followed in the case where the inoculation is successful by the maintenance of a *higher base line* of immunity.

2. We have seen that the inoculation of an excessive dose may involve a risk, in particular, the risk of an undue prolongation of the *negative phase*.

3. We have seen that the inoculation of a series of doses of a vaccine will, in the case where the inoculations are uncontrolled by intermediate blood examinations, involve the possibility of the production of a *cumulative negative phase*.

4. We have seen that the *cumulative positive phase* which is a desideratum either in itself or as leading to the maintenance of a high base line of resistance, is achieved only when the successive doses are properly adjusted and when the inoculations are appropriately interspaced.

5. We have seen that the success of a prophylactic inoculation process may be imperilled where sequence of negative and positive phase, and the cumulative effect of successive inoculations, is not taken into consideration.

6. We have seen that the success of serumtherapy in diphtheria and its comparative failure in the case of other diseases is explained by the fact that in the first case we are able to secure the elimination of all negative phase blood, and we are able to induce in the vicariously inoculated animals a cumulative positive phase of absolutely phenomenal dimensions. In the case of other diseases we have been unable to secure these prerequisites of a successful serumtherapy.

7. We have seen that in the case of patients who, though suffering from localized bacterial invasions, are possessed of a considerable balance of resisting power, it is possible without risk to undertake therapeutic inoculations of bacterial vaccines, provided always that the results of these inoculations are controlled by subsequent blood examinations.

Let us realize, in conclusion, that when all has been done that can be done in the way of guarding a patient against the risks attaching to the negative phase the success of a therapeutic inoculation cannot be guaranteed. The success must in each case depend upon the power of response which is possessed by the individual. In the case of a particular patient that power of response may fail us. It may fail us also in connexion with particular bacterial infections. But it may be predicted that success will in some cases be achieved. It seems to me, for instance, that it will certainly be achieved in the case of simple staphylococcus infections occurring in the young and otherwise robust. It seems to me very probable that it will be achieved also in the case of certain coli infections.

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ON EXALTATION OF BACTERIAL VIRULENCE BY PASSAGE IN VITRO.

By E. A. SHAW, M.B.CANTAB.,

THE appearance of Dr. E. W. Ainley Walker's paper on this subject in the BRITISH MEDICAL JOURNAL of October 18th, 1902, leads me to place on record the following series of observations made at Netley during the spring and summer of 1901, and not hitherto published because it was hoped that later more unimpeachable evidence of increased virulence by this method would be obtainable, and that the series might be extended by the inclusion of micrococcus Melitensis. Illness, the exigencies of the service, and other causes have conspired to prevent the realization of these desiderata, which, however, it is hoped may be attained ere long.

The medium selected for the "passages" was fresh human blood serum enumerated from myself; the methods of dilution of cultures, enumeration of cultures, etc., those devised by Wright and described by him in various papers in the Lancet during the last four years.

The fact that quite fresh serum has a bactericidal action on some pathogenic bacteria having been established on a firm basis by the work of many observers, the idea suggested itself that here was a means of increasing the virulence of these whenever that should be desirable, by bringing into play the operation of the principle of "the survival of the fittest." For,

a given volume of fresh serum having the power of killing off all the bacteria in a given volume of a bacterial emulsion of known strength, and failing to kill them all off in the same volume of an emulsion of greater strength, presumably the survivors would be the ones possessed of the greatest powers of resistance, the slain being those possessed of the least; and by allowing the survivors "the fittest" to propagate their species in serum, and so in each generation eliminating as before the weakly and unfit, one ought ultimately to arrive at a generation of increasingly healthy vigorous and resistant bacteria of the particular species subjected to this selective process, that is, a generation of increased virulence.

BACILLUS ANTHRACIS.

With this object in view the following series of experiments were undertaken.

