RÉSUMÉ.

1. The immunisation of mules with a polyvalent virus and an adequate or inadequate serum resulted in an immunity which was not complete. When tested later with the corresponding virus the immunity was broken to the extent of 14 per cent. reactions.

2. The immunity obtained with an inadequate serum and virus was broken by the same virus to the extent of 12 per cent. reactions.

3. The immunity obtained with an adequate serum and virus was broken by the same virus to the extent of 20 per cent. reactions.

4. The immunity obtained by the CD virus was broken by constituents to the extent of 22 per cent. reactions, and no deaths.

5. The immunity obtained by CD virus was broken by non-constituents to the extent of 7 per cent. deaths.

6. The immunity obtained by two injections of CD virus was broken by constituents of this virus to the extent of 82 per cent. reactions.

7. The immunity obtained by two injections of CD virus and one injection of a constituent was broken by constituents to the extent of 62 per cent. reactions.

CONCLUSIONS.

1. Animals immunised with a polyvalent virus and tested with the same virus show reactions when subsequently retested with the identical virus.

2. When the immunity was tested with constituents of this virus, breakdowns occurred to a large extent in the first test, and even in the second test, showing that the virus, considered to be polyvalent, did not contain all the constituents which were originally mixed. The fact remains, however, that none of the tested animals died, thus showing that the immunity resulting from the CD virus protected against any of the constituents.

3. For the purposes of the practice, where such severe tests are hardly encountered, the immunity given by this CD virus should prove sufficient.

E.—THE LOSS OF VIRULENCY OF HORSE-SICKNESS VIRUS IN PRACTICE.

On page 85 of my Annual Report for 1906–07 I quoted an instance where a virus (Tzaneen) which had proved virulent on the station became inert after it was introduced into practice. The virus was recalled and again tested on the station, when the virulency was proved beyond doubt, and since another virus, which had formerly given typical horse-sickness reactions, subsequently failed to do so on the station, a thorough investigation was made.

EXPERIMENT No 1.

With virus which became inert in vitro.

Virus 2199 (horse, origin Tzaneen, twelfth generation) had proved to be so virulent in the experiments with serum that it caused reactions in animals previously immunised against the first generation of the same strain.

"A." *Horse* 2148.—Injected intrajugularly on the 5th October, 1906, with 5 c.c. virus 2199, for the purpose of hyperimmunisation. On the 2nd day the temperature rose to 104 F., reaching 105 F. twenty-four hours later, and the animal died in the course of the 4th day. Blood was collected on the 9th October, 1906, and preserved in the usual way.

(*a*) Injections with virus 2148, dated 9th October, 1906.

"B." *Horse* 2805.—Injected intrajugularly on the 22nd June, 1907, with 5 c.c. blood of horse 2148 (257 days old).

No symptoms until the 14th day, when a small indication of a reaction appeared; the temperature, however, did not rise above 103 F. in the evening; the animal died on the 10th July, 1907—eighteen days after injection—but as it was in poor condition, and lesions of horse-sickness were absent, the cause of death is somewhat doubtful.

"C." *Horse* 2865.—Injected subcutaneously on the 12th July, 1907, with 5 c.c. virus 2148 (277 days old).

No reaction up to the 12th day, when the animal was injected intrajugularly with 5 c.c. virus 2199 (dated 5th October, 1906, or 293 days old, the same virus with which 2148 was originally injected). A reaction followed, and the animal died on the 7th day—31st July, 1907—from horse-sickness.

"D." *Horse* 2883.—Injected subcutaneously on the 20th July, 1907, with 5 c.c. blood 2148 (285 days old).

No reaction.

Tested on the 2nd September, 1907, by injection of 2 c.c. virus 2920 [(Tzaneen, thirteenth generation); horse 2920 had been injected with 2199.]

Reaction commenced on the 4th day after injection, and the animal died of horse-sickness on the 7th day—9th September, 1907.

Result.—The blood of horse 2199 produced horse-sickness in horse 2148 in October, 1906. The blood of 2148 injected in June and July, 1907 (257 to 285 days old) did not produce horse-sickness, and the animals thus injected succumbed to a virus of the same strain. This latter virus (2199, Tzaneen, twelfth generation), which produced horse-sickness in 2148, also did so in one of the animals (2865), and the following generation (2920, Tzaneen, thirteenth generation) in the other one (2883).

