

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.

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### “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.

3. *Mule 2377.*—

*Injected* as above.

*Result.*—Hardly any reaction noticeable.

*Tested.*—On the 27th November and 7th December with 10 c.c. virus 2287 strain O. There was a reaction which might be due to the injection of the 27th November. On the 18th December 2 c.c. virus 2268 Tzaneen strain was subcutaneously injected. No reaction ensued.

*Conclusion.*—The immunity obtained from the virus mixture O-T-B protected the animals against any individual strain of which the virus was composed.

EXPERIMENT No. 2.—With serum as above, injected simultaneously and subcutaneously.

Dose 400 c.c. with virus mixture O-T, viz., mule 2287 and mule 2268.

1. *Mule 2378.*—

*Injected* as above on the 6th November.

*Result.*—There was a slight and typical reaction.

*Tested* for its immunity on the 27th November by an intrajugular injection of 10 c.c. virus same mixture; on the 7th December injected with 10 c.c. virus mule 2268 strain Tzaneen. There was a slight reaction, which may be due to the injection on the 27th November. On the 18th December injected subcutaneously with 2 c.c. virus mule 2287 Ordinary strain. No reaction after this injection.

2. *Mule 2385.*—

*Injected* as above.

*Result.*—A short atypical reaction was noticed.

*Tested* on the 8th December by an intrajugular injection of 10 c.c. virus of the same mixture. A reaction took place not typical for horse-sickness. On the 18th December injected subcutaneously with 2 c.c. virus 2199 strain Tzaneen. A reaction typical for horse-sickness took place, although the diagnosis could not be verified by clinical examination. On the 12th January, 1907, the animal was subcutaneously injected with 2 c.c. virus 2287 strain O. Reaction, but not typical for horse-sickness.

3. *Mule 2386.*—

*Injected* as above.

*Result.*—There was no noticeable reaction due to this injection.

*Tested* for immunity on the 27th November by intrajugular injection of 10 c.c. virus same mixture, and this dose was repeated on the 7th December. There was a slight reaction which was probably due to the injection of the 27th November. On the 18th December an injection of 2 c.c. virus 1938 Ordinary strain was made. No reaction.

*Conclusion.*—The immunity obtained from the mixture O-T virus protected the mules against the subsequent injection of the same virus, and also against its components, but one

animal showed a reaction, which would indicate that the protection was not complete

4. *Mule 2384.*—

*Injected* as above.

*Result.*—Typical horse-sickness reaction.

*Tested* on the 27th November with intrajugular injection of 10 c.c. virus of the same mixture. The dose was repeated on the 7th December. A reaction ensued, probably due to the injection on the 27th November. On the 16th December this mule showed symptoms of dikkop, and died two days later.

5. *Mule 2387.*—

*Injected* as above.

*Result.*—A slight reaction took place.

*Tested* on the 8th December by intrajugular injection of 10 c.c. virus of same mixture. An atypical reaction was noted. On the 18th December an injection of 2 c.c. virus 2199 Tzaneen strain was made. Again a slight reaction was noted. On the 12th January, 1907, a further injection of 2 c.c. virus Ordinary strain took place. This injection was also followed by a reaction.

6. *Mule 2380.*—

*Injected* as above.

*Result.*—Hardly any reaction noticeable.

*Tested* on the 27th November with 10 c.c. virus of same mixture injected into the jugular vein. This dose was repeated on the 7th December. There was a slight reaction, but it was not typical. On the 18th December the animal was injected with 2 c.c. virus 2287 Ordinary strain. Again a reaction, which was atypical.

The immunity obtained through the inoculation of the virus O-T did not protect mule 2384 against the same virus mixture, and it died of horse-sickness. The other two animals also showed reactions, indicating that the immunity was not complete.

*Conclusion.*—By the simultaneous inoculation of a polyvalent serum composed of three different sera in equal quantities in conjunction with (a) the simultaneous injection of a mixture of the three corresponding virus, and (b) a mixture of two virus, an immunity was, generally speaking, established, but this immunity was not complete against subsequent inoculations of the same mixture, or of the components of the mixture. It must therefore be concluded that the immunity was only obtained against one component of the mixture, which immunity is, however, sufficient in the majority of cases to protect the animal against death due to another strain of virus.

EXPERIMENT No. 3.—Serum mixture of (1) mules hyperimmunised with Ordinary virus, (2) of horses hyperimmunised with Ordinary virus, (3) of horses hyperimmunised with Tzaneen virus, and (4) horses hyperimmunised with Bulawayo virus, mixed in equal quantities and injected simultaneously and subcutaneously with a virus mixture O-T-B.

(1a) Virus mixture of mules, viz., mule 2287 Ordinary virus, mule 2268 Tzaneen virus, and mule 1954 Bulawayo virus.

Dose of serum 400 c.c.

1. *Mule 2467.*—

*Injected* as above, on the 5th December, 1906.

*Result.*—A slight reaction.

*Tested* on the 23rd January, 1907, with 2 c.c. virus of same mixture. An atypical reaction took place.