On April 22nd, 1901, 4 sloped agar tubes (these throughout the series were all of the same length and diameter and contained identical quantities of agar sloped at the same angle) were inoculated as "mass" cultures of anthrax from an existing laboratory culture by making an emulsion of this with 1 per cent. peptone broth, allowing this emulsion to flow over the whole agar surface of each of the four sloped agar tubes, which were then inverted to allow as much emulsion to drain off as would, and these four tubes were then incubated at 37° C. for twenty-four hours, at the end of which, in order to get an index of its virulence, the resulting growths were all combined into one emulsion with 10 c.cm. of 1 per cent. peptone broth, which was injected into a guinea-pig of weight 320 grams, and at the same time another sloped agar tube was inoculated and placed in the incubator at 37° C., to be used as the basis of the succeeding experiments.

The guinea-pig just mentioned survived the administration of the four anthrax tubes and was alive some weeks after. Meanwhile the tube inoculated and incubated had its resulting plentiful growth of anthrax made into a thick emulsion with broth on April 25th, from which a series of broth dilutions were made as follows: $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{16}$, $\frac{1}{32}$, $\frac{1}{64}$, $\frac{1}{128}$, $\frac{1}{256}$. A specimen from each dilution was put up in a capillary tube (calibre of about $\frac{1}{16}$ mm.) with an equal volume of freshly-drawn serum obtained from myself, the two fluids intimately mixed; the tube then sealed off in a Bunsen flame, indexed, and the whole series incubated at 37° C. for twenty-four hours. Next day the contents of these capillary tubes were expelled on divisions of an agar surface contained in a Petri dish, which was in its turn incubated at 37° C. for twenty-four hours, with the result that in each area growth had taken place.

Second Generation.—Taking the growth from the weakest dilution $\frac{1}{256}$ —as there being fewer bacilli in this they would have been most acted on by the blood serum—it was emulsified in broth, the same dilutions made and the series repeated exactly as before, with the result that again there was growth from each dilution.

Third Generation.—Taking, as before, the growth from the weakest dilution $\frac{1}{256}$, a different series of dilutions was made, this time $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{16}$, $\frac{1}{32}$, $\frac{1}{64}$, $\frac{1}{128}$, the intention being to produce a dilution in which all the bacilli should be killed off by the bactericidal action of the serum, and then to make use of the growth from the next less weakest dilution for the production of the succeeding generation. This time it was found that though again there was growth from each dilution, that from the $\frac{1}{256}$ dilution was much more limited than from the others.

Fourth Generation.—Repeated as for third generation, using growth from $\frac{1}{256}$ dilution. This time it was found there was no growth from the $\frac{1}{256}$ and $\frac{1}{128}$ dilutions, but growth in all the others.

Fifth Generation.—Repeated as before, using the growth from $\frac{1}{64}$ dilution of fourth generation, and each dilution from $\frac{1}{2}$ to $\frac{1}{256}$ produced a growth. In order to test if any progress was being made, the growth from the $\frac{1}{256}$ dilution was utilized to prepare a "mass" cultivation in one sloped agar tube, which, after twenty-four hours at 37° C., was suspended in 5 c.cm. of broth, and on May 10th injected into a healthy guinea-pig weighing 250 grams. This animal's temperature on the 11th had risen to 39.8° C., on the 12th 40° C., and on the 13th it was found dead in its cage. A necropsy was made with the customary precautions, and B. anthracis was recovered from the heart's blood and spleen pulp whence, as the first guinea-pig had survived the administration of four exactly similar tubes of the original culture, it was deduced that some progress had been made in the direction of increasing virulence.

The growth from the $\frac{1}{256}$ dilution of the same generation was used for continuing the series on the lines laid down until the fourteenth generation had been reached. Concurrently with these generations passed through fresh blood serum a series of generations of anthrax commencing from the same original growth, but on agar alone, had been kept going; so that on May 30th one had for purposes of comparing (a) the fourteenth generation of a series which had been subjected to the bactericidal action of fresh blood stream, and was, on the hypothesis advanced, presumably increased in virulence; and (b) the fourteenth generation of a series which had grown on agar alone, and was presumably mild relative to the other; both (a) and (b) having originated from the same original growth; and all that remained to be done was to test them comparatively.

