

“F.”—IMMUNISATION OF MULES WITH INADEQUATE AND ADEQUATE SERUM AND VIRUS, AND THE IMMUNITY OBTAINED THEREFROM.

Under the phrase “Adequate Serum” is understood a serum which has been obtained from a horse injected with a corresponding virus. For instance, Ordinary serum is the adequate serum to Ordinary virus. An inadequate simultaneous inoculation would therefore be an inoculation of, say, Ordinary serum and Tzaneen virus.

The experiments were undertaken for the purpose of noting the extent to which adequate and inadequate inoculations can be made with safety, and to compare the immunity obtained in this way.

(See pages 89 and 90 for explanation of O, T, etc.)

A.—INADEQUATE SERUM AND VIRUS.

EXPERIMENT No. 1.—To note effect of injection of serum (O strain) together with virus Tzaneen (horse 1869, first generation).

(a) Serum 89/90 (2 parts horse serum 89 to 1 part mule serum 90). Dose 300 c.c., injected subcutaneously.

Virus 1869, dose 2 c.c., injected subcutaneously.

1. *Mule 2155.*—

Injected on 2nd August, 1906, with 300 c.c. serum, and immediately after with 2 c.c. virus.

Result.—Reaction 7 days later. Died on 16th August, 1906, from horse-sickness.

2. *Mule 2158.*—

Injected on 2nd August, 1906, with 2 c.c. virus, and immediately after with 300 c.c. serum.

Result.—Reaction 5 days later, lasting for 8 days.

Tested 1st September, 1906, with 5 c.c. virus 1964 Bulawayo. No reaction. *Tested* on 3rd January, 1907, 2 c.c. virus 2407 (horse 2407 Ord.), slight reaction. Hyperimmunised 13th September, 1907, virus mule 2266 Bulawayo. No reaction.

(b) Serum 89/90 (equal parts of horse and mule serum). Dose 400 c.c., injected subcutaneously.

Virus 1869, dose 2 c.c., injected subcutaneously.

3. *Mule 2161.*—

Injected on the 2nd August, 1906, with 400 c.c. serum mixture, and immediately after with 2 c.c. virus.

Result.—Reaction 8 days later, lasting until 18th August, 1906. Dikkop on 15th day.

Tested 1st September with 5 c.c. virus 1964 (Bulawayo). Atypical reaction. On 13th September hyperimmunised with virus mule 2266 (Bulawayo). No reaction. *Tested* on 3rd January, 1907, with virus horse 2407 Ord. Slight reaction.

4. *Mule 2164.*—

Injected on 2nd August, 1906, 2 c.c. virus 1869, and directly after with 400 c.c. serum mixture.

Result.—Incubation 7 days; reaction 9 days. Dikkop 17th day.

Tested 1st September with 5 c.c. virus 1964 (Bulawayo). Irregular reaction. Retested 16th October, 1906, with 20 c.c. virus horse 1938 (Ord.). Slight reaction.

Conclusion.—Ordinary serum (*viz.*, serum of horses and mules hyperimmunised with the strain O of the station) when injected simultaneously and subcutaneously with virus T of a horse give a reaction typical for horse-sickness. This immunity is not complete, as a subsequent inoculation of either virus B or virus O caused slight reactions in some instances.

EXPERIMENT NO. 2.—To note effect of injection of serum O together with virus Bulawayo (horse 2060, first generation).

(a) Serum 89/90 (2 parts of horse serum 89 to 1 part of mule serum 90). Dose 300 c.c., injected subcutaneously.

Virus 2060, dose 2 c.c., injected subcutaneously.

1. *Mule* 2156.—

Injected on 2nd August, 1906, with 300 c.c. serum mixture, and directly after subcutaneously with 2 c.c. virus.

Result.—Incubation 4 days; reaction 9 days; dikkop 17th day.

Tested on 1st September intrajugularly with 20 c.c. virus horse 1869 Tzaneen. No reaction. 13th September hyperimmunised with virus mule 2267 Tzaneen. No reaction. Retested 12th January, 1907, with 2 c.c. virus mule 2287 Ord. Typical and distinct reaction.

2. *Mule* 2159.—

Injected on 2nd August, 1906, 2 c.c. virus horse 2060, and directly after with 300 c.c. serum mixture.

Result.—Incubation 3 days; reaction lasted 8 days.

Tested on 1st September with 20 c.c. virus horse 1869 Tzaneen. Reaction.

(b) Serum 89/90 (horse and mule serum mixed in equal proportions). Dose 400 c.c., injected subcutaneously.

Virus 2060, dose 2 c.c., injected subcutaneously.

3. *Mule* 2162.

Injected on 2nd August, 1906, with serum mixture, and directly after subcutaneously with 2 c.c. virus 2060.

Result.—Incubation 5 days; dikkop 11th day. Died 13th day.

4. *Mule* 2119.—

Injected on 2nd August, 1906, with virus 2060 subcutaneously, and directly after with serum mixture.

Result.—Incubation 4 days; lasted 10 days; dikkop 16th day.

Tested on 1st September by injection of 20 c.c. virus horse 1869 Tzaneen. Irregular slight reaction. On the 13th September hyperimmunised with virus mule 2267 (T). Slight reaction. Retested on 12th January, 1907, with mule 2287 Ord. Typical and distinct reaction.

Conclusion.—Ordinary serum, *viz.*, serum of horses and mules hyperimmunised with O strain of the station, permits, when injected with virus B, a reaction typical for horse-sickness. This immunity is not complete, as a subsequent inoculation of virus O or virus B caused reactions.

The example given of mule 2119 is interesting, inasmuch as it showed reactions to all three vira.

EXPERIMENT No. 3.—To note effect of injection of serum O together with a mixture of Bulawayo and Tzaneen virus.

(a) Serum 89/90 mixed in proportion of 2 parts horse serum 89 to 1 part of mule serum 90. Dose 300 c.c., injected subcutaneously.

Virus mixture 2060 and 1869. Dose 2 c.c., injected subcutaneously.

1. *Mule 2157.*—

Injected on 2nd August, 1906, with serum mixture, and directly after with virus mixture (1869 and 2060).

Result.—Reaction after 5 days; dikkop 12th day. Died 14th day.

2. *Mule 2160.*—

Injected on 2nd August, 1906, with virus mixture, and directly after with serum mixture.

Result.—Incubation time 4 days, lasting 9 days. Dikkop 15th day.

Tested on 1st September by injection of 20 c.c. virus horse 1938 (O). Slight irregular reaction. Retested on 16th October by injection of 20 c.c. virus 1938 O. Reaction.

(b) Serum 89 and 90 mixed in equal parts of horse and mule serum. Dose 400 c.c., injected subcutaneously.

Virus as above.

3. *Mule 2163.*—

Injected on 2nd August, 1906, with serum mixture, and directly after with virus mixture.

Result.—Incubation 4 days; died on 11th day.

4. *Mule 2120.*—

Injected on 2nd August, 1906, with virus mixture, and directly after with serum mixture.

Result.—Incubation 4 days, lasting 7 days.

Tested on 1st September with 20 c.c. virus 1938 O. No reaction.

Conclusion.—Ordinary serum of horses and mules hyperimmunised with O strain of the station has not the same preventive action against a mixture of the virus T and B strains. Of 4 animals, 2 died of horse-sickness. In one animal the immunity obtained did not protect completely against a subsequent inoculation of virus O.

EXPERIMENT No. 4.—To note preventive value of serum mixture 91/92 (serum of horses and mules hyperimmunised with Ord. virus) and virus origin Tzaneen, injected simultaneously.

(a) Injections on 18th August, 1906, subcutaneously with 400 c.c. of serum mixture 91 and 92.

Virus 1869 (Tzaneen); dose 2 c.c., injected subcutaneously.

1. *Mule 2181.*—

Injected as above.

Result.—Reaction.

Tested on 20th September with 5 c.c. virus 2083 B. Slight reaction. Retested on 4th January, 1907, with 2 c.c. virus mule 2287 O. Irregular reaction.

2. *Mule 2182.*—

Injected as above.

Result.—Reaction.

Tested on 20th October with 5 c.c. virus 2083 B. Slight reaction. Retested on 4th January with 2 c.c. virus 2407 O. Slight reaction.

3. *Mule 2183.*—

Injected as above.

Result.—Reaction.

Tested on 20th September with 5 c.c. virus 2083 B. Reaction. Retested on 3rd January with virus mule 2287 O. Irregular reaction.

4. *Mule 2184.*—

Injected as above.

Result.—Reaction and dikkop on 14th day.

Tested on 20th September with 5 c.c. virus 2083 B. No reaction. Retested on 4th January, 1907, with 2 c.c. virus horse 2407 O. Typical and distinct reaction.

Conclusion.—The inoculation of 400 c.c. serum O of horses and mules simultaneously and subcutaneously with virus T of horse permits a reaction typical for horse-sickness. The immunity established by it does not prevent a subsequent reaction due to the injection of virus Bul. and Ord. strains; some animals showing reactions to each separate injection.

(b) Serum as above.

Virus 1965 Tz. (mule); dose 2 c.c.

Serum and virus injected subcutaneously and simultaneously.

5. *Mule 2185.*—

Injected on 18th August, 1906, with 400 c.c. mixture of serum 91/92, and subcutaneously with 2 c.c. virus 1965.

Result.—Reaction.

Tested on 20th September by an injection of 5 c.c. virus horse 2083 Bulawayo. Reaction, dikkop and death.

