THE RELATION OF THE LEUCOCYTIC BACTERIOLYSIN TO BODY FLUIDS.*

By WILFRED H. MANWARING, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research, New York.)

The discovery that certain cells of the body are capable of giving off bactericidal substances has given rise to the hope that eventually cellular derivatives may prove of therapeutic value. It has been conceived, for example, that an efficient antiseptic may thus be obtained, which can be safely injected into body cavities and tissue spaces.

The bactericidal properties of many cellular products[1] have therefore been studied, particularly those of leucocytic products.[2] The leucocytic bacteriolysin has usually been tested, either dissolved in distilled water, in physiological saline, or in culture media. From observations in a previous paper,[3] I was led to test its properties when placed under conditions more nearly approaching those in the animal body. Its action was therefore tested when mixed with various normal and pathological body fluids and tissue derivatives.

The influence of a number of foreign substances on this bacteriolysin has already been studied. Of particular interest in the present connection is the observation of Pettersson,[4] that the bactericidal power is diminished in the presence of certain colloids. Pettersson[5] and others have shown that the leucocytic and the serum bacteriolysins are distinct substances. Kling and others, however, have observed an activation of an inactive leucocytic extract by the addi-

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[1] Conradi, H., *Beitr. z. chem. Phys. u. Path.*, 1902, i, 193.

[2] For a summary of previous work with leucocytic extracts, see Kling, C. A., *Ztschr. f. Immunitätsforsch.*, 1910, vii, 1. A review of the literature is also given by Schneider, R., *Arch. f. Hyg.*, 1909, lxx, 40.

[3] Manwaring, W. H., *Jour. Exper. Med.*, 1912, xvi, 249.

[4] Pettersson, A., *Centralbl. f. Bakteriol., 1te Abt., Orig.*, 1911, lx, 286.

[5] Pettersson, A., *Ztschr. f. Immunitätsforsch.*, 1908–9, i, 52.

tion of serum. Weil, Schneider, and others have described the opposite effect, a diminution in bacteriolysis, as a result of serum addition.[6] Schattenfroh[7] found that the bactericidal power of an extract of rabbit leucocytes is independent of its salt content. Schneider[8] found that the addition of alkali diminishes the bactericidal power of the same extract.

MATERIAL AND TECHNIQUE.

The bacteriolysin studied in the present paper was obtained from horse leucocytes, according to the technique described in the previous communication. This extract had been heated to 57° C. for thirty minutes, had been dialyzed free from diffusible products, and then evaporated to dryness *in vacuo* at 45° C. It was tested after having been stored in a vacuum at 4° C. for about six months. The tests were made at room temperature.

EFFECTS OF SERUM ON THE BACTERIOLYSIN.

Inactive Serum.—The addition of a trace of inactive[9] homologous or foreign serum to an amount of leucocytic extract barely sufficient

TABLE I.

Effect of Inactive Serum on the Bacteriolysin.

The fluids to be tested were made up to equal volumes (1 c.c.) by the addition of ¼ physiological saline solution (0.22 per cent. sodium chloride). Each fluid was then inoculated with a loopful of an eighteen hour broth culture of *B. typhosus*. Plates were made from the resulting mixtures at the times indicated. The table records the number of colonies on the plates thus obtained. The serum tested in this table was horse serum that had spontaneously become inactive by long standing in the ice chest.

Material tested.	Time of plating.				
	1 min.	1 hr.	2 hrs.	5 hrs.	24 hrs.
Bacteriolysin alone	950	90	10	8	650
Bacteriolysin + 0.5% inactive serum	510	7	0	0	115
50% inactive serum alone	1,000	960	1,010	1,220	16,000

[6] Kling, C. A., *loc. cit.*, p. 30.

[7] Schattenfroh, A., *Arch. f. Hyg.*, 1899, xxxv, 135.

[8] Schneider, R., *loc. cit.*, p. 120.