In the case of anthrax, owing to the adhesion of the bacilli to each other, no such enumeration of individuals in an emulsion as can be undertaken in the case of typhoid bacilli is possible, and the best way of obtaining equivalent doses for administration seemed to be that of preparing twenty-four hours' mass cultivation in exactly similar sloped agar

tubes in the manner already described, and to regard the product of each sloped agar tube thus obtained as one dose. By means of a sterilized platinum needle and sterile broth poured on the surface of agar in each such sloped tube it was easy enough to get the whole of its growth of anthrax in suspension in the broth, which could then be injected into the animal selected.

On May 31st four such tubes of the presumably mild, and two tubes of the presumably virulent, were so prepared and placed in the incubators. On June 1st a series of normal healthy guinea-pigs of selected weights, all approximating as closely as possible to 300 grams, were injected subcutaneously with the customary precautions: one with four tubes of the "mild" anthrax; the others with, of the "virulent" anthrax, respectively $\frac{1}{2}$, $\frac{1}{3}$, $\frac{1}{4}$, $\frac{1}{5}$, $\frac{1}{6}$, and $\frac{1}{7}$ of tube. On June 5th all these guinea-pigs, including the one injected with four tubes mild, were alive, and apparently not one of them any the worse for its dose of anthrax, which in view of the results anticipated was somewhat disconcerting. So with a view to confirm or otherwise the result produced in the guinea-pig of May 11th, another guinea-pig (310 grams in weight), was on June 5th injected with one whole tube of the presumably virulent anthrax. On June 9th this last guinea-pig was found dead in its cage, and *post mortem* anthrax bacilli were recovered from its heart's blood and spleen pulp. On June 10th all the guinea-pigs of June 1st were still alive and well, as was the case on June 25th.

The minimum lethal dose of anthrax grown for fourteen generations on agar alone was more than four tubes; the minimum lethal dose of anthrax passed through fresh blood serum for fourteen generations was not more than one tube, an increase of virulence fourfold at least, a result attained in the fifth generation and not improved on in the succeeding nine generations.

CHOLERA.

On May 20th a precisely similar procedure was initiated with the spirillum of cholera.

Fresh blood serum obtained from Professor A. E. Wright was used for each generation in preference to that obtainable from others, his blood having been incidentally discovered to have a much greater bactericidal power on cholera spirilla than my own, and hence better results might be expected. In this series capillary tubes with mixing chambers of about $\frac{1}{2}$ c.cm. in volume were used for each passage, and after the intimately mixed equal volumes of each cholera dilution in broth and fresh serum in the capillary part of the tube had been in the incubator at 37° C. for twenty-four hours, the sealed end of the capillary portion of each tube was broken off under the surface of sterile broth, which was then drawn in until the mixing chamber was three-quarters filled, the capillary end then sealed up again in the flame, and the series then returned to the incubator at 37° C. for another twenty-four hours, at the end of which it was easy to ascertain at once in which mixing chamber there had been growth or the contrary.

A growth of cholera was taken, a sample of which had been found to be killed off in a $\frac{1}{2}$ broth dilution by an equal bulk of A. E. W.'s serum, dilutions made in series $\frac{1}{10}$, $\frac{1}{100}$, $\frac{1}{1000}$, $\frac{1}{10000}$, and $\frac{1}{100000}$ specimens of each of these were intimately mixed with an equal bulk of serum as mentioned, then incubated, and next day (May 21st) broth was drawn up with the mixture into the mixing chamber of each tube, and these sealed off and returned to the incubator, till examined on May 22nd, when no growth was found in any of them, all presumably killed off. Another series of dilutions of the original cholera growth was therefore made as follows— $\frac{1}{2}$, $\frac{1}{3}$, $\frac{1}{4}$, $\frac{1}{5}$, and similarly treated, and on May 24th it was found there was growth in all save the $\frac{1}{5}$, hence the growth in the $\frac{1}{4}$ dilution was used as the basis of the second series of dilutions of $\frac{1}{2}$, $\frac{1}{3}$, $\frac{1}{4}$, and $\frac{1}{5}$. On May 26th there was growth in all these, so on May 27th a different series of dilutions were made from the growth in the $\frac{1}{4}$ dilution of May 26th as follows— $\frac{1}{2}$, $\frac{1}{3}$, $\frac{1}{4}$, $\frac{1}{5}$, $\frac{1}{6}$, and on May 29th it was found that all these had grown; so now the $\frac{1}{5}$ was used as basis of following series— $\frac{1}{2}$, $\frac{1}{3}$, $\frac{1}{4}$, $\frac{1}{5}$, $\frac{1}{6}$, and $\frac{1}{7}$, which were similarly treated, the intention throughout being as before, to use as the progenitor of each succeeding generation the weakest dilution (that is, that containing the fewest micro-organisms), which survived the bactericidal action of an equal volume of fresh blood serum.