6. *Mule 2186.*—

Injected as above.

Result.—Reaction.

Tested on 20th September with 5 c.c. virus horse 2083 B. Reaction. Retested on 4th January with virus horse 2407 O. Distinct and typical reaction.

7. *Mule 2187.*—

Injected as above.

Result.—Reaction, with dikkop on the 14th day.

Tested on 20th September with 5 c.c. virus horse 2083 B. Doubtful reaction. On 4th January, 1907, retested with 2 c.c. virus mule 2287 O. Reaction.

8. *Mule 2188.*

Injected as above.

Result.—Reaction.

Tested on 20th September with 5 c.c. virus 2083 B. Slight reaction. Retested on 3rd January with 2 c.c. virus 2287 O. Slight reaction.

Conclusions.—The inoculation of mules with serum O and virus Tzaneen permits of a reaction typical for horse-sickness. The immunity obtained does not prevent a subsequent reaction due to the virus of Bul. and Ord. strains, all animals showing reactions, due to each injection of virus.

EXPERIMENT NO. 5.—To note value of various sera in connection with virus origin Tzaneen on mules, when injected simultaneously.

(a) Serum mixture 91/92 of horses and mules hyper-immunised with Ord. virus. Dose 400 c.c., injected subcutaneously.

Virus 1965 Tz. Dose 2 c.c., injected subcutaneously.

1. *Argentine Mule 2211.*—

Injected on 30th August, 1906, with 400 c.c. serum mixture 91/92, and simultaneously and subcutaneously with 2 c.c. virus mule 1965 (origin Tzaneen).

Result.—Slight reaction.

Tested with 2 c.c. virus Tzaneen 1965, injected intrajugularly on 19th September, 1906. Slight reaction in typical time for horse-sickness. Retested on 20th November with 5 c.c. virus horse 1938 O. Reaction. Retested on 8th December with 5 c.c. virus horse 2199 T. No reaction.

2. *Argentine Mule 2213.*—

Injected as above.

Result.—Reaction after incubation time of 4 days, lasting 10 days; dikkop 14th day. Not tested.

3. *Argentine Mule 2214.*—

Injected as above.

Result.—Reaction after incubation time of 6 days, lasting 8 days.

Tested on 26th October with 5 c.c. virus horse 1938 O. Reaction and dikkop on 10th day. Exposed at Onderstepoort farm, near Pretoria, and contracted horse-sickness spontaneously, accompanied with dikkop, and recovered.

4. *Argentine Mule 2215.*—

Injected as above.

Result.—Reaction after 4 days' incubation, lasting 12 days.

Tested on 26th October with 5 c.c. virus 1938 O. Severe reaction.

Conclusion.—The inoculation of mules with serum O and virus Tzaneen permits of a reaction typical for horse-sickness. The immunity obtained does not prevent a subsequent reaction when injected with virus O.

The instance of mule 2211 is interesting, inasmuch as a subsequent injection of virus 1965 caused a reaction. Mule 2214 had shown dikkop three times within seven months, the last one naturally contracted.

(b) Serum mixture 93 and 94, of horses and mules, hyper-immunised with Ordinary virus. Dose 400 c.c., injected subcutaneously.

Virus 1965 Tz., dose 2 c.c., injected subcutaneously.

1. *Argentine Mule 2216.*—
Injected on 30th August, 1906, with 400 c.c. serum mixture 93/94, simultaneously and subcutaneously with 2 c.c. virus mule 1965.
Result.—A typical reaction.
Tested on 19th September, 1906, with 2 c.c. virus 1965 injected into jugular vein. Slight but typical reaction. Retested on 20th November with 5 c.c. virus 1938 O. Reaction. Retested on 8th December with 5 c.c. virus horse 2199 T. No reaction.
2. *Argentine Mule 2217.*—
Injected as above.
Result.—Incubation time of 4 days; reaction 9 days.
Tested on 26th October with 5 c.c. virus 1938 O. Pronounced reaction.
3. *Argentine Mule 2218.*—
Injected as above.
Result.—Incubation time of 6 days; reaction lasting 11 days.
Tested on 26th October with 5 c.c. virus 1938 O. Reaction and dikkop on 10th day.
4. *Argentine Mule 2219.*—
Injected as above.
Result.—Incubation time of 5 days; reaction lasted 10 days.
Tested on 26th October with 5 c.c. virus 1938 O. Reaction.
Conclusions.—The inoculation of serum () and virus Tzaneen permits of a reaction for horse-sickness. The immunity obtained does not prevent a subsequent reaction due to the injection of virus O.

EXPERIMENT No. 6.—To note effect of Ordinary serum of various dates when injected 24 hours previous to virus Tzaneen or virus Bulawayo.

- (a) Serum 23/24, dated 11/7/05. Dose 400 c.c.
1. *Argentine Mule 2220.*—
Injected on 30th August, 1906, with 400 c.c. serum 23/24 and 24 hours later with 2 c.c. virus mule 1964 (origin Bulawayo).
Result.—Reaction after 5 days' incubation. Dikkop on 11th day. Died on 13th day.
 2. *Argentine Mule 2221.*—
Injected with 400 c.c. serum 23/24, and 24 hours later with 2 c.c. virus mule 1965 (origin Tzaneen).
Result.—Incubation time of 3 days. Reaction lasted 9 days.
Tested on 26th October with 5 c.c. virus horse 1938 O. Reaction; dikkop on 8th day, and died from horse-sickness on 10th day.
- (b) Serum 75/76 (dated 8th January, 1908). Dose 400 c.c.
3. *Argentine Mule 2222.*—
Injected on 30th August, 1906, with 400 c.c. serum 75/76, and 24 hours later with 2 c.c. virus mule 1964 (Bulawayo).
Result.—Incubation time of 5 days; reaction for 10 days.
Tested on 26th October with 5 c.c. virus 1938 O. Reaction.

4. *Argentine Mule 2223.*

Injected on 30th August, 1906, with 400 c.c. serum 75/76, and 24 hours later with 2 c.c. virus mule 1965 (Tzaneen).

Result.—Slight reaction.

Tested on 19th September, 1906, with 2 c.c. virus mule 1965, injected intrajugularly. Slight reaction. Retested on 20th November, 1906, with 5 c.c. virus mule 2287 O. Reaction. Retested on 8th December with 5 c.c. virus horse 2199 Tzaneen. Reaction.

Conclusions.—The injection of serum about 1 year old, 24 hours previous to injection of virus Bulawayo and virus T did not prevent a horse-sickness reaction, death resulting from B virus. Serum of about 6 months old when injected in the same way permitted a reaction. The immunity obtained from Tzaneen virus did not prevent a reaction due to a subsequent injection of O virus, neither did the immunity obtained from virus B.

The immunity obtained from 1965 did not protect against the same strain 2199 (12th) generation.

EXPERIMENT NO. 7.—To test serum mixture against virus Tzaneen (mule first generation and mule second generation; virus of a horse injected into a mule).

(a) Serum Nos. 93/94, dose 400 c.c. injected subcutaneously. Virus 1965 Tzaneen; dose 2 c.c. injected subcutaneously.

Note.—All mules 15 hands high.

1. *Mule 2288.*—

Injected on 3rd October, 1906, with 400 c.c. serum mixture subcutaneously and 2 c.c. virus 1965 mule (first generation) subcutaneously. Reaction, 6 days' incubation, lasting 10 days.

Tested on 26th October, 1906, with 5 c.c. virus O 1938 into jugular vein; reaction.

2. *Mule 2294.*—

Injected as above, on 3rd October, 1906. Very slight reaction.

Tested on 26th October, 1906, with 5 c.c. virus O 1938 intrajugularly. Reaction and developed dikkop on 8th day. Died on the 18th day from horse-sickness.

(b) Virus 1995 Tzaneen (second generation mule). Serum as above.

3. *Mule 2291.*—

Injected on the 3rd October, 1906, with 400 c.c. serum 93/94 and subcutaneously with 2 c.c. virus 1995 (Tzaneen mule, second generation). Reaction after six days incubation, lasting 6 days; dikkop 16th day.

Tested on 26th October, 1906, with 5 c.c. virus O 1938 intrajugularly. Reaction.

4. *Mule 2293.*—

Injected as above, on 3rd October, 1906. Reaction after 8 days' incubation, lasting 8 days. Dikkop on 13th day.

Tested on 31st October, 1906, with 5 c.c. virus O 1938 intrajugularly. Slight reaction.

Conclusions.—The injection of serum O and Tzaneen virus permitted horse-sickness reactions. The immunity obtained did not prevent reactions due to subsequent injections of virus O.

(c) Serum mixture 95/96. Dose 400 c.c. injected subcutaneously.

Virus 1965 Tzaneen (mule, first generation). Dose 2 c.c. injected subcutaneously.

5. *Mule 2289.*—

Injected on 3rd October, 1906, with 400 c.c. serum 95/96 and 2 c.c. virus 1965 (Tzaneen mule, first generation).

Very slight reaction.

Tested on 25th October, 1906, with 5 c.c. virus 1938 O. Reaction.

6. *Mule 2290.*—

Injected as above on 3rd October, 1906.

Result.—Slight reaction.

Tested on 25th October, 1906, with 5 c.c. virus O 1938 injected intrajugularly. Reaction.

(d) Virus Tzaneen (mule, second generation). Serum as above.