[9] Serum was usually inactivated, either by heating it to 57° C. for 30 min., or by allowing it to stand at room temperature till its bactericidal properties had disappeared.

for complete sterilization usually increases slightly the rapidity and the completeness of its bacteriolytic action (table I). It is possible that a more pronounced phenomenon of this nature is the phenomenon described by Kling and others as an activation of the extract.

The addition of larger amounts of inactive serum invariably leads to a more or less complete inhibition of the bactericidal properties (table II). Sera differ considerably in the amount of this antibactericidal action, rabbit serum, for example, having approximately four times the antibactericidal power of horse serum.

TABLE II.

Effect of Inactive Serum on the Bacteriolysin.

The tests were made as in table I, but with larger amounts of the inactive serum. Test organism, *B. typhosus*. Test fluids contained 0.22 per cent. sodium chloride.

Material tested.	Time of plating.				
	1 min.	1 hr.	2 hrs.	5 hrs.	24 hrs.
Bacteriolysin alone	600	6	0	0	0
Bacteriolysin + 1.5% inactive serum	600	5	0	0	0
Bacteriolysin + 3% inactive serum	800	20	2	0	30
Bacteriolysin + 6% inactive serum	850	50	10	0	2,000
Bacteriolysin + 12% inactive serum	850	400	250	200	3,000
Bacteriolysin + 25% inactive serum	950	550	500	400	4,000
Bacteriolysin + 50% inactive serum	1,020	850	820	800	16,000
100% inactive serum alone	1,050	1,000	950	1,020	20,000

Active Serum.—The effects of the addition of active serum are less easily determined, due to the bactericidal properties of the serum itself. By carefully adjusting the relative amounts of serum and extract, however, it is possible to show (table III) that active serum also is capable of inhibiting or destroying the bacteriolytic action. But what from the practical point of view is possibly of equal importance is the fact that the active serum as well as the extract loses its bactericidal power, as a result of such admixture. This gives the phenomenon of two bactericidal substances, active serum and active leucocytic extract, added to each other to produce a fairly good culture medium for bacteria.

TABLE III.

Effect of Active Serum on the Bacteriolysin.

Guinea pig serum was selected on account of its comparatively weak bactericidal action on *B. typhosus*. Tests as in table I. Test fluids contained 0.22 per cent. sodium chloride.

Material tested.	Time of plating.			
	1 min.	1 hr.	3½ hrs.	24 hrs.
10% guinea pig serum alone	1,300	450	0	0
Bacteriolysin alone	450	5	0	0
Bacteriolysin+10 % guinea pig serum	1,200	1,000	750	15,000

EFFECT OF NORMAL TISSUE FLUIDS ON THE BACTERIOLYSIN.

Cerebrospinal fluid was selected as the easiest obtainable normal tissue fluid. The addition of cerebrospinal fluid to leucocytic extract produces nearly as great an inhibition or destruction of its bacteriolytic properties as the addition of serum itself (table IV).

TABLE IV.

Effect of Cerebrospinal Fluid on the Bacteriolysin.

Human cerebrospinal fluid (apparently normal) obtained by lumbar puncture for diagnostic purposes was tested as in table II. Test organism, *B. typhosus*. Dilutions made with 0.22 per cent. sodium chloride. A similar action was obtained with the cerebrospinal fluid of the monkey and the dog.

Material tested.	Time of plating.			
	1 min.	1 hr.	4 hrs.	24 hrs.
Bacteriolysin alone	300	1	0	0
Bacteriolysin+ 2% cerebrospinal fluid	350	0	0	0
Bacteriolysin+ 4% cerebrospinal fluid	700	20	1	0
Bacteriolysin+ 8% cerebrospinal fluid	750	250	4	3,000
Bacteriolysin+16% cerebrospinal fluid	800	300	250	10,000
Bacteriolysin+33% cerebrospinal fluid	1,000	600	700	15,000
Bacteriolysin+66% cerebrospinal fluid	1,000	750	800	25,000
100 % cerebrospinal fluid alone	1,000	1,000	1,200	20,000

EFFECTS OF PATHOLOGICAL FLUIDS ON THE BACTERIOLYSIN.