Conclusion.—It is evident from the above that virus 2148 collected in October, 1906, had lost its virulency by June, 1907.

Experiment No. 2.

To note whether it is possible to destroy the virulency of a virus by adding inert to virulent virus in varying proportions, and keeping the mixture for different lengths of time at the ordinary room temperature.

(*a*) Injections with a mixture, dated 1st August, 1907, of inert and virulent virus mixed in equal proportions and kept for over four weeks.

Note.—This mixture was prepared on the 1st August, 1907, by mixing equal quantities of avirulent virus 2148 and virulent virus 2199 (Tzaneen strain, thirteenth and twelfth generations respectively). The mixture was kept at the ordinary room temperature.

"A." *Mule* 2991.—Injected subcutaneously on the 2nd September, 1907, with 2 c.c. of the above mixture (thirty-two days old).

A temperature elevation noted on the 2nd and 3rd days, otherwise no indication of a reaction.

On the 18th September, 1907—sixteen days after injection—the animal was tested on its immunity by the subcutaneous injection of 2 c.c. virus 2199. Typical horse-sickness reaction from the 5th to 11th days, the temperature reaching 105 F. on the 8th day, and the animal recovered.

"B." *Mule* 3050.—Injected subcutaneously on the 11th September, 1907, with 8 c.c. of the above mixture (forty-one days old). No reaction.

Tested on the 4th October, 1907 (twenty-three days after first injection) by a subcutaneous injection of 2 c.c. virus 2199.

Reaction commenced six days later, lasting until the 16th day, and the animal recovered.

Retested on the 8th February, 1908, by an intrajugular injection of 10 c.c. mixture of virus mule 3319 and horse 3095 (Potgietersrust strain).

No reaction, thereby proving immune.

(*b*) Injections with a mixture, dated 11th September, 1907, of inert and virulent virus mixed in the proportion of 1 : 9, and kept for over four weeks.

NOTE.—This mixture was made on the 11th September, 1907, by adding 5 c.c. inert virus 2148 to 45 c.c. virus 2199. The mixture was kept at the ordinary room temperature.

"C." *Horse* 3117.—Injected subcutaneously on the 12th October, 1907, with 20 c.c. of virus mixture, dated 11th September, 1907 (thirty-one days old).

No reaction up to the 19th day

Tested on the 31st October, 1907, by subcutaneous injection of 2 c.c. virus 2199.

After an incubation time of five days a reaction started, and the animal died on the 8th day after injection—8th November, 1907—from horse-sickness.

(*c*) Injections with virus mixture, dated 23rd October, 1907, of inert and virulent virus mixed in equal quantities and kept for less than four weeks.

NOTE.—This mixture was made on the 23rd October, 1907, by mixing equal quantities of virus 2199 to the former mixture (dated 1st August, 1907), and was kept at the ordinary temperature.

"D." *Horse* 3091.—Injected subcutaneously on the 30th October, 1907, with 10 c.c. of above mixture (seven days old).

After an incubation time of six days a reaction started, lasting until the 13th day ; dikkop present on the 12th day, and the animal recovered. Horse 3091 was utilised later for hyperimmunisation.

"E." *Horse* 3142.—Injected on the 12th November, 1907, subcutaneously with 3 c.c. of above mixture (twenty days old).

Reaction started six days later, and the animal died on the 9th day from horse-sickness.

(*d*) Injections with mixture, dated 30th October, 1907, of inert and virulent virus in the proportion of 1 : 5 and kept for fourteen days.

NOTE.—This mixture was made on the 30th October, 1907, by adding 5 parts of virulent virus 3051 (Tzaneen, fifteenth generation) to 1 part of the mixture, dated 23rd October, 1907, and keeping it at the ordinary room temperature.

"F." *Horse* 3135.—Injected subcutaneously on the 14th November, 1907, with 2 c.c. of above mixture (fourteen days old).

Reaction commenced after five days' incubation, and the animal died on the 9th day—23rd November, 1907—from horse-sickness.

(*e*) Injections with mixture, dated 27th December, 1907, of inert and virulent virus in the proportion of 1 : 20, and kept for at least nine weeks.