7. *Mule 2292.*—

Injected on 3rd October, 1906, with 400 c.c. serum 95/96 and 2 c.c. virus 1995 (Tzaneen mule, second generation).

Result.—Slight reaction.

Tested on 25th October, 1906, with 5 c.c. virus O 1938 injected intrajugularly. Reaction.

8. *Mule 2212.*—

Injected as above, on 3rd October, 1906.

Result.—Slight reaction.

Tested on 25th October, 1906, with 5 c.c. virus O 1938 intrajugularly. Reaction.

Conclusion.—The injection of serum O and virus T permitted a horse-sickness reaction. The immunity obtained did not prevent a reaction due to a subsequent inoculation of virus O.

EXPERIMENT No. 8.—Serum tests of various compositions (O strain).

(a) Virus Tzaneen, horse 2199 (12th generation). Dose 2 c.c. Serum mixture of Nos. I., II. and III. (of horses hyperimmunised once to three times) and ordinary mule serum (mules hyperimmunised once to three times); mixture No. 102; dose 400 c.c.

Virus and serum injected subcutaneously and simultaneously.

1. *Argentine Mule 2315.*—

Injected as above.

Result.—Incubation 5 days, reaction lasting 9 days.

Tested on 21st December with 2 c.c. virus 1938 O. Reaction.

2. *Argentine Mule 2316.*—

Injected as above.

Result.—Incubation 4 days; died on 9th day of horse-sickness.

3. *Argentine Mule 2317.*—
Injected as above. Second injection on 7th day of 200 c.c. same serum.
Result.—Incubation 5 days; died on 9th day.
4. *Argentine Mule 2318.*—
Injected as above. Second injection on 7th day with 200 c.c. same serum.
Result.—Incubation 6 days; died on the 10th day.
Conclusion.—The injection of O serum simultaneously and subcutaneously with virus Tzaneen of horse (12th generation) resulted in a mortality of 3 animals out of 4—75 per cent. The immunity obtained in the one surviving animal did not prevent a reaction due to a subsequent injection of virus O.
(b) Virus Tzaneen, horse 2199 (12th generation). Dose 2 c.c. Serum mixture of horse serum No. 4 (horses hyperimmunised four times) and mule serum 104 (1, 2 and 3) mixed in equal quantities.
 Virus and serum injected subcutaneously and simultaneously.
5. *Argentine Mule 2319.*—
Injected as above on the 18th October, 1906. Second injection on the 7th day with 200 c.c. of the same serum.
Result.—Incubation 5 days; dikkop on the 11th day Died on the 12th day.
6. *Argentine Mule 2320.*—
Treated as above. Second injection of serum on the 7th day.
Result.—Incubation 5 days; reaction lasting 12 days.
Tested on the 7th December with 5 c.c. mule virus 2295 (Tzaneen horse virus 2199 passed through mule). Slight reaction. Retested on 18th December with 2 c.c. virus 1938 (Ordinary virus). Reaction.
7. *Argentine Mule 2321.*—
Injected as above, but no second injection.
Result.—Incubation 5 days; reaction lasted 6 days.
Tested on 7th December with 5 c.c. virus 2295 (Tzaneen). Slight reaction. Retested on 18th December with 2 c.c. virus 1938 Ordinary. Slight reaction.
8. *Argentine Mule 2322.*—
Injected as above, and second injection on the 7th day with 200 c.c. same serum.
Result.—Incubation 5 days; reaction 9 days.
Tested on 12th January, 1907, with 2 c.c. virus mule 2287 O. Reaction.
9. *Argentine Mule 2323.*—
Injected as above, but no second injection.
Result.—Incubation 5 days; died on the 11th day.
10. *Argentine Mule 2324.*—
Injected as above, no second injection.
Result.—Incubation 5 days; reaction on the 11th day.

Tested on the 7th December with 5 c.c. virus 2295 Tzaneen; slight reaction. Retested on 18th December with 2 c.c. virus 2287, Ordinary, mule. No reaction.

Conclusion.—Serum O of horses hyperimmunised 4 times and of mules 1—3 times hyperimmunised permitted a typical reaction of horse-sickness with virus 2199 Tzaneen (12th generation). The immunity obtained did not prevent reactions to subsequent injections of virus of the same strain passed through a mule, nor against a subsequent inoculation of virus O.

(c) Virus 2199 (Tzaneen horse 12th generation); dose 2 c.c. Serum: Mixture in equal parts of serum 101 (horses hyperimmunised 1 to 3 times) and of mule serum (mules hyperimmunised 1 to 3 times).

Virus and serum injected subcutaneously and simultaneously.

11. *Argentine Mule 2325.*—

Injected as above.

Result.—Incubation 5 days; reaction 9 days; dikkop on the 17th day.

Tested on the 12th January, 1907, with 2 c.c. virus 2287 O. Reaction.

12. *Argentine Mule 2326.*—

Injected as above.

Result.—5 days' incubation; reaction lasted 8 days; dikkop on 12th day.

Tested on the 12th January with 2 c.c. virus 2287 O. Reaction.

13. *Argentine Mule 2327.*—

Injected as above; second injection of 200 c.c. same serum on the 7th day.

Result.—Incubation 5 days; reaction lasted 8 days.

Tested on 7th December, 1906, with 5 c.c. virus 2295 Tzaneen. Slight reaction. Retested on 18th December, 1906, with 2 c.c. virus 2287 (Ordinary virus mule). Distinct reaction.

14. *Argentine Mule 2328.*—

Injected as above; second injection on 7th day with 200 c.c. same serum.

Result.—Incubation 4 days; reaction 9 days.

Tested on 7th December with 5 c.c. virus 2295 (Tzaneen). Slight reaction. Retested on 18th December with 2 c.c. virus 2287 (Ordinary). Reaction.

Conclusion.—The injection of serum O simultaneously and subcutaneously with virus Tzaneen (12th generation) permitted of typical horse-sickness reactions. The immunity obtained did not prevent a reaction due to a subsequent injection of virus of the same strain or of the O strain.

(d) Virus 2199 (horse Tzaneen 12th generation). Dose 2 c.c. Serum: A mixture of serum 95 (horses hyperimmunised 1 to 3 times) and serum 96 (mules hyperimmunised 1 to 3 times) in equal parts. Dose 400 c.c.

Virus and serum injected subcutaneously and simultaneously.

15. *Mule 2329.*—

Injected as above.

Result.—Incubation 5 days; reaction lasting 7 days.

Tested on 7th December, 1906, with 5 c.c. virus 2295 (Tzaneen); reaction. Retested on 18th December, 1906, with 2 c.c. virus 1938 (Ordinary). Doubtful reaction.

16. *Mule 2330.*—

Injected as above, and second injection of 200 c.c. serum on the 7th day.

Result.—Incubation 5 days; reaction lasting 8 days.

Tested on 7th December, 1906, with 5 c.c. virus 2295 (Tzaneen); slight reaction. Retested on the 22nd December, 1906, with 2 c.c. virus 2287 (Ordinary mule); reaction.

17. *Mule 2331.*—

Injected as above; second injection of 200 c.c. same serum on 7th day.

Result.—Incubation 5 days; reaction 6 days.

Tested on the 20th November, 1906, with 2 c.c. virus 1938 (horse, ordinary); slight reaction. Retested on the 8th December, 1906, with 5 c.c. virus 2295 (Tzaneen, mule); no reaction.

18. *Mule 2332.*—

Injected as above.

Result.—Incubation 5 days; died on the 10th day.

Conclusion.—The simultaneous injection of serum O and virus Tzaneen 2199 (12th generation) resulted in a typical horse-sickness reaction. In two instances the immunity obtained did not prevent a reaction due to the subsequent injection of the same strain of virus, nor a reaction due to the O virus.

(e) Virus 2199 (horse Tzaneen, 12th generation); dose 2 c.c. Serum Nos. 97/98, mixed in equal parts (horses and mules hyperimmunised 1 to 3 times).

Virus and serum injected subcutaneously and simultaneously.

19. *Mule 2333.*—

Injected as above on the 18th October, 1906; second injection of 200 c.c. of same serum on the 7th day.

Result.—5 days' incubation; reaction lasting 7 days; dikkop on the 11th day.

Tested on the 20th November, 1906, with 2 c.c. virus O 1938 (horse). Reaction. Retested on the 8th December, 1906, with 5 c.c. virus 2295 (Tzaneen, mule); no reaction.

20. *Mule 2334.*—

Injected as above; no second injection of serum.

Result.—Incubation 5 days; dikkop on the 10th day; died on the 14th day.

21. *Mule 2335.*—

Injected as above; second injection of 200 c.c. same serum on the 7th day.

Result.—4 days' incubation; reaction lasting 8 days.

Tested on 20th November, 1906, with 5 c.c. virus horse 1938 Ordinary; reaction. Retested on 8th December, 1906, with 5 c.c. virus horse 2295 (Tzaneen); slight reaction.

22. *Mule 2336.*—

Injected as above; no second injection.

Result.—5 days' incubation; reaction 3 days; dikkop on the 9th day.

Tested on the 20th November, 1906, with 5 c.c. virus 1938 Ordinary; no reaction. Retested on the 8th December, 1906, with 5 c.c. virus 2295 (Tzaneen); slight reaction.

Conclusion.—The simultaneous injection of serum O and virus T resulted in a horse-sickness reaction. In two cases the immunity obtained did not prevent a reaction to a subsequent injection of virus of the same strain, nor a reaction due to the injection of O strain.