Pathological Effusion.—The action of a pathological effusion is also practically the same as that of serum (table V).

TABLE V.

Effect of Pathological Effusion on the Bacteriolysin.

The effusion here tested was obtained by injecting aleuronat into the pleural cavity of a dog. The resulting pleural exudate was aspirated, twenty-four hours later, was centrifuged free from cellular elements, and then inactivated by heating it to 57° C., for twenty minutes. Tests of its antibactericidal action were made as in table II. Test organism, *B. typhosus*. Test fluids contained 0.22 per cent. sodium chloride.

Material tested.	Time of plating.			
	1 min.	45 min.	3½ hrs.	24 hrs.
Bacteriolysin alone	300	0	0	0
Bacteriolysin + 1.5% pleural effusion	450	3	0	0
Bacteriolysin + 3% pleural effusion	500	20	0	0
Bacteriolysin + 6% pleural effusion	650	50	1	10
Bacteriolysin + 12% pleural effusion	800	60	2	40
Bacteriolysin + 25% pleural effusion	1,000	500	250	6,000
Bacteriolysin + 50 % pleural effusion	1,150	1,000	650	20,000
50% pleural effusion alone	1,100	1,100	900	2,000

Autolytic Products.—Substances given off during the aseptic autolysis of tissues are also strongly antibactericidal (table VI), as are also most of the products obtained by the bacterial decomposi-

TABLE VI.

Effect of Autolytic Products on the Bacteriolysin.

The kidney of a dog was removed with aseptic precautions, ground up in sterile sand, and the resulting finely divided tissue was washed three times by centrifugation with physiological saline solution. The serum-free tissue fragments were now suspended in three volumes of ¼ physiological saline solution, and the resulting suspension was incubated over night. The suspension was then freed from tissue fragments by centrifugation and its antibactericidal powers were tested. Test organism, *B. typhosus*. Test fluids contained 0.22 per cent. sodium chloride. A similar antibactericidal action was obtained with the autolytic products of liver, spleen, and heart muscle.

Material tested.	Time of plating.			
	1 min.	1 hr.	4 hrs.	24 hrs.
Bacteriolysin alone	600	20	0	0
Bacteriolysin + 0.75% autolytic products	850	100	0	0
Bacteriolysin + 1.5% autolytic products	850	150	6	0
Bacteriolysin + 3% autolytic products	850	350	150	25
Bacteriolysin + 6% autolytic products	900	500	350	600
Bacteriolysin + 12% autolytic products	950	600	400	2,000
Bacteriolysin + 25% autolytic products	950	700	600	15,000
Bacteriolysin + 50% autolytic products	980	850	850	30,000
50% autolytic products, alone	1,100	1,050	950	40,000

tion of tissues. None of the autolytic products thus far obtained have been in themselves bactericidal. A number of them have had the power of inhibiting bacterial multiplication, the property studied by Conradi.[10]

Of particular interest are the effects of products obtained by the prolonged autolysis of leucocytes themselves. On prolonged autolysis, both homologous leucocytes and foreign leucocytes give off strongly antibactericidal substances (table VII). The formation or liberation of these antibactericidal leucocytic products undoubtedly explains the fact, noted in the previous paper, that, in order to obtain an active leucocytic extract, the extraction process must be interrupted at a certain stage. Too prolonged an extraction usually gives an inactive extract.

TABLE VII.

Effect of the Products of the Prolonged Autolysis of Leucocytes on the Bacteriolysin.

Horse leucocytes were extracted at 37° C. for two hours in ¼ physiological saline solution, and were then freed from the supernatant fluid by centrifugation. The partially extracted leucocytes were now suspended in three volumes of ¼ physiological saline solution and the autolysis was allowed to continue at 37° C. over night. The resulting autolytic products were then freed from cellular elements and tested for their antibactericidal action. Test organism, *B. typhosus*. Test fluids contained 0.22 per cent. sodium chloride.