On May 31st it was found that there had been growth from each of the last-mentioned dilutions; so now, taking the growth from the $\frac{1}{7}$ dilution as the basis for the next series, the following series of dilutions was prepared— $\frac{1}{10}$, $\frac{1}{100}$, $\frac{1}{1000}$, $\frac{1}{10000}$, and treated as before. All grew save the $\frac{1}{10000}$, so the $\frac{1}{1000}$ was used as the basis of the next similar series of dilutions. On June 6th it was found there had been growth in the $\frac{1}{10}$ and $\frac{1}{100}$ dilutions of last series, but in none of the others; so the growth from the $\frac{1}{10}$ dilution was taken as the basis of the next series, which, proceeding by multiples of 5, was $\frac{1}{2}$, $\frac{1}{3}$, $\frac{1}{4}$, $\frac{1}{5}$, $\frac{1}{6}$, and $\frac{1}{7}$. On the 8th there was growth in the $\frac{1}{2}$, $\frac{1}{3}$, and $\frac{1}{4}$, but not in the others. Concurrently with these generations of serum-passaged cholera the same number of generations of the original growth had been inoculated from broth tube to broth tube. And now from the seventh generation of each, "mass" cultivations on sloped agar tubes were prepared. On June 10th a series of guinea-pigs of similar body-weights were intraperitoneally injected with these; one guinea-pig with one tube of solely agar-grown cholera (presumably mild relatively to the other); and the others with respectively $\frac{1}{2}$, $\frac{1}{3}$, and $\frac{1}{4}$ tubes of the serum-passaged cholera (presumably virulent relatively to the other).

Fourteen days later all of them were alive and well—an inconclusive result; and my ship having to quit Netley at this juncture, I was unable to proceed further with this series.

BACILLUS TYPHOSUS.

On May 20th also, a similar series of generations was initiated with a growth of typhoid which had been originally derived from a patient dead of typhoid, and kept going for months as a fresh young growth by inoculation and incubation from day to day of a fresh 10 c.cm. peptone broth tube.

In this series also, fresh blood serum obtained by bleeding Professor A. E. Wright at time of use thereof was employed; because, having subjected himself to repeated injections of typhoid vaccine, his blood serum had a high bactericidal power, usually killing off all the bacilli in a $\frac{1}{2}$ dilution of a 24-hour broth culture of *B. typhosus* (equal volumes of serum and dilution). Whereas blood serum obtained from myself, who may fairly be regarded as a normal healthy individual, but absolutely free of any suspicion of undue intimacy with *B. typhosus* or its toxins, would only kill off the same growth in a $\frac{1}{10000}$ dilution.

A series of dilutions of the original growth of typhoid was made— $\frac{1}{10}$, $\frac{1}{100}$, $\frac{1}{1000}$, $\frac{1}{10000}$, and $\frac{1}{100000}$, equal bulks of each were intimately mixed with an equal bulk of fresh serum (A. E. W.) in capillary tubes, with large mixing chambers, one for each dilution, and were all placed in incubator at 37° C. for 24 hours; after which each mixture was combined with 25 to 40 times its bulk of sterile broth in each large mixing chamber, the series replaced in incubator at 37° C. for another 24 hours, and then examined for growth.