(f) Virus 2199 (horse Tzaneen, 12th generation); dose 2 c.c. Serum 99/100 of horses and mules hyperimmunised 1 to 3 times; mixed in equal parts. Dose 400 c.c.

Virus and serum injected subcutaneously and simultaneously. Date, 18th October, 1906.

23. *Mule 2337.*—

Injected as above; second injection of 200 c.c. same serum on the 7th day.

Result.—Incubation 5 days; reaction 7th day; dikkop on the 9th day.

Tested on the 20th November, 1906, with 2 c.c. virus 1938; reaction. Tested on the 8th December, 1906, with 5 c.c. virus 2295; no reaction.

24. *Mule 2338.*—

Injected as above; second injection of 200 c.c. same serum on the 7th day.

Result.—Incubation 5 days; reaction lasted 9 days.

Tested on the 20th November, 1906, with 2 c.c. virus 1938; slight reaction. Retested on the 8th December, 1906, with 5 c.c. virus 2295; slight reaction.

25. *Mule 2339.*—

Injected as above; no second injection of serum.

Result.—Incubation 5 days; reaction lasted 9 days; dikkop on the 11th day.

Tested on the 20th November, 1906, with 2 c.c. virus mule 2287 (Ordinary); reaction. Retested on the 8th December, 1906, with 5 c.c. virus mule 2295; slight reaction.

Conclusion.—The simultaneous injection of serum O and virus T (12th generation) resulted in a horse-sickness reaction. In three instances the immunity obtained did not prevent a reaction, due to a subsequent injection of virus of the same strain, and in three cases not against the injection of virus O.

(g) Virus 2199 (horse Tzaneen, 12th generation); dose 2 c.c. Serum mixture 103/104, in equal parts, of horses and mules hyperimmunised 1 to 3 times; dose 400 c.c.

Virus and serum injected subcutaneously and simultaneously. Date, 18th October, 1906.

26. *Mule 2341.*—

Injected as above; second injection of 200 c.c. same serum on the 7th day.

Result.—5 days' incubation; reaction lasting 7 days.

Tested on 20th November, 1906, with 5 c.c. virus 2287; reaction. Retested on the 8th December, 1906, with 5 c.c. virus 2295; no reaction.

27. *Mule 2342.*—

Injected as above, but no second injection of serum.

Result.—Incubation 5 days; reaction 8 days.

Tested on the 20th November, 1906, with 2 c.c. virus 2287 into jugular vein; slight reaction.

28. *Mule 2343.*—

Injected as above; second injection of 200 c.c. same serum on 7th day.

Result.—Incubation 5 days; dikkop on the 11th day; died on the 13th day.

29. *Mule 2344.*—

Injected as above, but no second injection of serum.

Result.—Incubation 5 days; died on the 11th day.

Conclusion.—Simultaneous injection of serum O and virus Tzaneen (12th generation) resulted in reactions and two deaths. The immunity obtained did not prevent a reaction due to a subsequent injection of virus of the O strain.

(h) Virus 2199 (horse Tzaneen, 12th generation); dose 2 c.c. Serum mixture in equal parts of Nos. 105/106 of horses and mules hyperimmunised 1 to 3 times. Dose 400 c.c.

Virus and serum injected subcutaneously and simultaneously. Date, 18th October, 1906.

30. *Mule 2345.*—

Injected as above; second injection of 200 c.c. same serum on the 7th day.

Result.—5 days' incubation; reaction 8 days; dikkop on the 12th day.

Tested on the 20th November, 1906, with 2 c.c. virus 2287 O; reaction. Retested on the 8th December, 1906, with 5 c.c. virus 2295 T; reaction.

31. *Mule 2346.*—

Injected as above, but no second injection of serum.

Result.—Incubation 4 days; reaction 11 days.

Tested on the 20th November, 1906, with 5 c.c. virus 2287 O; reaction. Retested on the 8th December, 1906, with 5 c.c. virus 2295 T; reaction.

32. *Mule 2347.*—

Injected as above; second injection of 200 c.c. serum on the 7th day.

Result.—Incubation 5 days; reaction 8 days; dikkop on the 11th day.

Tested on the 20th November, 1906, with 5 c.c. virus 2287; no reaction. Retested on the 8th December, 1906, with 5 c.c. virus 2295; no reaction.

33. *Mule 2348.*—

Injected as above, but no second injection of serum.

Result.—Incubation 5 days; dikkop on the 10th day; died on the 12th day.

Conclusion.—The simultaneous injection of serum O and virus T (12th generation) resulted in horse-sickness reactions. The immunity obtained in two out of three cases did not prevent a reaction to a subsequent inoculation of virus of the same strain, nor to the O strain.

EXPERIMENT No. 9.—With serum O strain of various compositions, and virus Tzaneen, first generation.

(a) Virus 1965 Tzaneen mule, first generation; dose 2 c.c.

Serum mixture 107/108 mixed in equal parts of horses and mules hyperimmunised 1 to 3 times. Dose 400 c.c.

Virus and serum injected subcutaneously and simultaneously.

1. *Argentine Mule 2362.*—

Injected as above on the 1st November, 1906.

Result.—Slight reaction.

Tested on the 8th December, 1906, with 5 c.c. virus O mule 2287; reaction.

2. *Argentine Mule 2374.*—

Injected as above.

Result.—Slight reaction; died on the 16th November, 1906; dikkop on the 13th day.

3. *Argentine Mule 2375.*—

Injected as above.

Result.—Reaction after 6 days' incubation, lasting for 10 days.

Tested on the 8th December, 1906, subcutaneously with 5 c.c. virus O 2287 mule. Reaction.

Conclusion.—The simultaneous injection of serum O and virus Tzaneen mule (first generation) resulted in horse-sickness reactions. Two animals when tested later with virus O showed reactions.

(b) Virus 1965 Tzaneen mule (first generation); dose 2 c.c.

Serum mixture 109/110 in equal parts (of horses and mules hyperimmunised 1 to 3 times). Dose 400 c.c.

Virus and serum injected subcutaneously and simultaneously. Date, 1st November, 1906.

4. *Argentine Mule 2370.*—

Injected as above.

Result.—Slight reaction.

Tested on the 18th December, subcutaneously with 2 c.c. virus 2287; slight reaction.

5. *Argentine Mule 2365.*—

Injected as above.

Result.—Very slight reaction.

Tested on the 18th December, 1906, subcutaneously with 2 c.c. virus 2199; severe reaction. Retested on the

12th January, 1907, with 2 c.c. virus mule 2287 O.
Slight reaction.

6. *Argentine Mule 2366.*—

Injected as above.

Result.—Slight reaction.

Tested on the 18th December, 1906, with 2 c.c. virus 2199 (Tz. horse 12th generation); reaction. Tested on the 12th January with 2 c.c. virus 2287 O; reaction.

Conclusion.—The simultaneous injection of serum O and the virus Tzaneen (first generation) (mule virus 4 months old), resulted in but very slight reactions. When tested subsequently with virus of the same strain (12th generation), reactions—and even severe reactions—were noted. The immunity obtained did not prevent reactions due to subsequent injections of virus O.

(c) Virus 1965; Tzaneen mule (first generation); dose 2 c.c. Serum mixture 111/112 in equal parts (of horses and mules hyperimmunised 1 to 3 times). Dose 400 c.c.

Virus and serum injected subcutaneously and simultaneously.

Date, 1st November, 1906.

7. *Argentine Mule 2368.*—

Injected as above.

Result.—Doubtful reaction, if any.

Tested on the 18th December, 1906, with 2 c.c. virus 2287 O; distinct reaction.

8. *Argentine Mule 2369.*—

Injected as above.

Result.—Slight reaction.

Tested on the 18th December, 1906, with 2 c.c. virus 2287 O; injected subcutaneously; reaction, and died of horse-sickness on the 28th December, 1906.

9. *Argentine Mule 2364.*—

Injected as above.

Result.—Doubtful reaction.

Tested on the 18th January, 1906, with 2 c.c. virus 2199 (Tzaneen 12th generation); reaction, and died of dikkop on the 9th day—28th December, 1906.

Conclusion.—The simultaneous injection of serum O and virus T (first generation mule) resulted in but slight or doubtful reactions. One animal subsequently tested with virus of the same strain (12th generation) died; of two tested with O strain, one had a severe reaction and recovered, one dying.

It is probable that the virus 1965 had become attenuated.

EXPERIMENT No. 10.—With serum O strain of various compositions and virus Tzaneen (second generation).

(a) Virus 1996, Tzaneen mule, second generation. Dose 2 c.c., Serum mixture 113/114 in equal parts (of horses and mules hyperimmunised 1 to 3 times). Dose 400 c.c.

Virus and serum injected subcutaneously and simultaneously.

Date, 1st November, 1906.

1. *Argentine Mule 2371.*—

Injected on the 1st November, 1906, with 400 c.c. serum 113/114 and 2 c.c. virus mule 1996 subcutaneously.

Result.—Doubtful reaction.

Tested on the 18th December, 1906, subcutaneously with 2 c.c. virus T 2199 (12th generation). *Result:* Died of horse-sickness on the 30th December, 1906.

2. *Argentine Mule 2372.*—

Injected as above.

Result.—Doubtful reaction.

Tested on the 18th December, 1906, subcutaneously with 2 c.c. virus Tzaneen 2199 (12th generation); reaction; died on the 2nd January, 1907, of horse-sickness.

