum exerts on certain bacteria, *e.g. V. cholerae, B. dysenteriae.* When serum was weakly active "whole" blood and "serum-leucocytes" showed an enhanced bactericidal action, but on the other hand when a serum yielded a strong reaction "whole" blood and "serum-leucocytes" were definitely weaker; in fact, the presence of the leucocytes seemed to diminish the bactericidal action of the serum. In some cases with a serum of moderate activity, there was no quantitative difference between it and "whole" blood or a serum-leucocyte mixture. Thus the comparative bactericidal power of "whole" blood and serum depended on the degree of bactericidal activity of the serum *per se.*

The general results may be summarised as follows:

Gram-positive bacteria.

4 hours:	serum—	0,	blood and "serum-leucocytes"		weakly bactericidal.
	,,	+1 to +2,	,,	,,	more active than serum.
	,,	+3 or over,	,,	,,	less active than serum.
24 hours:	,,	growth-promoting,	,,	,,	inhibitory or weakly bactericidal.
	,,	0,	,,	,,	weakly bactericidal.
	,,	+1,	,,	,,	more active than serum.
	,,	+2 or over,	,,	,,	less active than serum.

https://doi.org/10.1017/S0022172400018210 Published online by Cambridge University Press

Gram-negative bacteria.

4 and 24 hours: serum—	growth-promoting,	blood and	"serum-leucocytes"	inhibitory or weakly bactericidal.
,,	0,	,,	,,	weakly bactericidal.
,,	+1 to +2,	,,	,,	more active than serum.
,,	+4 or over,	,,	,,	less active than serum.
,,	+3,	,,	,,	equal in activity to serum; sometimes more active, sometimes less active.

In exceptional cases, when serum gave a +4 effect, blood and "serum-leucocytes" were equally active or more active, *e.g.* with *B. abortus*.

It will be noted in the case of the Gram-positive bacteria that even when the serum was only moderately active (*e.g.* +2) at 24 hours the blood and "serum-leucocytes" were generally inferior to it in bactericidal action.

The bactericidal action of leucocytes *per se* is of special interest. This occurred more frequently with the Gram-positive than the Gram-negative organisms and with the latter was only occasionally noted. Among the Gram-positive types the effect was specially common with the staphylococci and streptococci.

EFFECT OF VARIATION IN NUMBER OF LEUCOCYTES IN SERUM-LEUCOCYTE MIXTURES.

Reference has been made to the fact that the results with serum-leucocyte mixtures did not apparently depend to any material degree on the number of cells present. A series of experiments was carried out in which the number of leucocytes in these mixtures varied, the bactericidal effects being compared. The results showed that the degree of bactericidal activity was not a function of the number of cells though a certain minimum concentration of leucocytes was necessary for the mixture to produce an enhanced effect as compared with the serum *per se*. Increased numbers did not further exalt the bactericidal action of the mixture (Table 10). An analogous observation

Table 10. *Bactericidal effects.*

Staphylococcus aureus.

	B	S	S-L	L	S-L	L
Ox blood; ox leucocytes	+2	0	0	0	+1	0
			2350		*5875*	
	S-L	L	S-L	L		
	+1	0	+1	0		
	11,750		*23,500*			

Streptococcus haemolyticus.

	B	S	S-L	L	S-L	L
Rabbit blood; ox leucocytes	+1	0	0	0	+1	0
	8500		*2900*		*5800*	
	S-L	L	S-L	L		
	+1	0	+1	0		
	11,600		*23,200*			

(Tests at 4 hours only.)

https://doi.org/10.1017/S0022172400018210 Published online by Cambridge University Press

has been made by Robertson and Sia (1924[b]) in regard to the growth-inhibitory action of serum-leucocyte mixtures on the pneumococcus.

EFFECT OF DILUTION OF THE SERUM IN SERUM-LEUCOCYTE MIXTURES.