May 22nd.—It was found there had been growth in the $\frac{1}{10}$, $\frac{1}{100}$, and $\frac{1}{1000}$ dilutions but not in the remainder, so the growth from the $\frac{1}{1000}$ dilution was used as the basis of the next similar series of dilutions.

May 24th.—Growth found in every dilution, series repeated, using growth in $\frac{1}{100000}$ dilution as basis for next series of dilutions.

May 27th.—Again there was growth in every dilution, but it was not nearly so marked in the highest dilution, the $\frac{1}{100000}$, as in the others, and this was used as basis of next similar series of dilutions.

May 29th.—Again growth in all, repeated as before.

May 31st.—Again growth in all, repeated as before.

June 3rd.—Again growth in all, repeated as before.

June 6th.—Again growth in every dilution, so now made dilutions of the growth from the $\frac{1}{100000}$ dilution of June 3rd as follows, $\frac{1}{2}$, $\frac{1}{3}$, $\frac{1}{4}$, $\frac{1}{5}$, $\frac{1}{6}$, $\frac{1}{7}$, $\frac{1}{8}$, $\frac{1}{9}$, $\frac{1}{10}$, $\frac{1}{11}$, $\frac{1}{12}$, $\frac{1}{13}$, $\frac{1}{14}$, $\frac{1}{15}$, $\frac{1}{16}$, $\frac{1}{17}$, $\frac{1}{18}$, $\frac{1}{19}$, and $\frac{1}{20}$.

June 8th.—Again growth in every dilution. This was the eighth generation and should have been presumably increased in virulence. To test this the growth from the $\frac{1}{1000000}$ dilution was used to inoculate a 10 c.cm. broth tube which, after growing twenty-four hours in the incubator at 37° C., was used to prepare "mass" cultivations in sloped agar tubes. The original growth having been kept going through the same number of generations in broth was also similarly prepared.

A series of guinea-pigs were now injected subcutaneously.

Date of Injection.	Weight of Guinea-pig.	Dose.	Result.
June 10th ...	500 grams	1 tube, mild	Alive and well June 24th.
" " ...	470 "	1 " virulent	Dead on June 28th.
" " ...	320 "	$\frac{1}{2}$ " "	Dead on June 11th.
" " ...	500 "	$\frac{1}{4}$ " "	Temperature rose, but was living on June 24th.

B. typhosus recovered *post mortem*.

As this series of guinea-pigs, though affording some information as to the "virulent" typhoid, was inconclusive as regarded the "mild," four sloped agar tube "mass" cultivations of "mild" and two of virulent were prepared on the 13th, and another series of guinea-pigs injected on June 14th.

Date of Injection.	Weight of Guinea-pig.	Dose.	Result.
June 14th ...	310 grams	2 tubes, mild	Alive on June 24th.
" " ...	310 "	1 tube, virulent	Dead on June 16th.
" " ...	330 "	$\frac{1}{2}$ " "	Dead on June 16th.
" " ...	320 "	$\frac{1}{4}$ " "	Alive on June 24th.

B. typhosus recovered *post mortem*.

This series, it will be observed, was confirmatory as regards the "virulent" of that of June 10th, in that half a tube of "virulent" was sufficient to quickly kill (two days) a 320-gram guinea-pig, but yielded no information as to the lethal dose of the "mild" typhoid. In giving larger doses of the latter suspended in broth, it was thought well to have some information as to any possible hurtful effects of peptone broth on guinea-pigs, so 20 c.cm. of this was injected into a healthy guinea-pig weighing 300 grams, which was unaffected and quite well one week later. As it was not intended to give nearly this bulk of typhoid emulsion, it was thereupon considered that this possibility might be safely ignored. And now what remained to be done was to ascertain the minimum lethal dose of the "mild" typhoid for guinea-pigs.