3. *Argentine Mule 2367.*—

Injected as above.

Result.—Doubtful reaction.

Tested on the 18th December, 1906, subcutaneously with 2 c.c. virus 1965; reaction; died on 8th January, 1907.

Conclusion.—The simultaneous injection of serum O and virus 1996 T resulted in a doubtful reaction, if any at all. There was a slight disturbance, but apparently no trace of immunity was caused by it. All three mules died, when subsequently injected with virus of the same strain, 12th generation.

(b) Virus 1996, Tzaneen mule, second generation. Dose 2 c.c.

Serum mixture 115/116 in equal parts of horses and mules hyperimmunised 1 to 3 times. Dose 400 c.c.

Virus and serum injected subcutaneously and simultaneously. Date, 1st November, 1906.

4. *Argentine Mule 2363.*—

Injected with 400 c.c. serum 115/116 and 2 c.c. virus mule 1996 (Tzaneen).

Result.—Doubtful reaction.

Tested on the 18th December, 1906, subcutaneously with 2 c.c. virus 1965; reaction: died on the 5th January, 1907.

5. *Argentine Mule 2382.*—

Injected as above.

Result.—Doubtful reaction.

Tested on the 8th December, 1906, by subcutaneous injection of 5 c.c. virus O 1938; severe reaction; died of horse-sickness on the 22nd December, 1906.

6. *Argentine Mule 2379.*—

Injected as above.

Result.—Doubtful reaction.

Tested on the 8th December, 1906, subcutaneously with 5 c.c. virus 1938 O (62nd generation); severe reaction; recovered.

Conclusion.—The simultaneous injection of serum O and virus 1996 T strain, resulted in a doubtful reaction; but little immunity was caused by it. A subsequent inoculation of virus O caused death in two animals, and a severe reaction in a third one.

(c) Virus 1965 Tz. mule, first generation. Dose 2 c.c.
Serum mixture Nos. I., II. and III. and mule 112. Dose
400 c.c.

Virus and serum injected subcutaneously and simultaneously.
Date, 1st November, 1906.

7. *Argentine Mule 2376.*—

Injected with 400 c.c. serum subcutaneously and simultaneously with 2 c.c. virus 1965.

Result.—Reaction; dikkop on the 21st November, 1906.

Tested on the 8th December, 1906, with 5 c.c. virus mule 2287; slight reaction.

(d) Virus 1996 Tz. mule, 2nd generation. Dose 2 c.c.
Serum as above.

Date, 1st November, 1906.

8. *Argentine Mule 2383.*—

Injected as above.

Result.—Doubtful reaction.

Tested on the 8th December, 1906, subcutaneously with 5 c.c. virus 1938 O; severe reaction; died on the 20th December, 1906, of horse-sickness, with dikkop.

Conclusion.—The simultaneous inoculation of a mule with virus Tzaneen 1965, mule 1st generation and serum O, resulted in a typical horse-sickness reaction; the immunity obtained still permitted a reaction due to a subsequent inoculation of virus O.

The simultaneous inoculation of serum O and virus 1996 gave a doubtful reaction, and no immunity was obtained against a subsequent inoculation of virus O strain.

ANALYSIS OF RESULTS.

DEATHS.

38 mules immunised with virus Tzaneen, 1st generation...	2 deaths.
9 mules immunised with virus Tzaneen, 2nd generation (attenuated or inert virus)	0 deaths.
34 mules immunised with virus Tzaneen, 12th generation	10 deaths.
	12 deaths.
6 mules immunised with virus Bulawayo, 2nd generation	2 deaths.
4 mules immunised with a mixture of virus Tzaneen 1st generation, and Bulawayo 2nd generation	2 deaths.

TESTS.

(a) *Tests with Ordinary Virus.*

18 mules immunised with Tzaneen, 1st generation.

12 tested with Ordinary, 62nd generation: 8 reactions, 2 reactions and dikkop, 2 deaths.

6 tested with Ordinary, 38th generation: 5 reactions and 1 death.

5 mules immunised with Tzaneen, 2nd generation (attenuated or inert virus).

Tested with Ordinary, 62nd generation: 3 reactions and 2 deaths.

5 mules immunised with Tzaneen, 12th generation

4 tested with Ordinary, 38th generation: 4 reactions.

1 tested with Ordinary, 62nd generation: 1 reaction.

1 mule immunised with a mixture of virus of Tzaneen 1st generation and Bulawayo 2nd generation.

Tested with Ordinary, 62nd generation: No reaction.

1 mule immunised with a mixture of virus of Tzaneen 1st generation and Bulawayo 2nd generation.

1st test with Ordinary, 62nd generation: Doubtful reaction.

2nd test with Ordinary, 62nd generation: Distinct reaction.

1 mule immunised with Bulawayo, 2nd generation.

Tested with Ordinary, 62nd generation: Reaction.

NOTE.—1 mule (2214) which was immunised with Tzaneen, 1st generation, and had a reaction with dikkop, again showed a reaction and dikkop with test of Ordinary, 62nd generation, and later contracted horse-sickness spontaneously, showing a third reaction and dikkop.

(b) Tests with Tzaneen Virus.

1 mule immunised with Tzaneen, 1st generation.

Tested with Tzaneen, 12th generation: Died.

4 mules immunised with Tzaneen, 2nd generation (inert or attenuated virus).

2 tested with Tzaneen, 1st generation: 2 reactions.

2 tested with Tzaneen, 12th generation: 2 deaths.

1 mule immunised with Bulawayo, 2nd generation.

Tested with Tzaneen, 1st generation: Reaction.

(c) 1st Test with Ordinary and 2nd Test with Tzaneen Virus.

12 mules immunised with Tzaneen, 12th generation.

6 tested with Ordinary, 62nd generation, and Tzaneen, 13th generation:

2 reactions on both tests.

3 reactions with Ordinary.

1 reaction with Tzaneen.

6 tested with Ordinary, 38th generation, and Tzaneen, 13th generation:

4 reactions on both tests.

1 reaction with Ordinary.

1 no reaction.

(d) 1st Test with Tzaneen and 2nd Test with Ordinary Virus.

7 mules immunised with Tzaneen, 12th generation.

3 tested with Tzaneen, 13th generation, and Ordinary, 62nd generation:

2 reactions on both tests.

1 distinct reaction with Tzaneen and a doubtful reaction with Ordinary.

4 tested with Tzaneen, 13th generation, and Ordinary, 38th generation:

3 reactions on both tests.

1 reacted only with Tzaneen.

2 mules immunised with Tzaneen, 1st generation.

Tested with Tzaneen, 12th generation, and Ordinary, 38th generation: Reactions on both tests.

(e) 1st Test with Tzaneen, 2nd Test with Ordinary, and 3rd Test with Tzaneen Virus.

3 mules immunised with Tzaneen, 1st generation.

1 mule tested with Tzaneen, 1st generation, Ordinary, 38th generation, and Tzaneen, 12th generation: Reaction on all three tests.

2 mules tested with Tzaneen, 1st generation, Ordinary, 62nd generation, and Tzaneen, 12th generation; reaction only with first and second tests.

(f) 1st and 2nd Test with Tzaneen and 3rd Test with Ordinary Virus.

2 mules immunised with Bulawayo, 2nd generation.

Tested with Tzaneen, 1st generation, Tzaneen, 3rd generation, and Ordinary, 38th generation:

1 reaction on each test; 1 had only a reaction with Ordinary.

(g) 1st Test with Bulawayo and 2nd Test with Ordinary Virus.

9 mules immunised with Tzaneen, 1st generation.

1 mule tested with Bulawayo, 2nd generation, and Ordinary, 62nd generation:

Doubtful reaction with the 1st, and distinct reaction with the 2nd test.

8 mules tested with Bulawayo, 4th generation, and Ordinary, 38th generation:

3 reactions on both tests.

2 distinct reactions with 1st, and a doubtful reaction with the 2nd test.

1 doubtful reaction with the 1st, and a distinct reaction with the 2nd test.

1 reaction only with the 2nd test.

1 died on the 1st test (Bulawayo).

(h) 1st and 2nd Test with Bulawayo and 3rd Test with Ordinary Virus.

2 mules immunised with Tzaneen, 1st generation.

Tested with Bulawayo, 2nd generation, Bulawayo, 3rd generation, and Ordinary, 38th generation:

1 gave a reaction on the 3rd test.

1 showed a doubtful reaction to 1st Bulawayo test, and a distinct reaction with Ordinary 3rd test.

1 mule was not tested.

TABULATED RESUME OF PREVIOUS EXPERIMENTS.
INADEQUATE SERUM AND VIRUS.