NOTE.—This mixture was made on the 27th December, 1907, by adding to the rest of the bottle which contained the mixture of 23rd October, 1907 [see Experiment 3 (*a*)], virus 2199 in the proportion of 1 : 20, and keeping it at the ordinary room temperature.

"G." *Horse* 3370.—Injected subcutaneously on the 29th February, 1908, with 2 c.c. of above mixture (sixty-three days old).

No reaction.

Tested on the 20th March, 1908—twenty days after the first injection—by subcutaneous injection of 2 c.c. virus 2884 (composite district).

Reaction commenced after an incubation time of four days, and the animal died on the 7th day—27th March, 1908—from horse-sickness.

"H." *Horse* 3409.—Injected intrajugularly on the 10th March, 1908, with 5 c.c. of above mixture (seventy-three days old).

Reaction noticed from about the 13th day, lasting for nine days, which might be interpreted as a retarded horse-sickness reaction.

On the 18th day after injection—28th March, 1908—the animal was tapped, and the blood injected into horse 3427, but with negative results.

Horse 3409 was tested on the 6th April, 1908, by a subcutaneous injection of 2 c.c. virus 2891 (Tzaneen, fourth generation).

Incubation time of three days; reaction for ten days; dikkop present on the 12th day; death occurred on the 13th day—19th April, 1908.

"I." *Horse* 3465.—Injected intrajugularly on the 30th March, 1908, with 2 c.c. of above mixture (ninety-three days old).

An indication of a very slight reaction noted from the 6th to the 12th days. The animal was bled on the 10th day, and 2 c.c. was injected into horse 3410. No reaction in 3410, the animal dying from horse-sickness in May in an experiment with virus Tzaneen (eighth generation).

"J." *Horse* 3488.—Injected intrajugularly on the 11th April, 1908, with 10 c.c. of above mixture (104 days old).

No reaction.

This animal was tested later with virus Tzaneen, fourth generation [see Experiment No. 6 (*a*)].

Results.—A mixture made by adding an inert virus mixture to virulent virus in equal proportions, and keeping it at a temperature of 24 C. for twenty days, produced horse-sickness in the two injected animals. A mixture made by adding 1 part of a mixture of inert virus to 5 parts of virulent virus, and keeping it at a temperature of 24 C. for fourteen days, produced horse-sickness in the injected horse. A mixture made by adding 1 part of a mixture of inert virus to 20 parts of virulent virus, and keeping it at a temperature of 24 C. for nine to fifteen weeks, did not produce horse-sickness in the four injected horses. In two of these injected horses a slight indication of a reaction

was noted, but blood collected at the time of the highest temperature record did not produce horse-sickness when injected into two other horses.

A mixture of inert and virulent virus in equal quantities, and kept for four to six weeks at a room temperature, did not produce horse-sickness in the injected animals.

A mixture made by adding 1 part of virulent virus to 9 parts of avirulent virus, and keeping it for thirty-one days at the ordinary room temperature, did not produce horse-sickness in the injected animal.

EXPERIMENT No. 3.

To note the influence of an inert virus mixture on a virus CD (or composite district, being a mixture of blood collected from all over the Transvaal).

(*a*) Injections with a mixture, dated 12th March, 1908.

NOTE.—This mixture was made on the 12th March, 1908, by adding 400 c.c. virus 2884 (CD) to 100 c.c. of the mixture, dated 27th December, 1907, and keeping it at the ordinary room temperature.

"A." *Horse* 3374.—Injected subcutaneously on the 20th March, 1908, with 2 c.c. of the above mixture (eight days old).

Incubation time of four days, followed by a reaction, and the animal died on the 8th day—28th March, 1908—from horse-sickness.

"B." *Horse* 3382.—Injected subcutaneously on the 30th March, 1908, with 2 c.c. of above mixture (eighteen days old).

No definite reaction; the animal was in very poor condition, frequently fell down, and had to be lifted up daily.

Death occurred on the 14th day, the cause of death being debility, and it is therefore doubtful whether a horse-sickness complication existed.

"C." *Horse* 3483.—Injected intrajugularly on the 11th April, 1908, with 10 c.c. of above mixture (thirty days old).

Reaction followed, and the animal was bled.

