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.

TABLE I.

No.	Description of Rabbit, etc.	Dates of Protection by Haffkinization or otherwise.	Dates of Infection with Live Plague and in what Doses.	Nature of Precipitins.	Nature of Bacteriolysis in Drop Cultures.
1	Black and white	By a special method, May 7th, 1902; May 12th, 1902; and May 26th, 1902	$\frac{1}{10}$ loopful of Pl., June 14th, 1902; $\frac{1}{2}$ loopful agar culture of B. pestis, July 4th, 1902	Very rich, August 4th, 1902	Great inhibition in the growth of B. pestis used for infecting the drop. Poor growth in clumps (bacteriolysis), July 10th, 1902.
2	White, with blue mark on the eyes	By another method giving attenuated live Pl., May 10th, 1902; special method, July 25th, 1902	$\frac{1}{10}$ loopful, August 28th, 1902; $\frac{1}{2}$ loopful, September 8th, 1902	Very rich, August 4th, 1902	Inhibition of growth in drop culture on September 3rd, 1902; September 12th, 1902; September 23rd, 1902, and those also in small clumps of 4 or 8 (bacteriolysis).
3	White	<i>Nil</i>	June 14th, 1902	—	Died on June 19th, 1902, of plague. Diffuse rich growth (no precipitins, no bacteriolysis).
4	White, with blue collar	Haffkinization, July 7th, 1902	$\frac{1}{10}$ loopful, August 7th, 1902; $\frac{1}{10}$ loopful, August 20th, 1902; $\frac{1}{2}$ loopful, September 1, 1902	Very rich, August 15th, 1902	Good inhibition on September 12th, 1902. Hardly any growth; here and there a chainlet, seen September 21st, 1902 (bacteriolysis).
5	Albino, with red collar	By special method, August 16th, 1902	$\frac{1}{2}$ loopful	<i>Nil</i> ; hardly any; Sept. 8th, 1902	Rich growth on September 8th, 1902; September 23rd, 1902 (hardly any bacteriolysis).
6	Albino, with two red dots on face	Haffkinization, July 7th, 1902; July 18th, 1902	$\frac{1}{10}$ loopful, August 8th, 1902; $\frac{1}{10}$ loopful, September 1st, 1902; $\frac{1}{2}$ loopful, September 8th, 1902	Hardly any, Sept. 5th, 1902	Rich growth in clumps, September 15th, 1902, and September 23rd, 1902 (no bacteriolysis, but precipitin demonstrable in drop culture though not in capillary tube).
7	Fawn, with a blue dot on face	Haffkinization, July 7th, 1902; July 18th, 1902	$\frac{1}{10}$ loopful, August 7th, 1902; $\frac{1}{10}$ loopful, September 1, 1902; $\frac{1}{2}$ loopful, September 8, 1902	No precipitin	Very rich and diffuse growth (no bacteriolysis).

PROPERTIES OF BLOOD SERUM OF IMMUNIZED RABBITS.

This leads me to the study of blood serums of animals immunized against plague, and I have been able to make my observations on a series of rabbits who were immunized by repeated injections of increasing doses of live Pl. bacilli, after receiving a preliminary protection by Haffkinization or otherwise. In this connexion it is of importance to note that the blood serums of such immunized rabbits show one or two points of fundamental difference from those of plague convalescents (human). These are in short (a) a protected rabbit surviving a lethal dose of live Pl. Bacilli may be quite immune to a hyperlethal dose of plague, and yet his blood serum may show no difference from that of a control; (b) when the blood serum is tested after frequent injections of increasing doses of Pl. bacilli it is found to be rich in "precipitins" and comparatively poor in "bacteriolysins," a character quite exceptional in the serums of human plague convalescents where the bacteriolysin or bactericidin is the most prominent and constant body, and where "precipitin" plays an exceptional and altogether a subordinate rôle; these and other facts have been set forth by me in the *Journal of the Bombay Medical and Physical Society*, August, 1902.

The behaviour of the immunized rabbits' blood serum towards the plague bacilli infecting it is seen in Table I.

From this table it is evident that the rabbits protected by Haffkinization or otherwise, though capable of standing lethal doses of live plague bacilli, may not show in their blood any evidence of their immunity to plague. However, by repeated dosage of increasing quantities of live plague bacilli each time, they may show in time (1) the presence of powerful "precipitins," (2) the presence of bacteriolysins, though these are in comparatively small quantities. It now remains to see how such blood serums behave towards plague bacilli when studied in mixtures—that is, how they compare with plague convalescents' serums studied under similar conditions, to which a reference is made on page 1076. I tabulate the result in Table II.

From that table I learn that in Rabbits 1, 2, and 4 a small amount of amboceptor is demonstrable, though the amount of it is not sufficient to make a suitable saturated combination with all the "complement" of the fresh drop of the control rabbit's serum, while the quantity in the others is scarcely appreciable *in vitro*. It is of interest to observe that the quantity is in the order of the number of injections each rabbit has undergone, and therefore it is quite possible to work up the system of the rabbit to produce a very potent serum by repeated injections of increasing doses of B. pestis.