As the comparative bactericidal action of "whole" blood and serum-leucocyte preparations seemed to depend essentially on the bactericidal strength of the serum, tests were made in which a strongly active serum was diluted to varying degrees, leucocytes in constant number being added to the various dilutions, and the serum-leucocyte preparations in each case being compared with serum acting alone. The results were specially interesting and confirmed in another way the rule previously stated: that the addition of leucocytes to a strongly acting serum diminishes the bactericidal effect whereas leucocytes enhance the action of a weakly active serum. Thus, as the serum was progressively diluted (Table 11) the bactericidal action was lessened and high dilutions were growth-promoting at 24 hours. The addition of leucocytes to undiluted serum diminished the effect from +5/+7 to +4/+5;

Table 11. *Bactericidal effects.*

Bacillus typhosus.

Ox serum; ox leucocytes	S	S-L
Serum undiluted	>+5/>+7	+4/+5
,, diluted 1 in 2	+3/+6	+5/+5
,, ,, 1 in 8	+3/+3	+5/+5
,, ,, 1 in 32	+1/−3	+4/−2
,, ,, 1 in 128	0/−3	+2/−3
,, ,, 1 in 512	0/−3	+1/−4
,, ,, 1 in 1024	0/−3	0/−4

Leucocytes 15,000 per c.mm. in each case.

in the 1 in 2 dilution of the serum, leucocytes increased the effect at 4 hours (from +3 to +5) but diminished it at 24 hours (from +6 to +5); in the 1 in 8 dilution leucocytes increased the bactericidal reaction both at 4 and 24 hours (from +3/+3 to +5/+5); in the high dilutions of serum (*e.g.* 1 in 512) leucocytes increased the growth-promoting effect at 24 hours though in lower dilutions (1 in 32) they exerted an inhibitory action. Probably in the higher dilutions the concentration of opsonin was insufficient to sensitise the organisms to the action of the leucocytes.

Similar results were obtained in other experiments of the same type. In one of these, a certain dilution of serum leucocytes neither enhanced nor diminished the bactericidal action of the serum (towards *B. typhosus*) which *per se* was +3/+3, whereas with the more active (lower) dilution the effect was reduced, and with the less active (higher) dilutions the bactericidal effect was increased.

EFFECT OF BACTERIAL EXTRACTS, LYSATES AND KILLED ORGANISMS.

An attempt was then made to explain by experimental methods the mechanisms involved in the phenomena described above. The greater bactericidal action of "whole" blood and "serum-leucocytes" as compared with

serum seemed in accord with the generally accepted immunological theory regarding the sensitisation of organisms by serum-opsonins to their phagocytosis by leucocytes and subsequent intracellular destruction. On the other hand, the diminished bactericidal action of blood and "serum-leucocytes" as compared with serum seemed theoretically paradoxical. This result, however, only occurred when the serum possessed bactericidal properties of a certain grade, and it seemed possible that it might be due to the products of the bacteriolytic or bactericidal reaction interfering when present in a certain concentration with the simultaneous or later killing of the organisms by the cells. Further, leucocytes by phagocyting bacteria without later killing them might protect them from the bactericidal action of the serum. Such effect has been described by Rous and Jones (1916) and alluded to also by Fiessinger and Cattan (1928). A possible explanation suggested by the data available was that in the presence of a strongly acting serum, the products of the reaction at a certain stage affected the vital activity of the cells and that the organisms already phagocyted were not killed intracellularly but protected from the serum.

This supposition was examined in a series of experiments in which bacterial extracts, lysates and dead organisms were added to the test mixtures and their effects on the bactericidal action of blood and serum respectively were compared.

Extracts of *B. typhosus* and *V. cholerae* were prepared by grinding dried bacteria in a flask containing large agate balls and mechanically rotated for 72 hours, then extracting with saline the bacterial débris and finally separating the latter by centrifuging.

The growth from three 6 in. agar plates was emulsified in a small quantity of saline and transferred to the flask in which it was dried at 50° C. (48 hours). The dried organisms were ground for about 72 hours and then mixed with 10 c.c. of saline. After an hour the bacterial débris was removed by centrifuging and the supernatant fluid constituted the extract. This fluid was heated at 60° C. for half an hour. 0·05 c.c. was added to each tube in the bactericidal test.

