ON THE BACTERICIDAL EFFECT EXERTED BY HUMAN
BLOOD ON CERTAIN SPECIES OF PATHOGENIC MICRO-
ORGANISMS AND ON THE ANTIBACTERICIDAL EFFECTS
OBTAINED BY THE ADDITION TO THE BLOOD IN
VITRO OF DEAD CULTURES OF MICRO-ORGANISMS IN
QUESTION.

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THE fact that the blood of ordinary laboratory animals exerts a very marked bactericidal effect upon the *Bacillus typhosus* and the *Spirillum cholerae asiaticae*, while it exerts little or no effect upon the *Staphylococcus* and *Streptococcus pyogenes*, has hardly received the attention which it would seem to merit in view of the circumstance that these facts involve the important problem as to whether the blood exerts its bactericidal action upon pathogenic organisms generally, or only upon certain species of such micro-organisms.

We have addressed ourselves to the task of re-investigating this general problem by the aid of the methods of bactericidal estimation which have been elsewhere described by one of us¹, conducting our experiments upon human blood, and drawing within the sphere of our observation, not only the micro-organisms particularized above, but also the *Micrococcus melitensis* of Bruce and the *Bacillus pestis*.

I. *Data with regard to the bactericidal power of the blood as affecting the Bacillus typhosus and Spirillum cholerae asiaticae.*

We may begin by setting forth certain data in connection with the bactericidal power of human blood upon the *Bacillus typhosus*, and

¹ *Lancet*, June 1, 1901, p. 1532; *Proc. Roy. Soc.* (this paper is about to appear).

Bactericidal Effects of Blood

TABLE I.

Exhibiting (a) the bactericidal action exerted by human serum on *Bacillus typhosus* and (b) the antibactericidal effect obtained by the addition of a sterilized typhoid culture to the mixture of serum and living culture.

Capillary testing pipettes were filled with mixture of									
Dilutions in which the living typhoid culture was employed	2 vols. F.N.W.'s serum 1 vol. dilution of living culture which contained 33,000,000 T.B. per c.c. and	1 vol. H.B.'s serum 1 vol. dilution of living culture which contained 220,000,000 T.B. per c.c. and	2 vols. A.E.W.'s serum 1 vol. dilution of living culture which contained 210,000,000 T.B. per c.c. and	1 vol. H.B.'s serum 1 vol. dilution of living culture which contained 220,000,000 T.B. per c.c. and	1 vol. A.E.W.'s serum 1 vol. dilution of living culture which contained 280,000,000 T.B. per c.c. and	1 vol. A.E.W.'s serum 1 vol. dilution of living culture which contained 540,000,000 T.B. per c.c. and			
	1 vol. sterile broth	1 vol. sterile broth	1 vol. sterile broth	1 vol. sterile broth	1 vol. sterile broth	1 vol. sterile broth	1 vol. sterile broth	1 vol. sterile broth	1 vol. sterile, lized typhoid culture
undiluted	— growth	— growth	— growth	— growth	— growth	— growth	growth	growth	growth
2-fold dilut.	"	"	"	"	"	"	"	"	"
5 "	"	"	"	"	"	"	"	"	"
10 "	sterile	"	sterile	"	"	"	sterile	sterile	"
25 "	"	"	"	"	"	"	"	"	"
50 "	"	sterile	"	"	"	"	sterile	sterile	"
100 "	"	"	"	"	"	"	"	"	"
1000 "	sterile	"	"	"	"	"	"	"	"
10,000 "	"	"	"	"	"	"	"	"	"
100,000 "	"	"	"	"	"	"	"	"	"

F.N.W. and H.B. were normal men. A.E.W. had 9 months previously, and also on previous occasions, undergone anti-typhoid inoculation.

In every case, both in this and in the subsequent tables, the serum was employed within 2 or 3 hours after the blood had been drawn off.

In every case, both in this and in the subsequent tables, the living cultures employed were, unless otherwise stated, 24 hour old broth cultures.

Here and in the subsequent tables the sterilized bacterial cultures employed had been sterilized by exposure to a temperature of about 60°–65° C. for 10–20 minutes.

Here and in the subsequent tables the sera were allowed to act upon the bacteria with which they were mixed for 18–24 hours at 37° C. before the effect was tested.

In every case the term "growth" denotes that the growth obtained presented the characters of a pure cultivation of the micro-organism employed in the test. In cases of doubt the purity of the culture was tested by subcultures and microscopical examination. Where contaminations were found the series of experiments was in almost every instance rejected. Where such a series is retained the fact that a contamination was found is in each case specifically mentioned.

TABLE II.

Exhibiting the antibactericidal effects produced by the addition to a mixture of serum and living typhoid culture of filtrates from typhoid cultures.

Dilutions in which the living typhoid culture was employed	Capillary testing pipettes were filled in with							
	1 vol. F. N. W.'s serum of living culture which contained 76,000,000 T.B. per c.c. and				1 vol. A. E. W.'s serum of living culture which contained 76,000,000 T.B. per c.c. and			
	1 vol. sterile broth	1 vol. filtrate from a 24 hours typhoid culture	1 vol. filtrate from a 5 months old typhoid culture	1 vol. same 5 months old typhoid culture unfiltered	1 vol. sterile broth	1 vol. filtrate from a 24 hour typhoid culture	1 vol. filtrate from a 5 months old typhoid culture	1 vol. filtrate from a 5 months old typhoid culture
	growth sterile * growth sterile " " " " " " " "	growth " sterile " " " " " " " "	growth " " sterile " " sterile " " " "	growth " " " " sterile " " " "	sterile " " " " " " " " "	growth " sterile " " " " " " "	growth " sterile " " " " contamination + sterile " "	growth " sterile " " " " contamination + sterile " "
2-fold dilut.								
5 "								
10 "								
25 "								
50 "								
100 "								
1000 "								
10,000 "								
100,000 "								
1,000,000 "								

With regard to F. N. W. and A. E. W. and general condition of the experiments see notes to Table I.