This leads me to give the reactions of such a serum (very rich in amboceptors), and I have availed myself for this purpose of Roux's serum from the Pasteur Institute. This serum when first studied by me by the drop culture method gave indifferent results, some samples giving a good growth of B. pestis in long chains of fine bacilli, while others at best gave an inhibition of the B. pestis growth with swollen pear-shaped involution forms.² Further, Roux's serum, when studied for the presence of precipitin, was found to be exceedingly rich in this property.

Considering Roux's serum is so rich in precipitin and also certainly inhibitory, though not quite bacteriolytic to the growth of B. pestis, the question suggested itself why such a

TABLE II.

Date and Observation.	Number of Rabbits whose Blood Serum is tested.	Heated or Unheated.	Nature of Admixture before infecting with Plague.	Result and Remarks.
1902. Sept. 30 (a)	Control (new rabbit)	Not heated	<i>Nil</i> + Pl.	Profuse growth and diffuse (no bacteriolysis).
Sept. 30 (b)	Rabbit (2)	Heated	"	Very scanty growth in small clumps in one or two places of the field (bacteriolysin and precipitin).
Sept. 30 (c)	"	Not heated	"	
Sept. 30 (d)	"	Heated	"	Profuse growth (complement destroyed).
Sept. 30 (e)	"	"	+ Control's fresh serum + Pl.	Growth more than in (c) but much less than in (d) (complement restored).
Sept. 30 (f)	Rabbit (6)	Not heated	<i>Nil</i> + Pl.	Very rich in growth. Profuse.
Sept. 30 (g)	"	Heated	"	"
Sept. 30 (h)	"	"	+ Control's fresh serum + Pl.	"
Oct. 2 (i)	Rabbit (4)	Not heated	<i>Nil</i> + Pl.	Fair growth.
Oct. 2 (k)	"	Heated	"	Rich and profuse growth.
Oct. 2 (l)	"	"	+ Control's fresh serum + Pl.	More than in (i) but less than in (k)
Oct. 2 (m)	Rabbit (7)	Not heated	<i>Nil</i> + Pl.	Very rich in control.
Oct. 2 (n)	"	Heated	"	"
Oct. 2 (o)	"	"	+ Control's fresh serum + Pl.	"
Oct. 2 (p)	Rabbit (1)	Not heated	<i>Nil</i> + Pl.	Very sparing growth.
Oct. 2 (q)	"	Heated	"	Profuse growth.
Oct. 2 (r)	"	"	+ Control's fresh serum + Pl.	Less than in (q) but more than in (p).

Note.

Pl. = saline emulsion of plague culture on agar (one loopful to 30 c.c.m. of normal saline) used as the infecting material in the "drop cultures."

serum was not potent enough for practical purpose. From the observations on the old Pl. convalescent's serum referred to, and also from the fact that fresh rabbit's serum, first distinctly bacteriolytic, lost its property towards B. pestis by exposure to heat, the idea naturally suggested that the potency of Roux's serum might have possibly lowered owing to age and exposure to the high temperature of the Red Sea, through which the serum had to pass before reaching Bombay. To put this idea to the experimental test the following experiments were undertaken, and here are the results:

Roux's serum taken direct from sample bottle + Pl. = growth of Pl. in isolated chains.

Fresh rabbit's serum + Pl. = diffuse rich growth of B. pestis.

Heated rabbit's serum + Pl. = very rich growth.

Heated Roux's serum + Pl. = very rich growth in long chains.

Heated Roux's serum + Pl. + fresh rabbit's serum = no growth.

From the above, the conclusion is inevitable that certain bodies from Roux's serum alter by age or exposure to heat,

and their place may be efficiently taken by admixture of fresh serum to make it perfectly bacteriolytic to *B. pestis*. In other words, the complements of Roux's serum are destroyed by heat, while the amboceptor remains unaltered, and for the formation of a potent antibody necessary to produce the bacteriolysis of *B. pestis* an introduction of fresh rabbit's serum rich in complement is essential. Further experiments have been made to show that the complement may be from the fresh blood of man, dog, rat, or monkey, and possibly from any other mammal. That Roux's serum is very rich in amboceptor is shown by the following experiment:

Heated Roux's serum was diluted with an indifferent serum; for example, heated rabbit's serum to the extent of 1 in 240, and a drop of this when mixed with a drop of fresh rabbit's serum has given results quite as good as a fresh plague convalescent's serum when infected with the plague culture.

On a perusal of the foregoing remarks the question naturally would suggest itself why a serum like Roux's serum, rich in precipitin and equally rich in amboceptor, does not yield such favourable result when applied to therapeutic use. One would at first sight expect that the complement or alexin naturally existing in a plague-infected individual would suffice to form a suitable antibody in combination with the amboceptor of Roux's serum, and a successful termination of the case would result. But the experience of all persons who have used this serum in India points no such hopeful issue. Could the complement of the plague patient at a certain stage of the disease be different from the normal complement? It is a well-known fact that after a certain stage of illness (in fatal cases) plague becomes septicaemic—that is, the blood of such patients shows *per se* bacilli, or at all events bacilli become demonstrable by culture. Here is an evidence of either a failure of amboceptor production or alteration of the complement or both, and this fact has been demonstrated by me *in vitro*—namely, that though a patient's blood may show marked production of bacteriolysis in the earlier stages of the disease, the blood from the same patient becomes a very fertile soil to *B. pestis* towards the fatal termination of the case, even though the blood may be free from *B. pestis*. What is the actual observation gained by experiment on the subject?