Lysates of *B. typhosus* and *V. cholerae* were obtained by allowing a strongly lytic serum (*e.g.* sheep serum) to act on these organisms for 4 hours and then centrifuging the mixture. The supernatant serum constituted the "lysate." Three agar-slope cultures were emulsified in 6 c.c. serum and incubated at 37° C. for 4 hours. The mixture was centrifuged to separate the bacterial bodies remaining in it and heated at 57° C. for 1 hour to ensure its sterility. 0·05 c.c. was added to each tube in the bactericidal test.

Extracts and lysates prepared by the methods described exerted an inhibitory influence on the bactericidal action of both serum and "whole" blood, the effect on blood being usually equal to that on serum, though in some cases more on blood than serum or *vice versa* (Table 12), but the results did not afford any clear indication that the phenomenon in question could be explained by the influence of the soluble products of bacteriolysis on the phagocytic cells.

It was also noted that when organisms were washed two or three times with normal saline solution, the bactericidal reaction with both serum (or

plasma) and "whole" blood was greater than with unwashed cultures (Table 13). Either the physical effect of such washing rendered them more susceptible to bactericidal effects or the extracellular products in the culture interfered with the bactericidal mechanism of the plasma. Washing, however, did not influence the bactericidal action of blood more than that of plasma or serum.

The question arose whether the dead organisms resulting from the bactericidal effect of a strongly acting serum might affect the killing properties of the cells as in "whole" blood or serum-leucocyte mixtures.

Table 12. *Bactericidal effects.*

	Bh	Ph	Bh *plus* extract	Ph *plus* extract	Extract
	V. cholerae "Bombay."				
Rabbit blood; *V. cholerae* extract	+4/+4	+5/+5	+3/+3	+5/+4	0/−3
	B. typhosus.				
Rabbit blood; *B. typhosus* extract	+5/+2	+6/>+6	+4/+1	+3/+5	0/−3
	V. cholerae "Bombay."				
Rabbit blood; *V. cholerae* lysate	+3/+4	+5/+3	+2/−3	+4/−3	0/−4
	B. typhosus.				
	B	S	B *plus* lysate	S *plus* lysate	Lysate
Rabbit blood; *B. typhosus* lysate	+3/+3	+3/+1	+2/+1	+1/0	0/−4

Table 13.

B. proteus X 19 and rabbit blood.

	Bh	Ph
Organisms: unwashed	+2/−2	0/+2
„ washed	+3/0	+3/+3

Streptococcus viridans and rabbit blood.

	B	S
Organisms: unwashed	+1/−2	0/+4
„ washed	+2/+2	+1/+5

A number of experiments were carried out in which bacteria killed at 60° C. (1 hour) were added to the test mixtures. With *V. cholerae* a striking difference was elicited in the reactions with blood and serum respectively. The addition of killed organisms lowered the bactericidal effect of serum acting *per se* but this inhibitory effect was more marked quantitatively in the case of "whole" blood (Tables 14 and 15).

Saline suspensions of agar-slope cultures were prepared and standardised to Brown's opacity standard No. 2. They were killed by exposure for 1 hour at 60° C. 0·05 c.c. was the amount added to the test mixtures. In some cases varying dilutions of the above standard were used, 0·05 c.c. being added in each case.

A similar result was obtained in tests with other organisms but not so uniformly nor to such marked degree. The difference was generally most

https://doi.org/10.1017/S0022172400018210 Published online by Cambridge University Press

pronounced at 24 hours. The effect was not specific; thus dead typhoid bacilli exerted the same influence as dead cholera vibrios in the reaction of blood and serum with *V. cholerae*.

On the basis of such findings it seemed possible that the bacteria killed by the initial action of a bactericidal serum when phagocyted in certain numbers interfered with the vital functions of the cells in such a way that they not only failed to exert their normal bactericidal effect but also protected the phagocyted living organisms from the action of the plasma.