Material tested.	Time of plating.			
	1 min.	45 min.	3½ hrs.	24 hrs.
Bacteriolysin alone	600	50	0	0
Bacteriolysin + 0.5% leucocytic products	750	60	0	0
Bacteriolysin + 1% leucocytic products	800	50	0	0
Bacteriolysin + 2% leucocytic products	800	150	0	5
Bacteriolysin + 4% leucocytic products	800	600	200	15
Bacteriolysin + 8% leucocytic products	750	700	350	300
Bacteriolysin + 16% leucocytic products	800	600	400	500
Bacteriolysin + 33% leucocytic products	950	850	500	1,000
Bacteriolysin + 66% leucocytic products	950	850	750	2,000
66 % leucocytic products alone	850	750	800	2,000

QUANTITATIVE RELATIONS.

Amount.—The relation between the amount of bacteriolysin tested and the amount of serum or other body fluids necessary to

[10] Conradi, H., *loc. cit.*, p. 222.

produce a given decrease in its bactericidal power is indicated in table VIII.

From this table it is seen that the amount of serum necessary to produce a given change in the bactericidal power increases with the amount of the bacteriolysin tested.

TABLE VIII.

Quantitative Relations.

Parallel tests of the antibactericidal power of horse serum, with three different amounts of the bacteriolysin. Test organism, *B. typhosus*. Test fluids contained 0.22 per cent. sodium chloride.

Material tested.	Serum necessary to reduce bacteriolysis.				Average.
	Slightly.	Markedly.	Nearly completely.	Completely.	
0.25 c.c. bacteriolysin.....	3%	5%	7%	11%	**6.5%**
0.35 c.c. bacteriolysin.....	7%	11%	16%	22%	**14.0%**
0.50 c.c. bacteriolysin.....	7%	16%	22%	33%	**19.5%**

Rate.—The neutralization, destruction, or binding of the bactericidal substance takes place instantaneously on the addition of the serum or body fluid. The reaction differs in this particular from a number of serum reactions, which require a certain length of time before becoming complete. Duplicate mixtures of serum and bacteriolysin tested immediately and after standing for various lengths of time show, within the limits of the experimental error, the same bactericidal powers.

ANTIBACTERICIDAL ACTION ANALYZED.

An effort was made to determine which of the components of serum and body fluids are responsible for the antibactericidal action.

Serum Colloids.—Serum colloids were obtained by dialyzing serum free from diffusible products. The precipitated globulins were put in solution by the addition of a minimum known amount of sodium chloride, and in testing the antibactericidal action the same amount of sodium chloride was used in all parallel and control tubes. The results of the analysis are shown in table IX. From this table it appears that about half the antibactericidal action of serum is due to the serum colloids.

TABLE IX.

Effect of the Serum Colloids on the Bacteriolysin.

The antibactericidal action of inactive horse serum was compared with that of the colloids obtained from the same serum. Test organism, *B. typhosus.* Test fluids contained 0.22 per cent. sodium chloride.

Material tested.	Time of plating.			
	1 min.	1 hr.	4 hrs.	24 hrs.
Bacteriolysin alone	950	60	0	0
Bacteriolysin + 2% whole serum	950	50	0	0
Bacteriolysin + 4% whole serum	700	0	0	0
Bacteriolysin + 8.5% whole serum	600	6	0	50
Bacteriolysin + 17% whole serum	800	350	70	8,000
Bacteriolysin + 33% whole serum	1,100	1,000	800	10,000
Bacteriolysin + 66% whole serum	1,150	1,000	1,000	30,000
Bacteriolysin + 2% serum colloids	900	2	0	0
Bacteriolysin + 4% serum colloids	700	15	0	0
Bacteriolysin + 8.5% serum colloids	600	1	0	0
Bacteriolysin + 17% serum colloids	600	30	0	60
Bacteriolysin + 33% serum colloids	700	400	100	1,200
Bacteriolysin + 66% serum colloids	1,050	650	750	2,000
66% whole serum alone	1,100	1,000	1,050	25,000
66% serum colloids alone	1,200	1,100	1,100	20,000

Serum Crystalloids.—The diffusible serum components were obtained by collecting the water from the dialysis above, and evaporating it to a small volume. The action of these diffusible products is shown in table X. This table confirms the previous finding, since

TABLE X.