* Sterility of the tube probably due to accidental overheating.

+ *Staphylococcus*.

with regard to the "antibactericidal effect" obtained by the introduction of a sterilized culture of the typhoid bacillus into human blood *in vitro*¹.

A point of incidental interest here suggests itself in connection with the question as to what is the element in the sterilized culture which exerts the antibactericidal effect exemplified in Table I.

The experiments subjoined in Table II. are typical examples of a number of experiments instituted with a view to the determination of this question.

These results show that a filtrate from a young culture of *B. typhosus* exerts little or no antibactericidal effect; while a filtrate from an old culture which contains in solution elements derived from the dissolution of the typhoid bacilli exerts a very marked antibactericidal effect. Of particular interest are the results in columns 3 and 4, which show that the filtrate derived from a culture in which the bacilli had been macerating at 37° C. for a period of 5 months, diminished the bactericidal power of the serum with which it was mixed to exactly the same degree as the unfiltered culture.

Passing to the consideration of the bactericidal effect exerted by human serum upon the cholera vibrio, we subjoin a selection of typical experiments illustrating on the one hand the bactericidal effect exerted upon the cholera vibrio, and on the other hand, the diminution of bactericidal power which is achieved by the addition of a sterilized cholera culture to a mixture of serum and living cholera culture.

It will be manifest from a comparison of the experiments in Table I. and Table III. that the bactericidal and antibactericidal effects proceed on precisely the same lines whether we are employing a culture of typhoid or a culture of cholera.

It becomes, therefore, a point of interest to determine whether a diminution of the bactericidal effect exerted on the typhoid bacillus is obtained by the addition of a sterilized cholera culture to the mixture of serum and living typhoid culture; and *vice versa* whether a diminution of the bactericidal effect exerted on the cholera vibrio is

¹ Data with regard to the first of these points have already been set forth by one of us in a paper published in the *Lancet*, Sept. 14th, 1901, p. 715, dealing with the changes produced in the blood by antityphoid inoculation. The second of these questions has also been briefly adverted to in the same Journal, June 1st, 1901, p. 1534, in connection with a suggestion that the antibactericidal effect exerted might serve as a criterion for the standardization of bacterial vaccines.

TABLE III.

Exhibiting (a) the bactericidal effect exerted by serum on a 24 hour old culture of the cholera vibrio, and (b) the antibactericidal effect achieved by the addition of a sterilized cholera culture to the mixture of serum and living cholera culture.

	Capillary tubes were filled with					
	1 vol. F.N.W.'s serum 1 vol. dilution of living culture which contained 240,000,000 cholera vibrios per c.c. and	1 vol. sterile broth	1 vol. steri- lized cholera culture	1 vol. A.E.W.'s serum 1 vol. dilution of living culture which contained 45,000,000 cholera vibrios per c.c. and	1 vol. sterile broth	1 vol. steri- lized cholera culture
Dilutions in which the living cholera culture was employed						
undiluted culture	growth	growth	growth	growth	growth	growth
2-fold dilution	"	"	"	"	"	"
5 "	"	"	"	"	"	"
10 "	sterile	"	"	"	"	"
25 "	"	"	"	"	"	"
50 "	"	"	"	"	"	"
100 "	"	"	"	"	"	"
1000 "	"	"	"	"	"	"
10,000 "	"	"	sterile	"	"	sterile
100,000 "	"	"	"	"	"	"

With regard to F.N.W. and A.E.W. see notes to Table I.
 Rabbit 1 had been inoculated with one tube of typhoid bacillus.
 Rabbit 2 " " " " cholera vibrio.

TABLE IV.

Exhibiting (a) the bactericidal effect exerted on a typhoid culture and (b) the diminution of that effect which is achieved by the addition of a sterilized cholera culture.

Dilutions in which the living typhoid culture was employed	Capillary tubes were filled with							
	1 vol. F.N.W.'s serum 1 vol. dilution of 24 hr. old living broth culture of the typhoid bacillus and		1 vol. A.E.W.'s serum 1 vol. dilution of 24 hr. old living broth culture of the typhoid bacillus and		1 vol. serum of Rabbit 1 1 vol. dilution of 24 hr. old living broth culture of the typhoid bacillus and		1 vol. serum of Rabbit 2 1 vol. dilution of 24 hr. old living broth culture of the typhoid bacillus and	
	1 vol. sterile broth	1 vol. steri- lized cholera culture	1 vol. sterile broth	1 vol. steri- lized cholera culture	1 vol. sterile broth	1 vol. steri- lized cholera culture	1 vol. sterile broth	1 vol. steri- lized cholera culture
undiluted culture	growth	growth	growth	growth	growth	growth	growth	growth
2-fold dilution	"	"	"	"	"	"	"	"
5 "	"	"	"	"	"	"	"	"
10 "	sterile	"	"	"	"	"	"	"
25 "	"	"	sterile	"	sterile	"	"	"
50 "	"	"	"	"	"	"	"	"
100 "	"	"	"	"	"	"	"	"
1000 "	"	"	"	"	"	"	"	"
10,000 "	"	"	"	"	"	"	"	"
100,000 "	"	sterile	"	sterile	"	"	sterile	"
1,000,000 "	—	—	"	"	"	"	"	"

With regard to F.N.W., A.E.W., and Rabbits 1 and 2, see notes to Table III.

TABLE V.

Exhibiting (a) the bactericidal effect exerted on a cholera culture, and (b) the diminution of that effect produced by the addition of a sterilized typhoid culture.