IMMUNISATION.					I. TEST.			II. TEST.			III. TEST.		
Mule.	Serum.	Virus.		Result.	Virus.		Result.	Virus.		Result.	Virus.		Result.
		Orig.	Gen.		Orig.	Gen.		Orig.	Gen.		Orig.	Gen.	
2155	Ord.	Tzn.	1	R†	—	—	—	—	—	—	—	—	—
2158	"	"	1	R	Bul.	2	—	Bul.	3	—	Ord.	38	R
2161	"	"	1	RD	"	2	?	"	3	—	"	38	R
2164	"	"	1	RD	"	2	?	Ord.	62	R	—	—	—
2156	"	Bul.	2	RD	Tzn.	1	—	Tzn.	3	—	Ord.	38	R
2159	"	"	2	R	"	1	R	—	—	—	—	—	—
2162	"	"	2	RD†	"	—	—	—	—	—	—	—	—
2119	"	"	2	RD	Tzn.	1	R	Tzn.	3	R	Ord.	38	R
2157	"	{Tzn. Bul.	1 2	RD†	—	—	—	—	—	—	—	—	—
2160	"	"	{1 2}	RD	Ord.	62	?	Ord.	62	R	—	—	—
2163	"	"	{1 2}	R†	—	—	—	—	—	—	—	—	—
2120	"	"	{1 2}	R	Ord.	62	—	—	—	—	—	—	—
2181	"	Tzn.	1	R	Bul.	4	R	Ord.	38	?	—	—	—
2182	"	"	1	R	"	4	R	"	38	R	—	—	—
2183	"	"	1	R	"	4	—	"	38	?	—	—	—
2184	"	"	1	RD	"	4	—	"	38	R	—	—	—
2185	"	"	1	R	"	4	RD†	—	—	—	—	—	—
2186	"	"	1	R	"	4	R	Ord.	38	R	—	—	—
2187	"	"	1	RD	"	4	?	"	38	R	—	—	—
2188	"	"	1	R	"	4	R	"	38	R	—	—	—
2211	"	"	1	R	Tzn.	1	R	"	62	R	Tzn.	12	—
2213	"	"	1	RD	Not	Tested.	—	—	—	—	—	—	—
2214	"	"	1	RD	Ord.	62	RD	Later	a	3rd	Dikkop	Spont.	—
2215	"	"	1	R	"	62	R	—	—	—	—	—	—
2216	"	"	1	?	Tzn.	1	R	Ord.	62	R	Tzn.	12	—
2217	"	"	1	R	Ord.	62	R	—	—	—	—	—	—
2218	"	"	1	R	"	62	RD.	—	—	—	—	—	—
2219	"	"	1	R	"	62	R	—	—	—	—	—	—
2220	"	Bul.	2	RD†	—	—	—	—	—	—	—	—	—
2222	"	"	2	R	Ord.	62	R	—	—	—	—	—	—
2221	"	Tzn.	1	R	"	62	RD†	—	—	—	—	—	—
2223	"	"	1	R	Tzn.	1	R	Ord.	38	R	Tzn.	12	R
2288	"	"	1	R	Ord.	62	R	—	—	—	—	—	—
2294	"	"	1	R	"	62	RD†	—	—	—	—	—	—
2291	"	"	2	RD	"	62	R	—	—	—	—	—	—
2293	"	"	2	RD	"	62	R	—	—	—	—	—	—
2289	"	"	1	R	"	62	R	—	—	—	—	—	—
2290	"	"	1	R	"	62	R	—	—	—	—	—	—
2292	"	"	1	R	"	62	R	—	—	—	—	—	—
2212	"	"	1	R	"	62	R	—	—	—	—	—	—
2315	"	"	12	R	"	62	R	—	—	—	—	—	—
2316	"	"	12	R†	—	—	—	—	—	—	—	—	—
2317	"	"	12	R†	—	—	—	—	—	—	—	—	—
2318	"	"	12	R†	—	—	—	—	—	—	—	—	—

R—Reaction. ?—Doubtful. RD—Reaction with Dikkop. R†—Reaction and died.
RD†—Reaction with Dikkop and died.

TABULATED RESUMÉ OF PREVIOUS EXPERIMENTS—(Continued).

INADEQUATE SERUM AND VIRUS—(Continued).

IMMUNISATION.					I. TEST.			II. TEST.			III. TEST.		
Mule.	Se- rum.	Virus.		Result.	Virus.		Result.	Virus.		Result.	Virus.		Result.
	Orig.	Orig.	Gen.		Orig.	Gen.		Orig.	Gen.		Orig.	Gen.	
2319	Ord.	Tzn.	12	RD†	—	—	—	—	—	—	—	—	—
2320	"	"	12	R	Tzn.	13	R	Ord.	62	R	—	—	—
2321	"	"	12	R	"	13	R	"	62	R	—	—	—
2322	"	"	12	R	Ord.	38	R	—	—	—	—	—	—
2323	"	"	12	R†	—	—	—	—	—	—	—	—	—
2324	"	"	12	R	Tzn.	13	R	Ord.	38	—	—	—	—
2325	"	"	12	RD	Ord.	38	R	—	—	—	—	—	—
2326	"	"	12	RD	"	38	R	—	—	—	—	—	—
2327	"	"	12	R	Tzn.	13	R	Ord.	38	R	—	—	—
2328	"	"	12	R	"	13	R	"	38	R	—	—	—
2329	"	"	12	R	"	13	R	"	62	?	—	—	—
2330	"	"	12	R	"	13	R	"	38	R	—	—	—
2331	"	"	12	R	Ord.	62	R	Tzn.	13	—	—	—	—
2332	"	"	12	R†	—	—	—	—	—	—	—	—	—
2333	"	"	12	RD	Ord.	62	R	Tzn.	13	—	—	—	—
2334	"	"	12	RD†	—	—	—	—	—	—	—	—	—
2335	"	"	12	R	Ord.	62	R	Tzn.	13	R	—	—	—
2336	"	"	12	RD	"	62	—	"	13	—	—	—	—
2337	"	"	12	RD	"	62	R	"	13	—	—	—	—
2338	"	"	12	R	"	62	R	"	13	R	—	—	—
2339	"	"	12	RD	"	38	R	"	13	R	—	—	—
2340	"	"	12	R	"	38	R	"	13	R	—	—	—
2341	"	"	12	R	"	38	R	"	13	—	—	—	—
2342	"	"	12	R	"	38	R	"	—	—	—	—	—
2343	"	"	12	RD†	—	—	—	—	—	—	—	—	—
2344	"	"	12	R†	—	—	—	—	—	—	—	—	—
2345	"	"	12	RD	Ord.	38	R	Tzn.	13	R	—	—	—
2346	"	"	12	R	"	38	R	"	13	R	—	—	—
2347	"	"	12	RD	"	38	—	"	13	—	—	—	—
2348	"	"	12	RD†	—	—	—	—	—	—	—	—	—
2362	"	"	1	R	Ord.	38	R	—	—	—	—	—	—
2374	"	"	1	RD†	—	—	—	—	—	—	—	—	—
2375	"	"	1	R	Ord.	38	R	—	—	—	—	—	—
2370	"	"	1	R	"	38	R	—	—	—	—	—	—
2365	"	"	1	R	Tzn.	12	R	Ord.	38	R	—	—	—
2366	"	"	1	R	"	12	R	"	38	R	—	—	—
2368	"	"	1	?	Ord.	38	R	—	—	—	—	—	—
2369	"	"	1	R	"	38	R†	—	—	—	—	—	—
2364	"	"	1	?	Tzn.	12	RD†	—	—	—	—	—	—
2371	"	"	2	?	"	12	R†	—	—	—	—	—	—
2372	"	"	2	?	"	12	R†	—	—	—	—	—	—
2367	"	"	2	?	"	1	R	—	—	—	—	—	—
2363	"	"	2	?	"	1	R	—	—	—	—	—	—
2382	"	"	2	?	Ord.	62	R†	—	—	—	—	—	—
2379	"	"	2	?	"	62	R	—	—	—	—	—	—
2376	"	"	1	RD	"	38	R	—	—	—	—	—	—
2383	"	"	2	?	"	62	RD†	—	—	—	—	—	—

R—Reaction. ?—Doubtful. RD—Reaction with Dikkop. R†—Reaction and died.
RD†—Reaction with Dikkop and died.

COMPLETE SUMMARY OF RESULTS.

Tests on mules previously immunised with virus Tzaneen 1 gen.

Mules.	Tested with Virus.	RESULT.			
		Reaction.	Reaction and Dikkop.	Doubtful Reaction.	Deaths.
10	Ordinary 38 gen. ..	8	—	2	1
20	Ordinary 62 gen. ..	18	2	—	2
3	Bulawayo 2 gen. ..	—	—	2	—
2	Bulawayo 3 gen. ..	—	—	—	—
8	Bulawayo 4 gen. ..	5	—	1	1
3	Tzaneen 1 gen. ..	3	—	—	—
5	Tzaneen 12 gen. ..	3	—	—	1

Tests on mules previously immunised with virus Tzaneen 2 gen.

Mules.	Tested with Virus.	RESULT.			
		Reaction.	Reaction and Dikkop.	Doubtful Reaction.	Deaths.
5	Ordinary 62 gen. ..	3	—	—	2
4	Tzaneen 12 gen. ..	2	—	—	2

Tests on mules previously immunised with virus Tzaneen 12 gen.

Mules.	Tested with Virus.	RESULT.			
		Reaction.	Reaction and Dikkop.	Doubtful Reaction.	Deaths.
14	Ordinary 38 gen. ..	12	—	—	—
10	Ordinary 62 gen. ..	8	—	1	—
19	Tzaneen 13 gen. ..	14	—	—	—

Tests on mules previously immunised with virus Bulawayo 2 gen. :

Mules.	Tested with Virus.	RESULT.			
		Reaction.	Reaction and Dikkop.	Doubtful Reaction.	Deaths.
2	Ordinary 38 gen. ..	2	—	—	—
1	Ordinary 62 gen. ..	1	—	—	—
3	Tzaneen 1 gen. ..	2	—	—	—
2	Tzaneen 3 gen. ..	1	—	—	—

Tests on mules previously immunised with a mixture of virus { *Tzaneen 1 gen.* }
 { *Bulawayo 2 gen.* }

Mules.	Tested with Virus.	RESULT.			
		Reaction.	Reaction and Dikkop.	Doubtful Reaction.	Deaths.
2	Ordinary 62 gen. ..	1	—	1	—

B.—ADEQUATE SERUM AND VIRUS.