Tested on the 7th May, 1908, with 2 c.c. virus mule 2415 (Tzaneen, fourth generation). Contracted horse-sickness and died.

Result.—A mixture made by adding 1 part of an inert virus mixture to 4 parts of virulent virus CD, and kept at the ordinary room temperature for eight days, produced horse-sickness in the injected animal. When this mixture was kept for thirty days, it produced an atypical reaction, but the animal died when tested later.

EXPERIMENT No. 4.

To find the influence of the temperature of an incubator on inert and virulent virus mixtures.

(*a*) Injections with mixture, dated 24th March, 1908.

NOTE.—This mixture was prepared on the 24th March, 1908, by putting into an Ehrlenmeyer flask about 270 c.c. virus 2884, after cultures of this virus had given negative results, and 10 c.c. of the old mixture, dated 27th December, 1907. One flask was kept in an incubator at a temperature of 37 C., and the other was kept at the ordinary room temperature 24 C.

(1) With above mixture kept in the incubator.

"A." *Horse* 3199.—Injected subcutaneously on the 6th April, 1908, with 2 c.c. of the above mixture (thirteen days old).

No reaction.

The animal was used later and died of horse-sickness [see Experiment No. 6 (*b*)].

(2) With mixture kept at room temperature.

" B." *Horse* 3363.—Injected subcutaneously on the 6th April, 1908, with 2 c.c. of above mixture (thirteen days old).

Reaction commenced after an incubation time of four days, and the animal died on the 8th day—14th April, 1908—from horse-sickness.

(*b*) Injections with mixture, dated 15th April, 1908.

NOTE.—This mixture was prepared on the 15th April, 1908, by adding to the original bottle containing the inert virus mixture, dated 23rd October, 1907, virus 2884 CD, after it had been tested on its purity in the proportion of 1 : 25.

(1) Above mixture kept at incubator temperature.

" C." *Horse* 3510.—Injected intrajugularly on the 7th May, 1908, with 10 c.c. of above mixture (twenty-two days old).

An atypical reaction.

This horse went into another experiment on the 30th June, 1908, when it was injected with 2 c.c. virus 3619 (Tzaneen, twentieth generation), and died of horse-sickness.

" D." *Horse* 3302.—Injected subcutaneously on the 7th May, 1908, with 10 c.c. of above mixture (twenty-two days old).

No reaction.

This horse was utilised for another experiment with virus Tzaneen, seventh, twentieth, and twenty-first generations, and recovered.

" F." *Horse* 3489.—Injected intrajugularly on the 21st May, 1908, with 10 c.c. of the above mixture (thirty-six days old).

Incubation time of three days; death from horse-sickness on the 6th day.

Result.—A mixture made by adding 1 part of an inert virus mixture to 27 parts of virulent virus 2884, and kept at a temperature of 37 C. for thirteen days, failed to produce horse-sickness in the injected animal. A similar mixture kept for the same length of time at the ordinary room temperature produced horse-sickness in the injected animal.

A mixture made by adding 1 part of an inert virus mixture to 25 parts of virulent virus 2884, and kept for twenty-two days, failed to produce horse-sickness in the two injected horses. The same mixture kept for thirty-six days produced horse-sickness on the 6th day after injection.

EXPERIMENT NO. 5.

With inert virus mixtures kept at a temperature of 37 C. to ascertain whether the injection of a large quantity of inert virus mixture would produce immunity.

NOTE.—The vira used for the following injections were mixed on the 12th March, 1908 [*vide* Experiment No. 3 (*a*)], and kept in an incubator (1 part inert virus to 4 parts virulent virus).

(*a*) Injections of 20 c.c. of virus mixture.

" A." *Horse* 3488.—Injected intrajugularly on the 20th April, 1908, with 20 c.c. of virus mixture (thirty-nine days old).

No reaction.

Tested on the 7th May, 1908, with virus 2415 (Tzaneen, fourth generation) and died of horse-sickness.

" B." *Horse* 3428.—Had been previously injected on the 20th March, 1908, with virus mule 3368 (Tzaneen, sixth generation), but this virus did not always produce horse-sickness, and 3428 had shown an atypical reaction.

Injected intrajugularly on the 20th April, 1908, with 20 c.c. of above mixture (thirty-nine days old).

No reaction.