Dilutions of the living cholera culture which were employed	Capillary testing pipettes were filled with							
	1 vol. A. E. W.'s serum 1 vol. dilution of living cholera culture which contained 466,000,000 chol. vibrios per c.c. and		1 vol. serum of Rabbit 1 1 vol. dilution of living cholera culture which contained 85,000,000 chol. vibrios per c.c. and		1 vol. serum of A. E. W. 1 vol. dilution of living cholera culture and		1 vol. W. G. L.'s serum 1 vol. dilution of living cholera culture containing 2,600,000 chol. vibrios per c.c. and	
	1 vol. sterile broth	1 vol. steri- lized typhoid culture	1 vol. sterile broth	1 vol. steri- lized typhoid culture	1 vol. sterile broth	1 vol. steri- lized typhoid culture	1 vol. sterile broth	1 vol. steri- lized typhoid culture
undiluted culture	growth	growth	growth	growth	sterile	growth	growth	growth
2-fold dilution	"	"	"	"	"	"	"	"
5 "	"	"	"	"	"	"	"	"
10 "	"	"	"	"	"	"	"	"
25 "	sterile	"	sterile	"	"	"	"	"
50 "	"	"	"	"	"	"	"	"
100 "	"	"	"	"	"	"	"	"
1000 "	"	"	"	"	"	"	"	"
10,000 "	"	"	"	"	"	sterile	sterile	sterile
100,000 "	"	"	"	"	"	"	"	"

With regard to A. E. W. and Rabbit no. 1, see notes to Table III.

TABLE VI.

Exhibiting the results of the blood examinations carried out on two rabbits which were inoculated with similar quantities, in the one case of a sterilized typhoid culture, in the other case of a sterilized cholera culture. These rabbits are in the table below denoted respectively as the typhoid rabbit and the cholera rabbit.

	Tests carried out immediately antecedent to inoculation				Tests carried out 24 hours after inoculation			
	Capillary testing pipettes were filled in with				Capillary testing pipettes were filled in with			
	1 vol. dilution of a culture of typhoid which contained 94,000,000 T.B. per c.c. and	1 vol. serum of typhoid rabbit	1 vol. dilution of a very thin culture of cholera and	1 vol. serum of cholera rabbit	1 vol. dilution of a culture of typhoid which contained 440,000,000 T.B. per c.c. and	1 vol. serum of typhoid rabbit	1 vol. dilution of a very thin culture of cholera and	1 vol. serum of cholera rabbit
Dilutions of the living cultures which were employed in the testings								
undiluted culture	growth	growth	growth	growth	growth	growth	growth	growth
2-fold dilution	"	"	"	sterile	"	sterile	sterile	"
5 "	"	"	"	"	"	"	"	"
10 "	"	"	"	"	"	"	"	"
25 "	"	"	"	"	"	"	"	"
50 "	sterile	"	"	"	"	"	"	"
100 "	"	"	"	"	"	"	"	"
1000 "	"	"	"	"	"	"	"	"
10,000 "	"	"	"	"	sterile	"	"	"
100,000 "	"	"	"	"	"	"	"	"

TABLE VI. (continued).

	Tests carried out 48 hours after inoculation			Tests carried out 14 days after inoculation		
	Capillary testing pipettes were filled in with					
Dilutions of the living cultures which were employed in the testings	1 vol. serum of typhoid rabbit	1 vol. serum of cholera rabbit and 324,000,000 T.B. per c.c.	1 vol. dilution of a culture of cholera which contained 400,000 C.V. per c.c.	1 vol. dilution of a culture of typhoid which contained 480,000,000 T.B. per c.c.	1 vol. dilution of a culture of cholera which contained 85,000,000 C.V. per c.c. and	1 vol. serum of cholera rabbit
undiluted culture	growth	1 vol. serum of cholera rabbit	1 vol. serum of typhoid rabbit	1 vol. serum of cholera rabbit	1 vol. serum of typhoid rabbit	1 vol. serum of cholera rabbit
2-fold dilution	sterile	growth	growth	growth	growth	sterile
5 "	"	"	"	"	"	"
10 "	"	"	sterile	"	"	"
25 "	"	"	"	"	sterile	"
50 "	"	"	"	"	"	"
100 "	"	"	"	"	"	"
1000 "	"	"	"	"	"	"
10,000 "	"	sterile	"	sterile	"	"
100,000 "	"	"	"	"	"	"

obtained by the addition of a sterilized typhoid culture to a mixture of serum and living cholera vibrios.

Tables IV. and V., which show the effect invariably obtained in our experiments, supply the answer to this question.

As shown in the Tables IV. and V., taken in conjunction with Tables I. and III., the antibactericidal effect which is in each case obtained, is obtained indifferently with either variety of sterilized culture. We must consequently assume either that the bactericidal substance in the serum which kills the typhoid bacillus is one and the same substance which kills the cholera vibrio, or alternatively, that the bactericidal substance which kills the cholera vibrio possesses an element in common with the bactericidal substance which kills the typhoid bacillus.

With a view to deciding between these alternatives, we have investigated the question as to whether the inoculation of a full dose of antityphoid vaccine, which produces in man a preliminary diminution and subsequent increase in the bactericidal effect exerted on the typhoid bacillus¹, brings about any similar diminution and increase in the bactericidal effect exerted upon the cholera vibrio.

The following observations bear on the question.

The bloods of three healthy men, who recently came up for prophylactic inoculation with antityphoid vaccine, were tested before inoculation and afterwards, at intervals of a few days, against both the typhoid bacillus and the cholera vibrio. In no case was any indication obtained of an alteration in the bactericidal effect exerted on the cholera vibrio, although the negative and positive phases of diminished and exalted bactericidal power with respect to the typhoid bacillus manifested themselves in a typical manner.

These results confirm those obtained by one of us on two previous patients.

We further investigated the point upon two rabbits inoculated respectively with sterilized cultures of cholera and typhoid.

The results of the blood examinations here made are subjoined in tabular form (Table VI.).