For this purpose, two series of experiments were undertaken: first, to show the absence of amboceptor in dying cases of plague; and, secondly, the alteration of the complement in the blood of such a case; and I tabulate the results as follows:

A.

Heated Pl. convalescent's blood serum + rabbit's fresh serum + Pl. = no growth.

Heated blood serum of a dying plague case + rabbit's fresh serum + Pl. = rich growth.

B.

Fresh blood serum from a dying case + Pl. = very rich growth.

Heated blood serum from a dying case + Roux's serum + Pl. = good growth.

Roux's serum + fresh rabbit's serum + Pl. = no growth.

Fresh blood serum from dying case + Roux's serum + fresh rabbit's serum + Pl. = rich growth.

The same result was obtained in 12 dying cases I have examined, and from this I am led to believe that the complement in a dying case of plague is not only inefficient, but is altered in composition to the extent of neutralizing the complement effect of the fresh rabbit's serum. And, lastly, by a study of the plague bloods as indicated above, it appears to me that changes in a dying plague case occur in the following order:

1. Appearance of amboceptor.
2. Disappearance of the amboceptor and commencement of septicaemia.
3. Disappearance of complement.
4. Alteration of complement in the form of possibly an anticomplement.

Further proof that the blood serum of such cases does undergo fundamental biochemical changes, and the causes underlying these changes are, I believe, possible to demonstrate *in vitro*; but as the experiments are yet incomplete, I propose to defer this part of the subject for a future occasion.

Before concluding I cannot pass over the most vitally important problem why a suitable complement should not be tried along with a serum rich in amboceptor for therapeutic purposes. It is true one has to bear in mind that an organism in its last stages and under the intoxication of a virulent infection may find it too much to react to a complement coming from another species—for example, the horse—and may break down in the effort to adjust itself on the entry

of such a complement foreign to it. But as one has to face the problem *in extremis*, such an attempt seems to me justifiable, and should an opportunity arise I propose to put it to the clinical use at an early date.

I summarize the above considerations as follows:

1. The lysogenic action of Pl. convalescent's blood to *B. pestis* is brought about by the combined action of two distinct bodies—
 - (a) One of the nature of amboceptor (not destroyed by heat up to 56° C.), and
 - (b) The other of the nature of complement (destroyed at 56° C.).
2. A serum from a plague convalescent loses its lysogenic action on *B. pestis* by age and exposure to light in a few days, and in a few minutes by exposure to 56° C. (probably due to the destruction of complement).
3. This lost property is restored by a mixture of a fresh serum; for example, from the rabbit's (addition of complement).
4. Rabbits when suitably immunized to plague produce a serum rich in precipitins, and containing a fair amount of bacteriolysis (to *B. pestis*).
5. By exposure to heat of 56° C. the bacteriolysis is rendered impotent (owing to destruction of complement); it is, however, to a certain extent restored by addition of fresh rabbit's blood serum (complement).
6. Roux's antipesteux serum is very rich in precipitin, but not particularly bacteriolytic to *B. pestis* as such.
7. This loss of bacteriolytic property is probably due to age, and is certainly produced by exposure to 56° C. (owing probably to loss of complement).
8. By addition of fresh rabbit's, man's, rat's, etc., fresh serum, however, the bacteriolytic property is restored (fresh complement).
9. Blood serum of dying cases of plague is deficient in amboceptor, and its complement is not only poor but of the nature of an anti-complement.

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THE USE OF ANTITOXIN IN THE TREATMENT AND PREVENTION OF DIPHTHERIA.

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THE object of this short communication is to give the results of our experience with diphtheria antitoxin in the isolation wards of the Victoria Hospital for Sick Children of Toronto (a) in the treatment, (b) in the prevention of diphtheria.

1. During 1901 there were an unusually large number of cases of diphtheria in the hospital. In spite of every possible care being taken, every now and then a case would be admitted while in the incubation period, and although suspicious cases were immediately isolated, the disease would spread. We had during 1901, then, about 100 cases of the disease, all proved bacteriologically to be diphtheria. All of them, except a few very mild ones, were treated from the outset with antitoxin, and only 3 died, and 1 of these was complicated with scarlet fever. Unfortunately, no actual note was kept of the number of cases during that year; and although I believe that 100 very nearly represents the total, still the percentage of deaths loses most of its value, and I only give it as a general statement. The number of deaths is accurate.

Since January 1st, 1902, we have kept a book in which all cases have been entered, with details as to age, sex, number of days ill before being admitted to the infectious wards, treatment, etc., and from this book I take the following facts:

Between January 1st and July 7th there were 42 cases of diphtheria admitted to the infectious wards, all of which showed the Klebs-Loeffler bacillus. In nearly all cases the