On June 26th a normal guinea-pig of 260 grams weight was injected with the total broth emulsion obtained from six sloped agar tubes of "mild" typhoid and was alive and quite well on the 29th.

On June 28th another normal guinea-pig of 255 grams weight was injected with 11 tubes of the "mild" typhoid emulsified in 6 c.cm. of broth. This animal died the same night; and *post mortem* in this as in all the other cases of death from these injections the pathogenic micro-organism was recovered by cultures from the heart's blood and spleen pulp.

It was consequently obvious that the minimum lethal dose of this mild typhoid for 300 grams of guinea-pig was therefore somewhere between six and eleven tubes; and hence estimating by tubes, as only half a tube of "virulent" had the same effect, that the method adopted had resulted, on this basis, in at least a 12-fold increase of virulence.

As enumerations of the typhoid bacilli in a given volume of a broth culture, according to the method described by Wright in the *Lancet* of June 1st, 1901, were being daily carried out in the Netley laboratory, the opportunity was taken of thus enumerating the broth emulsions of typhoid bacilli administered to the guinea-pigs mentioned. Omitting the mass of figures involved in the calculation, which have been carefully verified, the results may be summed up as follows:

June 14th.—182,000 millions of "virulent" typhoid bacilli—the half of the product of one sloped agar tube—killed 330 grams of guinea-pig in forty-eight hours.

June 14th.—280,000 millions of "mild" typhoid bacilli—the product of two tubes—failed to kill 310 grams of guinea-pig.

June 26th.—330,000 millions of "mild"—the product of six tubes—failed to kill 260 grams of guinea-pig.

June 28th.—552,000 millions of "mild"—the product of eleven tubes—killed 255 grams of guinea-pig in fifteen hours.

This enumeration method only takes count of living bacilli.

These figures imply also a distinct difference in virulence—about 1:2—but not to so great an extent as is given by taking the number of tubes as the basis of comparison.

On inspecting the figures of the dilutions which grew from generation to generation, it may be noticed that in all these cases, and markedly in the case of the typhoid and cholera growths, as each series progressed, a greater and greater dilution was required to obtain the result of "no growth" after contact for twenty-four hours with an equal bulk of fresh blood serum in the incubator. In the case of cholera, the series commenced with a growth which had to be diluted only to the extent of 1 in 5 to ensure this result, and ended with one which had to be diluted 1 in 1,250 to obtain it. In the case of typhoid, the series commenced with a growth which required to be diluted 1 in 250, and ended in one which required a greater dilution that 1 in 10,000,000 for the same object to be attained; a result which may, I think, be taken to imply that from generation to generation there was a gradually-increasing power of resistance of these micro-organisms to the bactericidal action of the blood serum used.

Again, looking at the enumeration figures, it will be seen that (June 14th) a sloped agar tube grew on its surface 364,000 millions of "virulent" typhoid, while of the mild 140,000 millions was the most ever found to have grown on the same sized areas of agar contained in other tubes. This may be taken to imply more vigorous growth, a greater prolific power, on the part of the "virulent."

Thus, while the exactitude of mathematical demonstration has been impossible—and in some of the comparisons made between the differing generations of the bacteria dealt with—a similarity of strength numerically has been assumed though impossible to prove; still, the general trend of the results obtained goes to show, I consider, that an increase of virulence is obtainable by this procedure.

In conclusion, I beg to tender my warmest thanks to Major Leishman, R.A.M.C., Captain Windsor, I.M.S., and especially to Professor A. E. Wright (who personally made the inoculations on guinea-pigs described) for many valuable suggestions and frequent material assistance during the course of these observations.

FURTHER OBSERVATIONS ON THE REACTION OF *BACILLUS PESTIS* IN PLAGUE.

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In former papers (^{1, 2}) I described the nature of the growth of *B. pestis* in the serums of various animals, and particularly pointed out the bacteriolytic property (to *B. pestis*) of the serums coming from the plague convalescents and plague patients at a certain stage of the disease. Since these observations were recorded a series of experiments have been

made to study in detail the properties of such serums, and I now propose to set forth the results of this inquiry, as they appear to be of some theoretical interest, and also as they seem to throw a certain amount of light on the causes which probably underlie the failure to a suitable reaction on the part of the plague patient when subjected to serumtherapeutics.