Effect of the Serum Crystalloids on the Bacteriolysin.

The serum crystalloids, separated by dialysis from the serum colloids tested in table IX, were tested for their antibactericidal action. Test organism, *B. typhosus.* Dilutions made with 0.2 per cent. sodium chloride.

Material tested.	Time of plating.			
	1 min.	1 hr.	4 hrs.	24 hrs.
Bacteriolysin alone	950	60	0	0
Bacteriolysin + 2% serum crystalloids	600	4	0	0
Bacteriolysin + 4% serum crystalloids	1,000	150	10	0
Bacteriolysin + 7.5% serum crystalloids	1,000	100	2	0
Bacteriolysin + 15% serum crystalloids	600	50	0	2
Bacteriolysin + 30% serum crystalloids	800	120	30	3,000
Bacteriolysin + 60% serum crystalloids	950	700	700	30,000
60% serum crystalloids alone	1,100	1,050	1,100	20,000

it shows that approximately half the antibactericidal action of serum is due to its non-colloidal or diffusible components. The fact that the diffusible serum components possess a considerable antibactericidal action agrees with the observation that cerebrospinal fluid is strongly antibactericidal. Normal cerebrospinal fluid contains only a trace of colloidal (proteid) material, but is rich in diffusible serum components.[11]

Ringer Solution.—The action of Ringer solution is similar to the action of the diffusible serum products (table XI).

TABLE XI.

Influence of Ringer Solution on the Bacteriolysin.

Equal amounts of the bacteriolysin were dissolved in distilled water, half strength Ringer solution, and full strength Ringer solution, and then tested for their bactericidal power. The Ringer solution used in these tests had the following composition: 2.5 c.c. *m/1* $NaHCO_3$ + 2 c.c. *m/1* $CaCl_2$ + 4 c.c. *m/1* KCl + 9 gm. NaCl + 990 c.c. H_2O. Test organism, *B. typhosus.*

Material tested.	Time of plating.			
	1 min.	1 hr.	4 hrs.	24 hrs.
Bacteriolysin in distilled water	500	30	0	0
Bacteriolysin in 50% Ringer solution	800	150	20	15,000
Bacteriolysin in 100% Ringer solution	950	600	500	40,000

Sodium Chloride.—It was pointed out in the previous paper that sodium chloride is antibactericidal for leucocytic extract.[12] Its antibactericidal action, however, is distinctly less than that of

[11] For analyses of normal and pathological cerebrospinal fluids, see Mott, F. W., *Lancet,* 1910, ii, 1, 79.

[12] The fact that an extract of horse leucocytes is decreased in its bactericidal power by the addition of sodium chloride does not agree with Schattenfroh's original observation (*loc. cit.*) that the action of the leucocytic bacteriolysin is independent of the salt content of the fluid tested. Schattenfroh's observation is of considerable importance, because it was cited as evidence that the leucocytic bacteriolysin and the serum bacteriolysin are two distinct substances, the bactericidal properties of serum being influenced by variations in its salt content.

The fact that an extract from horse leucocytes changes its bactericidal power on altering the amount of sodium chloride it contains does not, however, furnish evidence of the identity of the serum and leucocytic bacteriolysins. The serum bacteriolysin is active in the presence of sodium chloride, but inactive in distilled water. The leucocytic bacteriolysin is active in distilled water, but almost

Ringer solution (table XII). The antibactericidal action of sodium chloride is not due to a destruction or permanent injury of the bacteriolysin, since on removing the sodium chloride by dialysis the bactericidal power is restored quantitatively.

TABLE XII.