Tested on the 7th May, 1908, with virus 2415 (Tzaneen, fourth generation).

Slight reaction from 9th to 17th days.

When hyperimmunised later with Tzaneen virus, nineteenth generation, 3428 died of horse-sickness.

(*b*) Injections of 50 c.c. virus mixture.

" C." *Horse* 3199.—Injected intrajugularly on the 20th April, 1908, with 50 c.c. of above mixture (thirty-nine days old).

No reaction.

Tested on the 7th May with virus 2415 (Tzaneen, fourth generation): contracted horse-sickness after an incubation time of thirteen days; had a short reaction, and dikkop appeared on the 17th day.

When hyperimmunised later with Tzaneen virus (3606, nineteenth generation) 3199 contracted horse-sickness and died.

" D." *Horse* 3493.—Injected intrajugularly on the 20th April, 1908, with 50 c.c. of above mixture (thirty-nine days old).

No reaction.

Tested on the 7th May, 1908, with virus 2415 (Tzaneen, fourth generation).

Reaction with dikkop on the 14th day.

Again tested on the 19th June, 1908, with virus 3440 (Potgietersrust), and showed a slight reaction.

Hyperimmunised on the 11th July, 1908, with virus 3774 (Potgietersrust) and recovered.

Result.—The intrajugular injection of 20 c.c. of an inert virus mixture, thirty-nine days old, failed to produce horse-sickness in the two injected horses, and both died when tested later.

The intrajugular injection of 50 c.c. of an inert virus, thirty-nine days old, failed to produce horse-sickness in the two injected horses. When tested later, both showed reactions and one died.

RÉSUMÉ.

The experiments prove that a virus may become inert in practice; this avirulency is due to some foreign matter, inasmuch as inert virus added to virulent sterile virus promptly produces avirulency. It is probable that this avirulency is due to the presence of some germ, but the experiments have not been carried out to the extent necessary to determine the nature of this micro-organism.

CONCLUSIONS.

1. The avirulency of a virus takes place a certain time after mixing sterile to inert virus.

2. The avirulency takes place more rapidly when the mixture is kept in the incubator than when it is kept at room temperature.

3. The mixture of virulent and inert virus produces different results in injected animals according to the method of inoculation. The same virus

which proves inert after a subcutaneous injection may be virulent for an intrajugular injection.

4. The intrajugular injection of large doses of inert virus does not produce immunity.

5. It is clear that a certain virus may become inert, and therefore this fact influences the preparation and preservation of virus to be used in practice.

F.—ON THE VARIABILITY OF THE VIRULENCY OF A PARTICULAR STRAIN OF HORSE-SICKNESS VIRUS.

Hitherto it has been the experience that a virus of horse-sickness taken at random and injected into susceptible horses or mules, irrespective of quantity or method, has in every instance resulted in reactions, and in the great majority deaths followed. This is borne out by the fact that the simultaneous injections of mules with two or more vira and serum resulted in every instance with immunity against that particular virus, or at least in all instances which were tested with that particular strain (compare Annual Report, 1906–07). After the introduction of the Tzaneen virus into practice, and after the apparent failure of this virus in Natal, it became necessary to elucidate the cause, and a new feature was evident, namely, the variability of the virus. This formed the subject of an extended investigation, the details of which are given hereunder. The tables explain themselves, and I only need refer to the terms " Type " and " Strain."

All the different horse, mule, and donkey vira utilised for the following injections are of the same origin (that is of the same strain, Tzaneen), but owing to the numbers of animals used, I have divided them into six tables with the object of making the experiment more comprehensible.

Virus Tzaneen 1087 is the origin ; virus 1965 is the first generation, and the main injections with this virus appear under type 1965 (Experiment No. 1). From virus of the third generation of this type (Natal virus) a mule, 2415, was injected, and main injections with virus of this sub-origin form a separate type, 2415 (Experiment No. 2). A second branch was formed by this Natal virus, called type 2891 (Experiment No. 3). Type 2415 was again divided, the mule 2539 forming a type (Experiment No. 4). Similarly with type 2694 (Experiment No. 5) and with type 2732 (Experiment No. 6), these also being branches from 2415. The term " strain " is used to distinguish between the different kinds of vira—Ordinary, Tzaneen, Bulawayo, etc.