A comparison of the first and second testings of the cholera-inoculated rabbit² would seem to suggest that an initial reduction

¹ Wright, *Lancet*, Sept. 14, 1901, p. 715.

² The circumstance that a positive phase of increased bactericidal power was obtained in case of the typhoid rabbit without the intervention of a negative phase of diminished bactericidal power is in accordance with what occurs in man after the inoculation of a relatively small dose of typhoid vaccine (Wright, *Lancet*, Sept. 14, 1901, p. 715).

of the bactericidal power was exerted upon both species of micro-organisms. It would, in other words, seem to point to the comparability of the immediate effect exerted by the introduction of a sterilized culture of cholera into the animal organism with the effect exerted by the direct introduction of the culture into the serum *in vitro*.

On the other hand, a comparison of the results obtained in the first and last testings of both the typhoid and the cholera-inoculated rabbit will show that the increase of the bactericidal power which was achieved by inoculation was, in each case, an increase only with respect to the particular species of micro-organism which had been inoculated.

The latter datum is for our present purpose the essentially important one of the experiment. It seems to indicate clearly that the bactericidal effects of a serum, at any rate in the case of a serum derived from the immunized animal, is as is assumed by the theories of Ehrlich and Bordet respectively, achieved by the co-operation of two bactericidal elements, one of these being a chemical agent which exerts an action on more than one species of micro-organism, and the other a chemical agent which is specific for each particular species of micro-organism.

There is, however, nothing to forbid our explaining the bactericidal action of normal serum by the more simple assumption that the non-specific element referred to above ("complement" of Ehrlich, "alexin" of Bordet) suffices by itself to exert a bactericidal effect.

From the study of the action of the serum upon the typhoid bacillus and the cholera vibrio, we pass to the consideration of the action of the serum upon the *Staphylococcus pyogenes*.

II. *Data with regard to the bactericidal power of the blood as affecting the Staphylococcus pyogenes.*

As a preliminary to setting forth our results, we may observe that we have not in our numerous experiments found any difference of behaviour as between the different varieties of the *Staphylococcus pyogenes*. For this reason we have thought it unnecessary to encumber the tables given below by specifying in each case the particular variety of *Staphylococcus* employed. Suffice it to say that these were chiefly cultures of the *Staphylococcus aureus* and *albus* freshly cultivated from operation-wounds, furuncles and sycosis.

We set forth first a series of typical experiments conducted by mixing in capillary testing pipettes in each case one volume of serum

TABLE VII.

Exhibiting the results obtained on cultivating a mixture of equal volumes of serum and of a graduated dilution of Staphylococcus culture which had remained in contact for 18—24 hrs. at 37° C.

Dilutions in which the living Staphylococcus culture was employed	Capillary testing pipettes were filled with				
	1 vol. dilution of Staphylococcus culture and				
	1 vol. sterile broth	1 vol. F.N.W.'s serum	1 vol. A.E.W.'s serum	1 vol. W.B.L.'s serum	1 vol. J.A.'s serum
10-fold dilution	growth	growth	growth	growth	growth
100 " "	"	"	"	"	"
1000 " "	"	"	"	"	"
10,000 " "	"	"	"	"	"
100,000 " "	"	"	"	"	"
1,000,000 " "	"	"	"	"	"
10,000,000 " "	sterile	"	"	"	"

With regard to F.N.W., A.E.W., and the general conditions of the experiments see notes to Table I. W.B.L. was a man in normal health. J.A. had suffered from furunculosis and sycosis barbae for a period of 9 years, and had completely recovered after three successive inoculations of a sterilized culture of a *Staphylococcus aureus* cultivated from his boils.

and one volume of a progressively increasing dilution of a 24-hour-old *Staphylococcus* culture.

It will be manifest that the results set forth in Table VII. are in conformity with the results obtained with the blood of animals in the classical researches of Nuttall¹. They show that human serum does not exert any bactericidal effect whatever upon the *Staphylococcus*; nay more, they suggest, and this suggestion is confirmed by direct observation on the colonies grown² in capillary testing pipettes filled with equal volumes of serum and gelatine cultures of *Staphylococcus*, that additions of serum exert a favourable influence on the growth of this germ.

Not obtaining any indication of a bactericidal effect exerted in the case of the volume for volume mixture of serum and broth dilutions of *Staphylococcus* cultures, we experimented further, using dilutions of broth cultures made with the serum under examination. In the higher dilutions thus obtained, we are in point of fact dealing with practically undiluted serum.

The method of experimentation adopted was as follows:—Two series of progressive dilutions of the culture were made, the diluents employed being in the one case sterile nutrient broth, and in the other case human serum.

A series of equal volumes of each dilution was measured off into capillary testing pipettes. These measured volumes were in the case of the broth dilutions immediately transferred to the surface of the nutrient agar with a view to the enumeration of the contained *Staphylococci*. The serum dilutions, on the contrary, were before implantation upon agar digested for 24 hours at 37° C. with a view to allowing the serum to exert its full effect upon the micro-organisms.

The results are set forth in Table VIII.

An arithmetical calculation based upon the data set forth in Table VIII., indicates that in the first experiment 10 c.mm. of practically undiluted serum failed to kill 0.4, and in the second experiment the same quantity of practically undiluted serum failed to kill 3 of the *Staphylococci* employed.

From the fact that the serum does not exert any bactericidal effect upon the *Staphylococci*, we surmised that no bactericidal substances

¹ *Zeitschrift f. Hygiene*, 1888, vol. iv. pp. 353—394.

² The technique employed in connection with the observations here in question was that which was described by one of us in the *Lancet*, Dec. 1, 1900, pp. 1556—1560.

TABLE VIII.
Exhibiting the results of the cultivations undertaken in the case of Staphylococcus cultures diluted (a) with sterile broth, and (b) with undiluted serum.