Comparative Antibactericidal Action of Physiological Saline Solution and Ringer Solution.

Equal amounts of the bacteriolysin were dissolved in distilled water, physiological saline solution (0.9 per cent. sodium chloride), and Ringer solution, and then tested for their bactericidal power. Test organism, *B. typhosus.*

Material tested.	Time of plating.			
	1 min.	1 hr.	4 hrs.	24 hrs.
Bacteriolysin in distilled water	1,000	350	0	0
Bacteriolysin in physiological saline solution	1,000	600	400	1,500
Bacteriolysin in Ringer solution	1,000	900	900	5,000

Alkalies.—The addition of alkali to the bacteriolysin causes a rapid decrease in its bactericidal power (table XIII). The bacteriolytic action, for example, is nearly completely abolished in the

TABLE XIII.

Influence of Alkali on the Bacteriolysin.

Equal amounts of the bacteriolysin were tested alone and in the presence of increasing amounts of sodium hydroxide. Test organism, *B. typhosus.* Test fluids contained 0.22 per cent. sodium chloride.

Material tested.	Time of plating.			
	1 min.	1½ hrs.	4 hrs.	24 hrs.
Bacteriolysin alone	650	100	2	10
Bacteriolysin + 0.0004% sodium hydroxide	800	200	20	50
Bacteriolysin + 0.0008% sodium hydroxide	760	250	30	70
Bacteriolysin + 0.0016% sodium hydroxide	860	550	300	350
Bacteriolysin + 0.0033% sodium hydroxide	780	500	600	550
Bacteriolysin + 0.0067% sodium hydroxide	750	520	500	400
0.0067% sodium hydroxide alone	770	600	600	650

inactive in the presence of sodium chloride. The observation therefore tends to strengthen Schattenfroh's conclusion that the two lysins are distinct. It is probable that the differences in the results are due to differences in the methods of preparing the leucocytic extracts.

presence of 0.003 per cent. sodium hydrate. A similar, though possibly slightly less pronounced inhibition is produced by sodium carbonate. The antibactericidal action of alkalies is not due to a destruction or permanent injury of the bactericidal agent, since on neutralizing an inactive mixture of bacteriolysin and alkali the bactericidal power is restored quantitatively.

Acids.—The determination of the effects of acids on the bacteriolysin is limited by the toxicity of most acids. Hydrochloric acid, within the limits of its toxicity, apparently has no effect. The range of the test is somewhat greater with acetic acid (table XIV), but within the limits of the experimental error, acetic acid also is without a distinct action on the leucocytic bacteriolysin.

TABLE XIV.

Influence of Acetic Acid on the Bacteriolysin.

Equal amounts of the bacteriolysin were tested alone and in the presence of increasing amounts of acetic acid. Test organism, *B. typhosus*. Test fluids contained 0.22 per cent. sodium chloride.

Material tested.	Time of plating.			
	1 min.	1½ hrs.	4 hrs.	24 hrs.
Bacteriolysin alone	650	100	2	7
Bacteriolysin + 0.0003% acetic acid	750	90	2	8
Bacteriolysin + 0.0006% acetic acid	800	100	4	50
Bacteriolysin + 0.0012% acetic acid	900	90	2	30
Bacteriolysin + 0.0025% acetic acid	700	60	6	0
Bacteriolysin + 0.0050% acetic acid	650	60	3	0
0.0050% acetic acid alone	1,000	850	750	700

Boric acid is less toxic for bacteria than acetic acid, and can be tested in much higher concentrations (table XV). When added to leucocytic extract in small amounts, boric acid also is without any distinct action on bacteriolysis. It is only when the amount reaches 0.2 per cent. that a slight slowing in the rate of the bacteriolysis is observed. It is questionable, however, whether this is really due to acid action, or may not result from the necessary changes in osmotic condition. With 1.25 per cent. boric acid, bacteriolysis is distinctly reduced.

TABLE XV.

Influence of Boric Acid on the Bacteriolysin.