EXPERIMENT No. 11.—With serum mixture 119 O strain injected subcutaneously; dose 400 c.c. and 2 c.c. virus horse 2407 O strain.

Serum and virus injected subcutaneously and simultaneously on 8th January, 1907.

1. *Mule 2566.*—

Immunised as above.

Result.—Slight reaction.

Tested on the 22nd January with 2 c.c. same virus; no reaction.

2. *Mule 2567.*—

Immunised as above.

Result.—Slight reaction.

Tested on the 22nd January with same virus; no reaction.

3. *Mule 2568.*—

Immunised as above.

Result.—Distinct reaction.

Tested on the 22nd January with same virus; no reaction.

4. *Mule 2569.*—

Immunised as above.

Result.—Slight reaction.

Tested on the 22nd January with same virus; no reaction.

5. *Mule 2570.*—

Immunised as above.

Result.—Distinct reaction.

Tested on the 22nd January with same virus; no reaction.

6. *Mule 2571.*—

Immunised as above.

Result.—Distinct reaction.

Tested on the 22nd January; no reaction.

7. *Mule 2572.*—

Immunised as above.

Result.—Distinct reaction.

Tested on the 22nd January; no reaction.

8. *Mule 2573.*—

Immunised as above.

Result.—Reaction.

Tested on the 22nd January; no reaction.

9. *Mule 2574.* —
Immunised as above.
Result.—Slight reaction; dikkop on the 14th day.
Tested on the 22nd January, same virus; no reaction.
10. *Mule 2575.*—
Immunised as above.
Result.—Slight reaction.
Tested on the 22nd January; no reaction.
11. *Mule 2576.*—
Immunised as above.
Result.—Reaction.
Tested on the 22nd January; no reaction.
12. *Mule 2577.*—
Immunised as above.
Result.—Reaction.
Tested on the 22nd January, same virus; no reaction.
- Conclusion.*—The simultaneous inoculation of serum O and virus O 2407 resulted in typical horse-sickness reactions. The immunity obtained proved to be complete to a subsequent inoculation of the same virus.

EXPERIMENT No. 12.—With serum mixture 119 O strain; dose 300 c.c. and virus 2287 mule O strain; dose 2 c.c.

Virus and serum injected subcutaneously and simultaneously.
 Date, 12th January, 1907.

1. *Mule 2525.*—
Immunised as above.
Result.—Distinct reaction.
Tested on the 21st February with 2 c.c. virus mule 2268 T; distinct reaction. Retested on the 13th March, 2 c.c. virus 1954 B; distinct reaction.
2. *Mule 2526.*—
Immunised as above.
Result.—Slight reaction.
 Not tested.
3. *Mule 2527.*—
Immunised as above.
Result.—Slight reaction.
 Not tested.

Conclusion.—The simultaneous inoculation of serum O and virus O (38th generation) was succeeded by typical horse-sickness reactions. The immunity obtained was tested in one instance, and proved not to be able to prevent a subsequent reaction due to the injection of Tzaneen virus and Bulawayo virus.

EXPERIMENT No. 13.—With serum mixture No. 121 (O strain).

Dose 300 c.c.; virus 2287 O strain (38th generation mule).

Dose 2 c.c., virus and serum injected subcutaneously and simultaneously.

Date, 12th January, 1908.

1. *Mule 2528.*—
Immunised as above.
Result.—Reaction and dikkop.

Tested on the 21st February with 2 c.c. virus 1954 B; reaction and dikkop on the 13th day. Retested on the 13th March with 2 c.c. virus mule 2268 T; no reaction.

2. *Mule 2529.*—

Immunised as above.

Result.—Reaction and dikkop on the 14th day.

Tested on the 21st February with virus mule 2268 T; reaction. Retested on the 13th March with 2 c.c. virus 1954 B; no reaction.

3. *Mule 2530.*—

Immunised as above.

Result.—Reaction and dikkop on the 13th day.

Not tested.

Conclusion.—The simultaneous inoculation of serum O and virus O resulted in typical reactions. When tested subsequently, in the first instance with Bulawayo virus, a reaction ensued, and the immunity now established protected against a subsequent inoculation of Tzaneen strain. In the second instance reaction was noticed due to a subsequent inoculation of Tzaneen virus, and the immunity established afterwards protected against an injection of Bulawayo strain.

EXPERIMENT No. 14.—With serum mixture No. 123 O strain, and virus mule 2287 O strain.

Dose of serum 300 c.c., and dose of virus 2 c.c.

Virus and serum injected subcutaneously and simultaneously.

Date, 12th January, 1907.

1. *Mule 2531.*—

Immunised as above.

Result.—Reaction.

Not tested.

2. *Mule 2532.*—

Immunised as above.

Result.—Reaction.

Tested on the 21st February with virus 1954 B; reaction, dikkop and death on the 18th day.

3. *Mule 2533.*—

Immunised as above.

Result.—Reaction and dikkop on the 14th day.

Not tested.

Conclusion.—The simultaneous inoculation of serum O and virus of the same strain resulted in typical horse-sickness reactions. One of the mules was tested with virus of Bulawayo strain, when it contracted horse-sickness and died at an unusual late date after inoculation.

EXPERIMENT No. 15.—With serum mixture No. 125 O strain and virus mule 2287 O strain.

Dose of serum 300 c.c., and dose of virus 2 c.c.

Serum and virus injected subcutaneously and simultaneously.

Date, 18th January, 1907.

1. *Mule 2508.*—
Immunised as above.
Result.—Slight reaction.
 Not tested.
2. *Mule 2509.*—
Immunised as above.
Result.—Slight reaction.
 Not tested.
3. *Mule 2511.*—
Immunised as above.
Result.—Reaction.
 Not tested.
4. *Mule 2512.* —
Immunised as above.
Result.—Reaction.
 Not tested.

Conclusion.—Typical reactions due to simultaneous inoculation of serum O and virus O.

EXPERIMENT No. 16.—With serum mixture No. 127 O strain and virus mule 2287 O strain.

Serum dose 300 c.c., virus dose 2 c.c.

Serum and virus injected subcutaneously and simultaneously.

Date, 18th January, 1907.

1. *Mule 2534.*—
Injected as above.
Result.—Doubtful reaction.
2. *Mule 2535.*—
Injected as above.
Result.—Slight reaction.
3. *Mule 2536.*—
Injected as above.
Result.—Slight reaction.
4. *Mule 2537.*—
Injected as above.
Result.—Slight reaction.

Conclusion.—Typical reactions due to simultaneous inoculation of serum O and virus O.

EXPERIMENT No. 17.—With serum mixture No. 129 O strain and virus mule 2287 O strain.

Serum dose 300 c.c., virus dose 2 c.c.

Serum and virus injected subcutaneously and simultaneously.

Date, 18th January, 1907.

1. *Mule 2538.*—
Immunised as above.
Result.—Slight reaction.
 Not tested.
2. *Mule 2540.*—
Immunised as above.
Result.—Distinct reaction.
 Not tested.

3. *Mule 2541.*—*Immunised* as above.*Result.*—Reaction.

Not tested.

4. *Mule 2542.*—*Immunised* as above.*Result.*—Reaction.

Not tested.

Conclusion.—Typical reactions due to simultaneous inoculation of O serum and O virus.

EXPERIMENT No. 18.—With serum mixture No. 131 O strain and virus mule 2287 O strain.

Serum dose 300 c.c., virus dose 2 c.c.

Serum and virus injected subcutaneously and simultaneously.

Date, 18th January, 1907.

1. *Mule 2543.*—*Immunised* as above.*Result.*—Reaction.

Not tested.

2. *Mule 2544.*—*Immunised* as above.*Result.*—Reaction.

Not tested.

3. *Mule 2545.*—*Immunised* as above.*Result.*—Reaction.

Not tested.

4. *Mule 2546.*—*Immunised* as above.*Result.*—Reaction.

Not tested.

Conclusion.—Typical reactions due to simultaneous inoculation of O serum and O virus.

EXPERIMENT No. 19.—With serum mixture No. 133 O strain and virus mule 2287 O strain.

Serum dose 300 c.c., virus dose 2 c.c.

Serum and virus injected subcutaneously and simultaneously.

Date, 18th January, 1907.

1. *Mule 2547.*—*Immunised* as above.*Result.*—Reaction.

Not tested.

2. *Mule 2548.*—*Immunised* as above.*Result.*—Distinct reaction.

Not tested.

3. *Mule 2549.*—*Immunised* as above.*Result.*—Reaction and died of horse-sickness on the 10th day.*Conclusion.*—Typical reactions due to simultaneous inoculation of O serum and O virus.

EXPERIMENT No. 20.—With serum mixture No. 135 O strain and virus mule 2287 O strain.

Serum dose 300 c.c., virus dose 2 c.c.

Serum and virus injected subcutaneously and simultaneously.

Date, 22nd January, 1907.

1. *Mule 2578.*—

Result.—Reaction, dikkop on the 10th day, and death on the 11th day from horse-sickness.