Table 14. *Bactericidal effects.*

	V. cholerae "Bombay"			
	B	S	B *plus* killed organisms	S *plus* killed organisms
(*a*) Rabbit blood; killed *V. cholerae* (Opacity Standard 2)	+2/+3	+2/+3	0/−3	+2/0
(*b*) Rabbit blood; killed *V. cholerae* (Standard 2). Diluted 1 in 2	+3/+3	+4/+4	+1/0	+3/+2
,, 1 in 4			+2/+1	+4/+3
,, 1 in 8			+3/+1	+4/+4
(*c*) Rabbit blood; killed *V. cholerae* (Standard 2). Diluted 1 in 2	+3/+5	+4/+3	0/−1	+2/−2
,, 1 in 4			+2/+1	+3/0
killed *B. typhosus* (Standard 2). Diluted 1 in 2			+3/+2	+4/+2
,, 1 in 4			+3/+2	+4/+3

Table 15. *Bactericidal effects.*

	B. typhosus.			
	B	S	B *plus* killed organisms	S *plus* killed organisms
Rabbit blood; killed *B. typhosus* (Standard 2). Diluted 1 in 2	+4/+3	+4/+4	+3/+2	+4/+4
	B. proteus X 19.			
Rabbit blood; killed *B. proteus* (Standard 2). Diluted 1 in 4	+3/1	+2/0	0/−2	1/−1
	B. dysenteriae Shiga.			
Rabbit blood; killed *B. dysenteriae* Shiga (Standard 2). Undiluted	+4/+4	+4/+4	1/−5	1/−1
Diluted 1 in 4			+3/2	+3/+4

DISCUSSION.

The observations recorded are of special interest in the information they afford regarding the combined action *in vitro* of the various factors concerned in the natural antibacterial defences of the blood. The results illustrate clearly the bactericidal power possessed by "whole" blood in the absence of any such effect produced by the separated serum (or plasma) and show further that when the serum is active *per se* "whole" blood and serum-leucocyte mixtures may, under certain conditions, possess a still greater bactericidal power. These findings are in accord with the long-accepted view that the serum-opsonins

sensitise bacteria to the phagocytic action of the leucocytes and their subsequent intracellular destruction. In some cases, however, it was found that the cells *per se* exerted a bactericidal effect within a short period of time (4 hours). This was most noticeable with the Gram-positive bacteria, and the bactericidal action of serum-leucocyte mixtures was sometimes not greater than the sum of the effects of the serum and leucocytes acting independently. Leucocytes *per se* were almost invariably growth-promoting during longer periods of incubation (24 hours) even when they exerted an initial bactericidal action. Apparently this initial effect was not sustained or progressive and was followed by the growth of the surviving organisms. On the other hand the enhanced bactericidal action of "whole" blood and serum-leucocyte mixtures as compared with serum was not restricted to the short period of incubation. The question arises whether this independent effect of leucocytes is due to "spontaneous phagocytosis" (as described by Neufeld and Hüne, 1907; and others) and subsequent intracellular killing or to some bactericidal principle liberated from the cells. We have found it is annulled by heating the leucocytes at 55° C. for half an hour and would seem to be dependent either on their vital action or on a highly thermolabile product. The adjuvant bactericidal action of leucocytes along with serum was also annulled when they had been heated at 55° C. Further study, however, is required regarding the part which the products of leucocytes and platelets may play in these processes independently of the phagocyting of the organisms.

There has been a tendency to regard the humoral defences as of minor importance in natural and acquired immunity as compared with the destruction of parasitic organisms by the phagocytic cells. In our experiments the bactericidal power of "whole" blood and "serum-leucocytes" in the absence of killing properties in the serum *per se* were relatively weak as compared with the bactericidal power a serum may exert on certain bacteria. The same applied to the adjuvant action of leucocytes along with an active serum. As regards the natural immunity mechanism of the blood the cellular defence does not predominate quantitatively over the humoral.