Equal amounts of the bacteriolysin were tested alone and in the presence of increasing amounts of boric acid. Test organism, *B. typhosus*. Test fluids contained 0.22 per cent. sodium chloride.

Material tested.	Time of plating.			
	1 min.	1½ hrs.	4 hrs.	24 hrs.
Bacteriolysin alone	550	30	0	0
Bacteriolysin+0.025% boric acid	450	1	0	0
Bacteriolysin+0.05% boric acid	700	5	0	0
Bacteriolysin+0.1% boric acid	800	40	0	0
Bacteriolysin+0.2% boric acid	800	300	6	0
Bacteriolysin+0.4% boric acid	750	300	3	0
Bacteriolysin+0.8% boric acid	750	300	0	0
Bacteriolysin+1.25% boric acid	750	400	100	20
Bacteriolysin+2.5% boric acid	800	300	200	50
2.5% boric acid alone	750	750	700	650

Quantitative Relations.—The relative amount of the destruction or inhibition of bacteriolysis that is due to each of the above components has not been determined. With serum, however, it is probable that about half the antibactericidal action is due to the serum colloids, about a quarter to the neutral diffusible products, and a quarter to the diffusible alkalies.

MECHANISM OF THE ANTIBACTERICIDAL ACTION.

The manner in which the various substances above enumerated antagonize or overcome the bactericidal action has not been determined. Two methods are theoretically possible. First, the antibactericidal substances may enter into a direct chemical relation with the bacteriolysin, with the formation of non-bactericidal combination products or split products. Or second, the antibactericidal substance may enter into direct chemical relation with the bacteria, making them in some way insusceptible to the action of the bacteriolysin. This second method might conceivably be somewhat similar to the antagonistic salt actions studied by Loeb[13] and his co-workers, in which one chemical substance so alters the permeability of the limiting cell membrane as to prevent the entrance into the cell of the toxic agent. It is quite possible, and even probable,

[13] Loeb, J., The Dynamics of Living Matter, New York, 1906, 70.

that the antagonistic action of any one of the antibactericidal body fluids is a combination of a direct action of certain components on the bacteriolysin and of other components on the bacteria.

METHODS TO PREVENT THE ANTIBACTERICIDAL ACTION.

Quantity.—An attempt was made to devise a method to overcome or prevent the antibactericidal action of serum and body fluids. The first method that suggested itself was to exhaust the antibactericidal power of the inhibiting fluid by increasing the amount of bacteriolysin added to it. It is possible, under certain conditions, to overcome the antibactericidal action of sodium chloride by this means (table XVI).

TABLE XVI.

Attempt to Exhaust the Antibactericidal Action of a Neutral Salt.

Parallel tests with multiple doses of the leucocytic bacteriolysin dissolved in physiological saline (0.9 per cent. sodium chloride). Test organism, *B. typhosus.*

Material tested.	Time of plating.			
	1 min.	1 hr.	4 hrs.	24 hrs.
Bacteriolysin in distilled water	650	40	5	0
Bacteriolysin in physiological saline	600	550	600	25,000
2 ×bacteriolysin in physiological saline	600	350	300	20,000
4 ×bacteriolysin in physiological saline	400	10	5	2,000
8 ×bacteriolysin in physiological saline	400	5	0	0

It is also possible to overcome part of the antibactericidal action of Ringer solution by the same means (table XVII).

TABLE XVII.

Attempt to Exhaust the Antibactericidal Action of Ringer Solution.

Parallel tests with multiple doses of the leucocytic bacteriolysin dissolved in Ringer solution. Test organism, *B. typhosus.*

Material tested.	Time of plating.			
	1 min.	1 hr.	4 hrs.	24 hrs.
Bacteriolysin in distilled water	1,000	70	1	0
Bacteriolysin in Ringer solution	1,000	950	1,000	50,000
2 ×bacteriolysin in Ringer solution	1,000	700	600	50,000
4 ×bacteriolysin in Ringer solution	1,000	300	70	20,000
8 ×bacteriolysin in Ringer solution	950	150	20	2,500

TABLE XVIII.