Dilutions of the cultures which were employed	6 day broth culture of Staphylococcus		2 day broth culture of Staphylococcus		diluted with M.G.'s serum, digested with this 24 hours at 37° C. and then cultivated in nutrient broth
	diluted with sterile broth, then transferred to nutrient agar and incubated	diluted with A. E. W.'s serum, digested with this 24 hours at 37° C. and then cultivated in nutrient broth	diluted 1,000,000-fold with sterile broth, then transferred to nutrient agar and cultivated	diluted with A. E. W.'s serum, digested with this 24 hours at 37° C. and then cultivated in nutrient broth	
10-fold dilut.		growth obtained from circ. 10 c.mm.		growth obtained from circ. 5 c.mm.	growth obtained from circ. 5 c.mm.
100 "		" "	9 colonies from 25 c.mm.	" "	" "
1000 "		" "	6 " 15 "	" "	" "
10,000 "		" "	6 " 15 "	" "	" "
100,000 "	40 colonies developed from 10 c.mm.	" "	2 " 10 "	" "	" "
1,000,000 "	0 colonies developed from 20 c.mm.	" "	0 " 5 "	" "	" "
10,000,000 "		" "	0 " 5 "	" sterile	" "

M.G. who had been a martyr to furunculosis had recently undergone 3 successive therapeutic inoculations with sterilized Staphylococcus cultures.

TABLE IX.

Exhibiting the results obtained on adding a sterilized culture of Staphylococcus to a mixture of serum and living typhoid culture.

		Capillary testing pipettes were filled in with							
Dilutions in which the living typhoid culture was employed		1 vol. F. N. W.'s serum 1 vol. dilut. of living typhoid culture	1 vol. A. E. W.'s serum 1 vol. dilut. of living typhoid culture	1 vol. E. A. S.'s serum 1 vol. dilut. of living typhoid culture	1 vol. W. G. L.'s serum 1 vol. dilut. of living typhoid culture	1 vol. W. G. L.'s serum 1 vol. dilut. of living typhoid culture	1 vol. W. G. L.'s serum 1 vol. dilut. of living typhoid culture	1 vol. W. G. L.'s serum 1 vol. dilut. of living typhoid culture	1 vol. W. G. L.'s serum 1 vol. dilut. of living typhoid culture
		1 vol. sterile broth	1 vol. sterile broth	1 vol. sterile broth	1 vol. sterile broth	1 vol. sterile broth	1 vol. sterile broth	1 vol. sterile broth	1 vol. sterile broth
2-fold dilut.		growth	growth	growth	growth	growth	growth	growth	growth
5	"	"	sterile	"	"	"	"	"	"
10	"	"	"	"	"	"	"	"	"
25	"	"	"	"	"	"	"	"	"
50	"	"	"	"	"	"	"	"	"
100	"	sterile	"	sterile	"	sterile	"	sterile	"
1000	"	"	"	"	"	"	"	"	"
10,000	"	"	"	"	"	"	"	"	"
100,000	"	"	"	"	"	"	"	"	sterile

E. A. S. was a man in normal health.

TABLE X.

Exhibiting the results obtained when a sterilized culture of Staphylococcus is added to a mixture of serum and living cholera culture.

Dilutions in which the living cholera culture was employed	Capillary testing pipettes were filled with							
	1 vol. F. N. W.'s serum 1 vol. dilution of living cholera culture		1 vol. A. E. W.'s serum 1 vol. dilution of living cholera culture		1 vol. W. G. L.'s serum 1 vol. dilution of living cholera culture containing 2 600,000 cholera vibrios per c.c.		1 vol. W. B. L.'s serum 1 vol. dilution of living cholera culture containing 21 500,000 cholera vibrios per c.c.	
	1 vol. sterile broth	1 vol. sterilized Staphylococcus culture	1 vol. sterile broth	1 vol. sterilized Staphylococcus culture	1 vol. sterile broth	1 vol. sterilized Staphylococcus culture	1 vol. sterile broth	1 vol. sterilized Staphylococcus culture
undiluted	—	—	—	—	growth	growth	growth	growth
2-fold dilution	growth	growth	growth	growth	"	"	"	"
5 "	sterile	sterile	"	"	"	"	"	"
10 "	"	"	sterile	sterile	"	"	sterile	sterile
25 "	"	"	"	"	"	"	"	"
50 "	"	"	"	"	"	"	"	"
100 "	"	"	"	"	"	"	"	"
1000 "	"	"	"	"	sterile	sterile	"	"
10,000 "	"	"	"	"	"	"	"	"
100,000 "	—	—	—	—	"	"	"	"

would be extracted from the serum *in vitro* by the addition of a sterilized culture of *Staphylococcus*.

The substantial correctness of this inference was tested by means of the experiment set forth in Tables IX. and X. It must be noted that in these experiments we employed, not as in the experiments set forth in Tables I., III. and IV., a sterilized broth culture, but a very dense bacterial suspension made from one or more agar cultures.

It will be seen that with the exception of experiments 3, 4 and 5 in Table IX., where the difference is in each case a very small one, the bactericidal effect exerted was in no case less in the case of the serum which had received an addition of sterilized *Staphylococcus* culture than in the case of the serum which had received only an addition of sterile nutrient broth.

On reviewing the results obtained, we cannot fail to be struck with the sharp contrast between those obtained with the *Staphylococcus* and those obtained with the typhoid bacillus and cholera vibrio.

We have seen (a) that the typhoid bacillus and the cholera vibrio are killed off in very large numbers by the normal serum.

(b) That sterilized cultures of these micro-organisms when added to the serum *in vitro* extract from this last a bactericidal element.

(c) That the introduction of sterilized cultures of these bacteria into the human and animal organism, confers upon the animal an increased bactericidal power, with respect to the particular species of micro-organisms inoculated.