EFFECTS OF AGE AND TEMPERATURE ON PLAGUE CONVALESCENTS' SERUM.

These observations were made with the object of ascertaining if an old serum coming from a plague convalescent would behave in any way different from that in a fresh condition; in other words, to determine if the bacteriolytic property of a fresh plague convalescent's serum (to *B. pestis*) would alter by age. As these observations formed the starting point of the present research I give the details of the experiment. Thus an old blood serum of a plague convalescent exposed to the laboratory temperature and light in a sealed tube, and which in a fresh condition was found to be bacteriolytic to *B. pestis*, was infected with plague bacilli, and made into a hanging-drop culture according to the method described elsewhere,¹ and on examination after twenty-four hours was found to have lost all the old property. In fact, it behaved like an old serum from a control.

Subsequent experiments on various samples of plague convalescent's serum have confirmed these observations, and I come to the conclusion that age and exposure to light deprive the serum of "something" whose presence gave the serum in a fresh condition the bacteriolytic characters. Further, it has been experimentally determined by me that this loss of potency begins to manifest itself from the third day onwards, and by the end of a week the serum by itself behaves as any ordinary nutrient serum.

The same remarkable loss of the character of a plague convalescent's serum is brought about by a short exposure to 56° C., as the following experiment will show:

Fresh plague convalescent's serum + infecting plague drop.	Plague convalescent's serum heated to 56° C.+infecting plague drop.
	<i>24 Hours' Growth.</i>

No growth in hanging drop	Rich growth in hanging drop.
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In another communication,² while describing the probable factors concerned in the bacteriolysis of *B. pestis* in certain serums from plague patients, I had thrown out a hint (partly from the study of a variety of blood serums in healthy as well as plague-infected men and animals, and partly from a review³ of analogous phenomena in parallel processes—for example, haemolysis, cytotoxicity, and bacteriolysis of other bacilli—for example, *Bacillus typhosus*, and cholera in their respective serums), that probably a combined action of at least two distinct bodies was necessary to produce the lysogenic action of *B. pestis* used to infect such serums, one of these bodies being of the nature of "amboceptor" (not altered by heat up to 56° or 60° C.) and the other of the nature of "complement"; and seeing that the latter body is destroyed by heat, it appeared to me the loss of the bactericidal property of plague convalescent's serum when subjected to heat or age and exposure to light was caused by the destruction of the complement present in the fresh blood serum of the plague convalescent, and the following experiments were undertaken to see if the plague serum could be further utilized to demonstrate some other broad facts which have been recently brought to light in the study of parallel phenomena of cytotoxicity, haemolysis, etc.⁴ I summarize the result of my observations in the following table. It may be noted that the observations were made twenty-four hours after infecting the blood serum with a plague bacillary saline emulsion, which is designated throughout this paper by "Pl."

1. Fresh plague convalescent's blood serum + Pl. = no growth.
2. Two weeks' old Pl. convalescent's blood serum + Pl. = good growth of *B. pestis*.
3. Pl. convalescent's blood serum heated to 56° C. for 5 min. + Pl. = good growth.
4. (Fresh) rabbit's serum + Pl. = rich growth.
5. Rabbit's serum heated to 56° C. for 5 min. + Pl. = very rich growth.
6. Heated Pl. convalescent's blood serum + fresh rabbit's blood serum + Pl. = no growth.
7. Heated Pl. convalescent's blood serum + heated rabbit's blood serum + Pl. = good growth.

Thus it will be seen that the heated Pl. convalescent's serum and (fresh) rabbit's serum—each in itself a good culture medium for the plague bacilli—when infected in mixtures yield results as good as fresh Pl. convalescent's blood serum. It appears that what is lost by heating a plague convalescent's serum is restored by the addition of fresh rabbit's serum.