This study elicited the specially interesting fact that under certain conditions "whole" blood and serum-leucocyte mixtures were inferior to serum in bactericidal power: the phagocytes not only failed to augment the bactericidal action of the blood or serum-leucocyte preparation as compared with serum or plasma but in some way actually interfered with the action of the serum. A similar phenomenon has been observed by Fiessinger and Cattan (1928) in relation to the bactericidal action of the blood and serum in typhoid infection. In discussing their findings they quote the work of Rous and Jones (1916) on the protection of pathogenic organisms by body cells. It has, of course, been long recognised that phagocytosed organisms may, under certain conditions, remain viable, and it has also been supposed that the phagocytic cell may be devitalised by the ingested organisms if present in certain numbers and, failing to kill them, afford protection against other bactericidal agencies.

Rous and Jones (1916) showed how phagocytes protected organisms from bactericidal agents in the surrounding fluid.

In our experiments this diminished effect of "whole" blood as compared with serum (or plasma) was associated generally with a strongly bactericidal action of the serum. Experiments in which varying dilutions of a strongly acting serum were tested along with added leucocytes indicated that this paradoxical reaction depended on the strength of the serum effect: the presence of leucocytes in the undiluted serum diminished its activity while they augmented the effect of the diluted and more weakly reacting serum. Such results might suggest that the bactericidal action of the cells is dependent on an optimal amount of the serum, but the phenomenon in question is not merely an inhibition of this reaction but includes also a diminished bactericidal action of the serum or plasma in the presence of cells, conditioned by the degree of independent activity of the bactericidin. The question was considered whether fixation of complement associated with the bacteriolytic reaction might lead to an inhibition of opsonisation and phagocytosis, but the phenomenon occurred with Gram-positive bacteria which are killed by a serum principle acting independently of complement (see Mackie and Finkelstein, 1932).

The inhibition could not be explained as a result of the action of cytoxic substances liberated by the bacteriolytic reaction. Bacterial extracts and lysates exerted marked inhibitory effects, but to an approximately equal degree, on the bactericidal action of serum and of "whole" blood. On the other hand dead organisms in certain numbers while producing inhibition of the serum reaction exhibited a quantitatively greater reduction of the bactericidal action of "whole" blood. This suggested that when the plasma is strongly bactericidal, the dead organisms resulting from its initial effect on being phagocyted in certain numbers along with living organisms interfere with the intracellular destruction of the latter, which then remain viable and are protected from the effect of the bactericidin in the surrounding fluid. This must be regarded as only a tentative explanation of the phenomenon; as indicated above, certain aspects of these combined bactericidal reactions still require further study.

These observations have been elicited in experiments with a wide variety of bacteria and the general rules drawn are applicable apparently to all types of bacteria irrespective of group or species. It is interesting also to note that the behaviour of the leucocytes was not apparently altered when associated with a heterologous serum. Their bactericidal effects along with sera did not bear any quantitative relationship to their numbers though a certain minimum was required.

In these tests heparinised blood gave on the whole similar results to those of defibrinated blood and the same applied to the plasma of the former and the serum separated from the latter. In some cases, however, heparinised blood was more actively bactericidal than a defibrinated specimen and corre-

spondingly the plasma was more active than serum. Occasionally defibrinated blood was more active than a heparinised specimen.

It might be said that the serum-leucocyte preparation, being artificial and containing leucocytes altered perhaps as a result of the treatment and manipulation to which they had been subjected in their separation, might not react similarly to their natural behaviour *in vivo*; but it was specially noticeable how closely the reactions of these preparations corresponded to those of "whole" blood when tested in parallel.

The results also contribute further information regarding the bactericidal mechanism of serum towards various bacteria. With some organisms the maximum reaction generally occurred after a short period of incubation (3 to 4 hours), whereas with others there was a progressive effect between 4 and 24 hours. In some cases a bactericidal reaction observable after a short period of incubation was followed by a growth-promoting effect and multiplication of the surviving organisms. The behaviour of organisms in these respects depended to some extent on their biological type. For example, the staphylococci with an active serum showed an initial bactericidal reaction followed by active growth in the serum; with the streptococci, generally there was a progressive reaction between 4 and 24 hours. Some species in their behaviour varied with the sample of serum tested.