On the other hand, we have seen in the case of the *Staphylococcus* :

(a) That this micro-organism is favourably, rather than unfavourably, affected by contact with the normal serum.

(b) That sterilized cultures of this micro-organism added to the serum *in vitro* do not, unless possibly to a very small extent, diminish its bactericidal action upon the typhoid bacillus and the cholera vibrio.

Lastly, it would seem from the experiment in the last column of Table VIII. and from certain other observations which will be discussed elsewhere :

(c) That the introduction of sterilized cultures of the *Staphylococcus* into the human organism does not confer upon the serum any bactericidal power.

In view of the important bearing of facts such as those just disclosed in connection with the theory of immunity and in connection with protective inoculation, we now proceeded to draw within the scope of our enquiry, on the one hand, the *Bacillus pestis*, and on the other hand, the *Micrococcus melitensis*.

TABLE XI.
Exhibiting the results obtained by cultivating mixtures of one volume of a graduated dilution of a culture of the bacillus of plague and one volume of broth or of serum.

Dilutions in which the living plague culture was employed	Capillary testing pipettes were filled with			
	1 vol. dilution of living plague culture (cultivated 4 days at 37° C.) and			1 vol. A. E. W.'s serum
	1 vol. sterile broth	1 vol. F. N. W.'s serum	1 vol. A. E. W.'s serum	
10-fold dilution	growth	growth	growth	growth
25 "	"	"	"	"
50 "	"	"	"	"
100 "	"	"	"	"
1000 "	"	"	"	"
10,000 "	sterile	"	"	"
100,000 "	"	sterile	"	"

A. E. W. had undergone an inoculation with 'half a dose' of Haffkine's plague vaccine 2 years previously.

TABLE XII.

Exhibiting the results obtained by making graduated dilutions of a 2 day old living plague culture, with sterile broth and serum respectively; and incubating one, or in most cases two 10 c.mm. volumes of each dilution after transference to the surface of nutrient agar. This transference was in case of the serum dilution postponed for 24 hours. The capillary testing pipettes were in the interval kept at a temperature of 37° C.

Dilutions of the living plague culture which were employed	Number of colonies which developed on the nutrient agar in case of the			
	Dilution made with sterile broth	Dilution made with F. N. W.'s serum	Dilution made with A. E. W.'s serum	Dilution made with E. A. S.'s serum
1000-fold dilut.	60	innumerable	innumerable	innumerable
{10,000 " (a)	{12	{ " "	{ " "	{ " "
{10,000 " (b)	{14	{ " "	{ " "	{ " "
{100,000 " (a)	{ 0	{60	{40	{ " "
{100,000 " (b)	{ 2	{50	{35	{35
{1,000,000 " (a)	{ 1	{ 1	{ 1	{50
{1,000,000 " (b)	{ 1	{ 4	{ —	{50

With regard to A. E. W. see note to Table XI.

III. *Data with regard to the bactericidal power of the blood as affecting the Bacillus pestis.*

The observations recorded below suggest that, as in the case of the *Staphylococcus*, a favourable rather than an unfavourable influence is exerted upon the plague bacillus by human serum when mixed in equal volumes with a plague culture (see Table XI.).

The effect of the serum was further investigated by comparing the number of living plague colonies obtained from equal volumes of progressive dilutions of a plague culture made (a) with sterile nutrient broth, and (b) with human serum.

The results obtained are set forth in Table XII.

It will be manifest that the results bear testimony to the absence of a bactericidal effect and to a multiplication of the plague bacilli in almost all the serum tubes.

Following out the plan pursued in the case of the other micro-organisms treated of above, we now sought to determine whether any bactericidal element was extracted when a sterilized plague culture was added to a mixture of serum and living typhoid or living cholera culture. The method of investigation was the same as in the *Staphylococcus* experiments (Tables IX. and X.), a very dense bacterial suspension being made from one or more agar cultures. The results obtained are given in Tables XIII. and XIV.

It will be seen that the bactericidal power was practically unaffected by the addition of a sterilized plague culture.

IV. *Data with regard to the bactericidal power of the blood and the Micrococcus melitensis.*

The data obtained in the case of the Malta fever micrococcus hardly seem to require anything in the way of verbal comment. They are subjoined in the form of Tables XV. to XIX. inclusive.

Tables XV. and XVI. show that human serum, when mixed volume for volume with cultures of *Micrococcus melitensis*, is without action upon this micro-organism.

Table XVII. establishes that even the undiluted serum is entirely without bactericidal action, and that a multiplication of the micro-organism may take place in this medium.

Tables XVIII. and XIX. establish that the antibactericidal effect exerted by the addition of a dense suspension of *Micrococcus melitensis* upon human serum is quite insignificant.

TABLE XIII.

Exhibiting the results obtained on adding a sterilized culture of the plague bacillus to a mixture of serum and living culture of typhoid.

Dilutions of the living typhoid culture which were employed	Capillary testing pipettes were filled in with							
	1 vol. F. N. W.'s serum 1 vol. of a living typhoid culture containing 3000,000,000 <i>T.B.</i> per c.c. and		1 vol. A. E. W.'s serum 1 vol. of a living typhoid culture containing 3000,000,000 <i>T.B.</i> per c.c. and		1 vol. F. N. W.'s serum 1 vol. of a living typhoid culture and		1 vol. A. E. W.'s serum 1 vol. of a living typhoid culture containing 1100,000,000 <i>T.B.</i> per c.c. and	
	1 vol. sterile broth	1 vol. steri- lized plague culture	1 vol. sterile broth	1 vol. steri- lized plague culture	1 vol. sterile broth	1 vol. steri- lized plague culture	1 vol. sterile broth	1 vol. steri- lized plague culture
undiluted culture	growth	growth	growth	growth	growth	growth	growth	growth
2-fold dilution	"	"	"	"	"	"	"	"
5 "	"	"	"	"	"	"	"	"
10 "	"	"	"	"	"	"	"	"
25 "	"	"	"	"	"	"	"	"
50 "	"	"	"	"	"	"	"	"
100 "	"	"	"	"	"	"	"	"
1000 "	"	"	"	"	"	"	"	"
10,000 "	sterile	sterile	sterile	sterile	sterile	sterile	sterile	sterile
100,000 "	"	"	"	"	"	"	"	"

* The irregularity was probably due to an accidental overheating of the tube.