Attempt to Exhaust the Antibactericidal Action of Body Fluids.

Parallel tests with multiple doses of the leucocytic bacteriolysin dissolved in human cerebrospinal fluid. Test organism, *B. typhosus*. Dilutions made with 0.22 per cent. sodium chloride. A practically identical result was obtained with inactivated pathological transudate and with inactive serum.

Material tested.	Time of plating.			
	1 min.	1 hr.	4 hrs.	24 hrs.
Bacteriolysin in distilled water	1,100	350	0	0
Bacteriolysin in cerebrospinal fluid	1,100	1,000	1,000	6,000
2 ×bacteriolysin in cerebrospinal fluid	1,050	1,000	1,000	8,000
4 ×bacteriolysin in cerebrospinal fluid	1,050	950	1,000	8,000
8 ×bacteriolysin in cerebrospinal fluid	1,100	1,000	1,000	6,000

Attempts to overcome the antibactericidal action of cerebrospinal fluid (table XVIII), of pathological transudates, and of serum by this means, however, have thus far been unsuccessful. Apparently no amount of leucocytic bacteriolysin added to these fluids, when tested in their full concentration, is able to exhaust their antibactericidal capacity. If the fluids, however, are diluted, their action is more nearly that of Ringer solution.

TABLE XIX.

Attempt to Diminish the Antibactericidal Action of Body Fluids.

Parallel samples of a non-bactericidal mixture of the leucocytic bacteriolysin and serum were tested alone and in the presence of increasing amounts of boric acid. Test organism, *B. typhosus*. Test fluids contained 0.22 per cent. sodium chloride. A practically identical result was obtained with a non-bactericidal mixture of the bacteriolysin and cerebrospinal fluid. A similar restoration of part of the original bactericidal power can be obtained with acetic acid.

Material tested.	Time of plating.			
	1 min.	1 hr.	4 hrs.	24 hrs.
Bacteriolysin alone	1,200	60	0	0
Bacteriolysin+serum	1,100	1,050	1,100	50,000
Bacteriolysin+serum+0.017% boric acid	1,050	1,000	1,000	8,000
Bacteriolysin+serum+0.035% boric acid	1,100	900	850	6,000
Bacteriolysin+serum+0.07% boric acid	1,050	850	800	1,200
Bacteriolysin+serum+0.15% boric acid	1,050	750	800	850
Bacteriolysin+serum+0.3% boric acid	1,000	600	600	350
Bacteriolysin+serum+0.6% boric acid	1,000	600	600	400
Bacteriolysin+serum+1.25% boric acid	1,100	550	500	350
Bacteriolysin+serum+2.5% boric acid	1,050	600	400	300

Acidulation.—A second method that suggested itself was to neutralize the antibactericidal alkalies. This method is partially successful. The addition, for example, of boric acids to a non-bactericidal mixture of the bacteriolysin and body fluids occasionally restores part of the original bactericidal power (table XIX). In no case thus far tested, however, has more than a small fraction of the original power been restored by this means. The result differs in this respect from the result reported by Lamar,[14] who was able to restore completely the inhibited bactericidal power of certain soaps by adding boric acid to an inactive mixture of the soap and serum.

SUMMARY.

1. An extract of horse leucocytes is strongly bactericidal when dissolved in distilled water; it has considerable bactericidal power when dissolved in physiological saline; but it loses its bactericidal properties when mixed with blood serum or with normal or pathological tissue fluids.

2. About half the antibactericidal action of blood serum is due to the serum colloids, about a quarter to the neutral serum crystalloids, and a quarter to the diffusible alkalies. Diffusible acids have no antibactericidal action.

3. The addition of boric acid to an inactive mixture of leucocytic extract and serum or other body fluid occasionally restores part of the original bactericidal power, but never more than a small fraction of that power.

[14] Lamar, R. V., *Jour. Exper. Med.*, 1911, xiii, 1, 380; xiv, 256.