The comparative bactericidal action of "whole" blood and serum-leucocyte mixtures on the one hand and serum or plasma on the other, after the short and long period of incubation depended essentially on the degree of bactericidal action of the serum or plasma at the particular time. Thus in the case of the streptococci which showed only a weak reaction at 4 hours and a pronounced reaction at 24 hours, blood was initially more bactericidal than serum, but after the longer interval the position was reversed, serum being more active than blood. Thus the bactericidal activity of the serum determined the comparative action of "whole" blood and this rule applied, irrespective of the type of bacterium, among the considerable variety tested.

These observations contribute further data relative to the mechanism of natural immunity but must of course be interpreted with caution in their application to conditions *in vivo*. They illustrate, however, the complex biological interactions involved in the bactericidal action of the plasma and phagocytes of the blood.

Summary and conclusions.

1. Normal "whole" blood and serum-leucocyte mixtures may exert a bactericidal effect when the serum (or plasma) is inactive in this respect, and may possess quantitatively greater bactericidal properties than serum (or plasma), when the latter is active *per se*, but such effects are relatively weak.

2. Leucocyte suspensions may exhibit an initial bactericidal action on certain bacteria, but this is usually weak and is followed by a pronounced growth-promoting influence on the surviving organisms.

3. Under certain conditions normal "whole" blood and serum-leucocyte mixtures are inferior to serum (or plasma) in bactericidal power. This is observed when the serum (or plasma) is strongly active *per se* and is due not merely to an inhibition of the bactericidal action of the leucocytes, but also to a lessened effect of the serum-bactericidins.

4. The comparative bactericidal power of normal "whole" blood or serum-leucocyte mixtures on the one hand and serum or plasma on the other depends on the activity of the serum; when this is relatively weak, blood shows an enhanced action; when strong, blood is less active.

5. The above findings apply to a wide variety of bacteria irrespectively of biological species.

6. Bacterial extracts possess marked inhibitory properties on the bactericidal action of serum (or plasma), but not to any extent on the bactericidal properties of the leucocytes as evidenced by the approximately equal influence of such extracts on whole blood and serum respectively.

7. Dead bacteria in certain numbers produce inhibition of the bactericidal action of serum (or plasma), but exert a greater inhibitory influence on the reaction of "whole" blood.

8. It is suggested tentatively that when the serum is strongly bactericidal the killed organisms on being phagocyted along with living bacteria interfere with the intracellular destruction of the latter which at the same time are protected from the serum-bactericidins.

9. The paper also includes certain data regarding the time of maximum bactericidal action by serum towards various organisms and the occurrence of growth-promoting action following an initial bactericidal effect.

ACKNOWLEDGEMENTS. One of us (T. J. M.) received a grant for the expenses of this research from the Moray Fund of Edinburgh University; another (M. H. F.) while engaged in the work was in receipt of a grant from the Medical Research Council.

REFERENCES.

FIESSINGER, N. and CATTAN, ROGER (1928). *Presse Médicale*, **36**, 1185.
HEIST, G. D., SOLIS-COHEN, S. and SOLIS-COHEN, M. (1918). *J. Immunol.* **3**, 261.
MACKIE, T. J. and FINKELSTEIN, M. H. (1931). *J. Hyg.* **31**, 35.
—— —— (1932). *Ibid.* **32**, 1.
NEUFELD, F. and HÜNE (1907). *Arb. K. Gesundh. Amt., Berlin*, **25**, 164.
ROBERTSON, O. H. and SIA, R. H. P. (1924[a]). *J. Exper. Med.* **39**, 219.
—— —— (1924[b]). *Ibid.* **40**, 467.
ROUS, P. and JONES, F. S. (1916). *Ibid.* **23**, 601.
WRIGHT, A. E., COLEBROOK, L. and STORER, E. J. (1923). *Lancet*, **1**, 365, 417, 473.

(*MS. received for publication* 22. IV. 1932—Ed.)