TABLE XIV.

Exhibiting the results obtained by the addition of a sterilized plague culture to a mixture of serum and living culture of cholera.

Dilutions in which the living culture of cholera was employed	Capillary testing pipettes were filled in with							
	1 vol. F. N. W.'s serum 1 vol. living cholera culture containing 18,000,000 chol. vibrios per c.c. and		1 vol. A. E. W.'s serum 1 vol. living cholera culture containing 18,000,000 chol. vibrios per c.c. and		1 vol. F. N. W.'s serum 1 vol. living cholera culture containing 44,000,000 chol. vibrios per c.c. and		1 vol. A. E. W.'s serum 1 vol. living cholera culture containing 44,000,000 chol. vibrios per c.c. and	
	1 vol. sterile broth	1 vol. sterilized plague culture	1 vol. sterile broth	1 vol. sterilized plague culture	1 vol. sterile broth	1 vol. sterilized plague culture	1 vol. sterile broth	1 vol. sterilized plague culture
undiluted culture	growth sterile	growth sterile	growth sterile	growth sterile	growth sterile	growth sterile	growth sterile	growth
2-fold dilution	sterile	sterile	sterile	sterile	sterile	sterile	sterile	sterile
5 "	"	"	"	"	"	"	"	"
10 "	"	"	"	"	"	"	"	"
25 "	"	"	"	"	"	"	"	"
50 "	"	"	"	"	"	"	"	"
100 "	"	"	"	"	"	"	"	"
1000 "	"	"	"	"	"	"	"	"
10,000 "	"	"	"	"	"	"	"	"

TABLE XV.

Exhibiting the results obtained on cultivating equal volumes of serum and diluted Micrococcus melitensis culture which had remained in contact at 37° C. for 24 hours.

Dilutions in which the living Malta fever culture was employed	Capillary testing pipettes were filled in with				
	1 vol. of a 4 day old broth culture of <i>Micrococcus melitensis</i>				
	1 vol. sterile broth	1 vol. F. N. W.'s serum	and 1 vol. W. B. L.'s serum	1 vol. A. B.'s serum	1 vol. J. W.'s serum
10-fold dilution	growth	growth	growth	growth	growth
25 "	"	"	"	"	"
50 "	"	"	"	"	"
100 "	"	"	"	"	"
1000 "	"	"	"	"	"
10,000 "	"	"	"	"	"
100,000 "	"	"	"	"	"
1,000,000 "	sterile	sterile	sterile	sterile	sterile

TABLE XVI.
Exhibiting the results obtained by cultivating at 25° C. in capillary tubes equal volumes of serum and diluted gelatine culture of the Micrococcus melitensis.

Dilutions of the gelatine culture which were employed	Capillary tubes were filled in with 1 volume of the culture of <i>Micrococcus melitensis</i> diluted with nutrient gelatine (15% gelatine) and		
	1 vol. sterile broth	1 vol. F. N. W.'s serum	1 vol. A. E. W.'s serum
	no. of colonies which developed in the tubes	no. of colonies which developed in the tubes	no. of colonies which developed in the tubes
10-fold dilution	innumerable	innumerable	innumerable
100 "	"	"	"
1000 "	100 (circ.)	100 (circ.)	100 (circ.)
10,000 "	17	20	14
100,000 "	15	23	20
1,000,000 "	3	6	4

The tubes were filled in and the colonies in the capillary tubes were counted under the microscope by the technique described by one of us in the *Lancet*, Dec. 1st 1900, pp. 1556—1560.

TABLE XVII.

Exhibiting the results obtained by diluting 4 day old culture of the Micrococcus melitensis with sterile broth and human serum respectively, and by cultivating 10 c.mm., or, where specified, 5 c.mm., of each dilution on nutrient agar. The transference to nutrient agar was in the case of the serum dilutions postponed for 24 hrs. During this interval the capillary testing pipettes were kept at 37° C.

Dilutions in which the culture was employed	Number of colonies which developed in the case of the			Dilutions of culture no. 2 made with A. E. W.'s serum
	Dilutions of culture no. 1 made with sterile broth	Dilutions of culture no. 1 made with F. N. W.'s serum	Dilutions of culture no. 2 made with sterile broth	
100-fold dilution				
1000 "	—	innumerable	—	—
{10,000 " "	{innumerable	"	—	{innumerable
{10,000 " "	{—	"	—	{"
{100,000 " "	{innumerable	"	{113	{100 (circ.)
{100,000 " "	{—	"	{68	{—
{1,000,000 " "	{50	"	{15	{50 (5 c.mm.)
{1,000,000 " "	{—	"	{1	{—
10,000,000 "	1	80	—	—

TABLE XVIII.

Exhibiting the results obtained by the addition of a sterilized dense suspension of the Micrococcus melitensis to a mixture of serum and living typhoid culture.