2. *Mule 2579.*—

Immunised as above.

Result.—Distinct reaction.

Not tested.

3. *Mule 2580.*—

Immunised as above.

Result.—Distinct reaction.

Not tested.

Conclusion.—Typical reactions due to simultaneous inoculation of serum O and virus O.

EXPERIMENT No. 21.—With serum mixture No. 137 O strain and virus mule 2287 O strain.

Serum dose 300 c.c., virus dose 2 c.c.

Serum and virus injected subcutaneously and simultaneously.

Date, 22nd January, 1907.

1. *Mule 2582.*—

Immunised as above.

Result.—Reaction, dikkop on the 10th day, and died on the 11th day.

2. *Mule 2583.*—

Immunised as above.

Not tested.

Result.—Reaction.

3. *Mule 2584.*—

Immunised as above.

Result.—Reaction.

Not tested.

Conclusion.—Typical reactions due to simultaneous inoculation of serum O and virus O.

EXPERIMENT No. 22.—With serum mixture No. 139 O strain and virus mule 2287 O strain.

Serum dose 300 c.c., virus dose 2 c.c.

Serum and virus injected subcutaneously and simultaneously.

Date, 22nd January, 1907.

1. *Mule 2585.*—

Injected as above.

Result.—Distinct reaction.

2. *Mule 2586.*—

Injected as above.

Result.—Slight reaction.

3. *Mule 2587.*—

Injected as above.

Result.—Slight reaction.

Conclusion.—Typical reactions due to simultaneous inoculation of serum O and virus O.

TABULATED RÉSUMÉ FROM PREVIOUS EXPERIMENTS.
ADEQUATE SERUM AND VIRUS.

Mice.	IMMUNISATION.				I. TEST.			II. TEST		
	Se- rum.	Virus.		Result.	Virus.		Result.	Virus.		Result.
		Orig.	Orig.		Gen.	Orig.		Gen.	Orig.	
2566	Ord.	Ord.	38	R	Ord.	38	—	—	—	—
2567	"	"	38	R	"	38	—	—	—	—
2568	"	"	38	R	"	38	—	—	—	—
2569	"	"	38	R	"	38	—	—	—	—
2570	"	"	38	R	"	38	—	—	—	—
2571	"	"	38	R	"	38	—	—	—	—
2572	"	"	38	R	"	38	—	—	—	—
2573	"	"	38	R	"	38	—	—	—	—
2574	"	"	38	RD	"	38	—	—	—	—
2575	"	"	38	R	"	38	—	—	—	—
2576	"	"	38	R	"	38	—	—	—	—
2577	"	"	38	R	"	38	—	—	—	—
2525	"	"	38	R	Tzn.	1	R	Bul.	1	R
2526	"	"	38	R	—	—	—	—	—	—
2527	"	"	38	R	—	—	—	—	—	—
2528	"	"	38	RD	Bul.	1	RD	Tzn.	1	—
2529	"	"	38	RD	Tzn.	1	R	Bul.	1	—
2530	"	"	38	RD	—	—	—	—	—	—
2531	"	"	38	R	—	—	—	—	—	—
2532	"	"	38	R	Bul.	1	RD†	—	—	—
2533	"	"	38	RD	—	—	—	—	—	—
2508	"	"	38	R	—	—	—	—	—	—
2509	"	"	38	R	—	—	—	—	—	—
2511	"	"	38	R	—	—	—	—	—	—
2512	"	"	38	R	—	—	—	—	—	—
2534	"	"	38	?	—	—	—	—	—	—
2535	"	"	38	R	—	—	—	—	—	—
2536	"	"	38	R	—	—	—	—	—	—
2537	"	"	38	R	—	—	—	—	—	—
2538	"	"	38	R	—	—	—	—	—	—
2540	"	"	38	R	—	—	—	—	—	—
2541	"	"	38	R	—	—	—	—	—	—
2542	"	"	38	R	—	—	—	—	—	—
2543	"	"	38	R	—	—	—	—	—	—
2544	"	"	38	R	—	—	—	—	—	—
2545	"	"	38	R	—	—	—	—	—	—
2546	"	"	38	R	—	—	—	—	—	—
2547	"	"	38	R	—	—	—	—	—	—
2548	"	"	38	R	—	—	—	—	—	—
2549	"	"	38	R†	—	—	—	—	—	—
2578	"	"	38	RD†	—	—	—	—	—	—
2579	"	"	38	R	—	—	—	—	—	—
2580	"	"	38	R	—	—	—	—	—	—
2582	"	"	38	RD†	—	—	—	—	—	—
2583	"	"	38	R	—	—	—	—	—	—
2584	"	"	38	R	—	—	—	—	—	—
2585	"	"	38	R	—	—	—	—	—	—
2586	"	"	38	R	—	—	—	—	—	—
2587	"	"	38	R	—	—	—	—	—	—

R—Reaction. ?—Doubtful. RD—Reaction with Dikkop. R†—Reaction and died.
RD†—Reaction with Dikkop and died.

ANALYSIS OF RESULTS.

Deaths.

49 mules immunised with virus Ordinary, 38th generation: 3 deaths.

*Tests.*A.—*Tests with Ordinary Virus.*

12 mules tested with Ordinary, 38th generation, gave no results.

B.—*Tests with Bulawayo Virus.*

1 mule tested with Bulawayo, 1st generation, had reaction, dikkop, and died.

C.—*1st Test with Bulawayo and 2nd Test with Tzaneen Virus.*

1 mule tested with Bulawayo, 1st generation, and afterwards with Tzaneen, 1st generation, had reaction and dikkop with 1st test and no reaction from the 2nd test.

D.—*1st Test with Tzaneen and 2nd Test with Bulawayo Virus.*

2 mules tested with Tzaneen, 1st generation, and later with Bulawayo, 1st generation:

1 mule reacted with both tests and

1 mule only with 1st test (Tzaneen).

30 mules were not tested.

INADEQUATE AND ADEQUATE SERUM AND VIRUS.

Résumé of Conclusions.

1. The simultaneous injection of serum O and adequate virus resulted in typical horse-sickness reactions, and the immunity obtained therefrom prevented a reaction when the adequate virus is subsequently reinjected.

2. The immunity obtained by a reaction after injection of serum O and virus O (adequate serum and virus) does not prevent a reaction due to a subsequent inoculation of virus of a different strain (Tzaneen and Bulawayo).

3. The simultaneous injection of serum O and virus Tzaneen (inadequate serum) is succeeded by reactions which proved not to be more fatal to animals than that due to virus O (adequate serum).

4. The immunity obtained by this reaction was in no way complete. It did not prevent a reaction either due to a subsequent inoculation of a different strain (O or Bulawayo), nor did it prevent reactions when the same strain of Tzaneen virus of a later generation was utilised.

5. The fact that a reaction was noted due to the subsequent inoculation of virus in an animal immunised with the same strain of virus can be explained either by accepting that the test virus, being of a higher generation and derived from horses, is of a greater virulency, or that the virus Tzaneen is already of a complex nature containing certain constituents of the O virus which are deviated by the O serum and accordingly during the immunisation leave no impression on the system of the animal. A subsequent inoculation of the same strain would then not meet the corresponding antibodies and a reaction would result.

6. In some of the animals a single injection of virus, together with adequate or inadequate serum, produced complete immunity against subsequent inoculations, hence a factor in the animal has also something to do with the creation of immunity.

7. In the foregoing experiments it has been noted that a virus can attenuate and completely lose its virulency.

8. Virus T of the 12th generation has increased enormously in virulency, which shows itself in the immunisation and in the tests.

“G.”—INOCULATION OF MULES WITH POLYVALENT VIRUS AND SERUM.

Under the term “polyvalent serum” in this article is understood either a serum which is composed of various monovalent sera, obtained by mixing them together, or by a serum obtained from a horse previously injected with a mixture of three or more vira.

The object of the experiments was to determine whether such a polyvalent serum can be utilised with greater advantage in practice, and if the immunity afforded by the polyvalent virus would be a better protection against horse-sickness than that given by a monovalent virus.

Serum O-T-B.—This is a mixture made on the 5th November, 1906, and consisting of serum O (that is of horses immunised with the virus hitherto used on this station), serum T strain (of virus obtained from the Tzaneen Estate, Zoutpansberg), and serum B or Bulawayo (of horses hyperimmunised with a strain of virus obtained from Bulawayo).

(See also explanations on pages 89 and 90.)

EXPERIMENT No. 1.—On the 6th November, 1906, 400 c.c. serum O-T-B to be injected simultaneously and subcutaneously with virus O-T-B respectively of mules 2287 and 2268 and horse 2083.

1. Mule 2388.—

Injected as above.

Result.—A slight reaction.

Tested on its immunity on the 27th November, with 10 c.c. virus 2268 Tzaneen strain, injected intrajugularly. On the 7th December the same dose was repeated. Slight reaction, the character of which was, however, doubtful. On the 18th December 2 c.c. virus mule 2287 strain O was injected subcutaneously. No reaction due to this injection.

2. Mule 2381.—

Injected as above.

Result.—A slight but distinct typical reaction ensued.

Tested for immunity on the 27th November with 10 c.c. Bulawayo virus horse 2083, injected into jugular vein. This dose was repeated on the 7th December. There was a reaction which might be due to the injection of the 27th November, but which was not typical. Injected on the 18th December with 2 c.c. virus mule 2268 Tzaneen strain. There was an atypical reaction.