Dilutions in which the living typhoid culture was employed	Capillary testing pipettes were filled in with							
	1 vol. living typhoid culture 1 vol. F.N.W.'s serum and		1 vol. living typhoid culture 1 vol. E.A.S.'s serum and		1 vol. living typhoid culture 1 vol. W.B.L.'s serum and		1 vol. living typhoid culture 1 vol. F.N.W.'s serum and	
	1 vol. sterile broth	1 vol. sterilized M. m. culture	1 vol. sterile broth	1 vol. sterilized M. m. culture	1 vol. sterile broth	1 vol. sterilized M. m. culture	1 vol. sterile broth	1 vol. sterilized M. m. culture
undiluted culture	growth	growth	growth	growth	growth	growth	growth	growth
2-fold dilut.	"	"	"	"	"	"	"	"
5 "	"	"	"	"	"	"	"	"
10 "	sterile	"	"	"	"	"	"	"
25 "	"	"	"	"	"	"	"	"
50 "	"	"	"	"	"	"	"	"
100 "	"	sterile	"	"	"	"	"	"
1000 "	"	"	sterile	"	sterile	"	sterile	sterile
10,000 "	"	"	"	"	"	"	"	"
100,000 "	"	sterile	"	sterile	"	sterile	"	"
	"	"	"	"	"	"	"	"

TABLE XIX.

Exhibiting the results obtained by the addition of a sterilized dense suspension of the Micrococcus meliensis to a mixture of serum and living cholera culture.

		Capillary testing pipettes were filled with							
Dilutions in which living cholera culture was employed		1 vol. living cholera culture 1 vol. E. A. S.'s serum	1 vol. living chol. culture containing 18,000,000 c. vibrios per c.c. 1 vol. F. N. W.'s serum	1 vol. living chol. culture containing 18,000,000 c. vibrios per c.c. 1 vol. A. E. W.'s serum	1 vol. living chol. culture containing 44,000,000 c. vibrios per c.c. 1 vol. F. N. W.'s serum	1 vol. living chol. culture containing 44,000,000 c. vibrios per c.c. 1 vol. A. E. W.'s serum	1 vol. living chol. culture containing 44,000,000 c. vibrios per c.c. 1 vol. A. E. W.'s serum	1 vol. living chol. culture containing 44,000,000 c. vibrios per c.c. 1 vol. A. E. W.'s serum	1 vol. living chol. culture containing 44,000,000 c. vibrios per c.c. 1 vol. A. E. W.'s serum
		and 1 vol. sterile broth	1 vol. sterile broth	1 vol. sterile broth	1 vol. sterile broth	1 vol. sterile broth	1 vol. sterile broth	1 vol. sterile broth	1 vol. sterile broth
undiluted culture		growth	growth	growth	growth	growth	growth	growth	growth
2-fold dilut.		"	sterile	sterile	sterile	sterile	sterile	sterile	sterile
5		"	"	"	"	"	"	"	"
10		"	"	"	"	"	"	"	"
25		"	"	sterile	"	"	"	sterile	sterile
50		"	"	"	"	"	"	"	"
100		"	"	"	"	"	"	"	"
1000		"	"	"	"	"	"	"	"
10,000		"	"	"	"	"	"	"	"
100,000		"	"	"	"	"	"	"	"

CONCLUSIONS.

On reviewing the experimental data which we have set forth, it would seem clear that—

(1) Human serum has a powerful bactericidal effect upon the typhoid bacillus, and the cholera vibrio, while it is without bactericidal action upon the *Staphylococcus pyogenes*, *B. pestis*, *Micrococcus melitensis* (and so far as we have gone, upon the *Streptococcus pyogenes*, and *B. diphtheriae*).

(2) Sterilized cultures of those species of pathogenic micro-organisms which are killed by the serum, appear, in contradistinction to those species of micro-organisms which are not affected by the serum, to possess the power of directly abstracting a bactericidal element from the blood.

The first of these generalizations appears to possess a far-reaching significance in connection with the general theory of immunity.

(a) It has an obvious bearing on the question of the mechanism by which bacteria are destroyed in the organism.

(b) It also bears on the question as to whether the bactericidal action is acquired only after withdrawal from the organism, and after the disintegration of leucocytes.

For it would seem difficult to assume that the bactericidal power of the serum is only a particular manifestation of a digestive power or originally resident in the leucocyte, when we have realized that the serum exerts a bactericidal action only on particular species of micro-organisms while the leucocyte exerts a digestive action on bacteria generally.

The second of the generalizations arrived at above would seem to point to the bactericidal effects being the result of definite chemical combinations occurring between the bactericidal substance or substances in the blood and the affected bacteria.

In conclusion, reference may be made to a possible relation between the danger or relative absence of danger associated with the hypodermic inoculation of different species of bacteria, and the effect or absence of effect of the blood upon these micro-organisms. A notable contrast obtains in this respect between the event of inoculations of cholera and typhoid on the one hand, and plague and Malta fever on the other hand.

While inoculation with living cultures of cholera is, as has been

shown in connection with Haffkine's anticholera inoculations, practically unassociated with risk, and while inoculations with small quantities of living typhoid bacilli are—judging from the event of an experimental inoculation undertaken by one of us, and from the immunity from accident which has attended wholesale manipulations with this micro-organism—associated with only slight risk, the results are quite other in the case of even minimal inoculations of plague and Malta fever cultures.

That extreme risk attaches to the inoculation of even minimal quantities of living plague bacilli is attested by the numerous cases of plague which have supervened upon the accidental inoculation of infected material into small superficial scratches.

The risk attaching to even minimal inoculations of the *Micrococcus melitensis* is less well known. Six cases of the disease have occurred in connection with bacteriological work on Malta fever undertaken at Netley, and two further cases have originated at the Royal Naval Hospital, Haslar, and in the Philippines respectively, in connection with bacteriological work.

Of the cases occurring at Netley, one originated from an accidental prick with a needle of a syringe containing a Malta fever culture; a second arose in connection with an experimental inoculation; and a third has recently occurred in connection with the accidental projection of the end of a contaminated capillary sedimentation tube into the eye. The three other cases at Netley arose apart from a recognized inoculation in the case of observers working with living cultures. It would seem difficult to conceive of inoculations with quite minimal quantities of cultures being so effectual in the case of micro-organisms subject to the bactericidal action of the blood and lymph.