2. *Mule 2466.*—

*Injected* as above.

*Result.*—A distinct reaction.

*Tested* with 2 c.c. virus mixture O-T-B of horses 2407 (Ordinary), 1869 (Tzaneen), and 2359 (Bulawayo). Typical reaction ensued, and the animal died on the 2nd February, 1907, from horse-sickness.

3. *Mule 2472.*—

*Injected* as above.

*Result.*—A distinct and typical reaction ensued.

*Tested* on the 23rd January, with a mixture O-T-B of mule virus. An atypical reaction.

4. *Mule 2474.*—

*Injected* as above.

*Result.*—A slight reaction was noticed.

*Tested* on the 23rd January with a mixture O-T-B of horse virus. A typical reaction, and mule died of horse-sickness on the 3rd February.

5. *Mule 2468.*—

*Injected* as above.

*Result.*—Reaction.

*Tested* on the 23rd January with virus horse 2418. This virus is called OTB, obtained from the horse 2418 which was injected simultaneously with virus O horse 1938, with virus T horse 2199, and with virus B mule 1964. No reaction noted.

6. *Mule 2471.*—

*Injected* as above.

*Result.*—A distinct and typical reaction ensued.

*Tested* on the 23rd January by subcutaneous injection of 2 c.c. virus 2418 OTB. A reaction ensued due to this inoculation.

7. *Mule 2475.*—

*Injected* as above.

*Result.*—A typical reaction ensued, from which it recovered.

The animal died on the 12th January, 1907, of debility.

*Conclusion.*—The inoculation of the mixture of mule virus of the three various strains produced a reaction, and accordingly immunity had to be expected. When tested with virus of the same mixture, only a slight reaction took place, thus proving that the required immunity was sufficient to protect the animal against the subsequent inoculation. When tested with horse virus of the same strains two animals

succumbed. When tested with OTB virus of one horse the immunity proved sufficient, one animal showing a slight reaction.

(1b) Serum and virus as above.

Dose of serum 300 c.c.

8. *Mule 2473.*—

*Injected* as above on the 5th December, 1906, with virus O of mule 2287, T of mule 2268, and B of mule 1954.

*Result.*—There was a slight reaction.

*Tested* on the 23rd January, 1907, with 2 c.c. virus subcutaneously injected of the same mixture. There was again a reaction.

9. *Mule 2461.*—

*Injected* as above.

*Result.*—Reaction.

*Tested* on the 23rd January by injection separately of the three different virus. Nothing particular noted.

10. *Mule 2464.*—

*Injected* as above.

*Result.*—Distinct reaction.

*Tested* on the 23rd January with virus 2418. Nothing particular was noted.

11. *Mule 2470.*—

*Injected* as above on the 5th December.

*Result.*—Reaction.

*Tested* on the 23rd January by an injection of 2 c.c. virus horse 2406. This virus was derived from a horse which had been injected with virus O, with virus T, with virus B, and a virus of immunised mules which in practice had relapses. There were in addition to the foregoing mules one from Lydenburg, one from Piet Retief, one from Warmbaths, and two from Tzaneen (Altenroxel); this virus is called OTBLPW. There was no reaction due to this test.

12. *Mule 2450.*—

*Injected* as above.

*Result.*—Reaction.

*Tested* on the 3rd January with 2 c.c. virus of mule 2287 Ordinary strain. There was a slight reaction—not certain whether due to this injection. On the 23rd January tested again by 2 c.c. virus mule 1954 Bulawayo strain; no reaction.

13. *Mule 2448.*—

*Injected* as above on the 5th December.

*Result.*—Reaction.

*Tested* on the 3rd January with 2 c.c. virus mule 2268 Tzaneen strain; reaction not typical. Tested again on the 23rd January with 2 c.c. virus mule 2287 Ordinary strain. There was a retarded reaction, not likely due to this injection.

14. *Mule 2469.*—

*Injected* as above on the 5th December.

*Result.*—Reaction.

*Tested* on the 3rd January with 2 c.c. virus mule 1954 Bulawayo strain; a retarded reaction, probably not due to this injection. On the 23rd January tested with 2 c.c. virus mule 2268 Tzaneen strain. Doubtful horse-sickness reaction.

*Conclusion.*—The inoculation of O-T-B virus protected against a subsequent inoculation of the same virus, of virus OTBLPW, and against the constituents of the virus mixture. There were, however, reactions in some instances, not typical for horse-sickness, and therefore it is uncertain whether they were produced by the test virus.

IIa.—Virus mixture of horses (2407 O; 1869 Tz.; 1959 B). Serum as before, but dose of 400 c.c.

15. *Mule 2449.*—

*Injected* on the 5th December with 400 c.c. serum and 2 c.c. of above virus mixture.

*Result.*—Slight reaction.

*Tested* on the 23rd January with 2 c.c. virus mixture O-T-B of mule 2287 Ord., mule 2268 Tzaneen, and mule 1954 Bulawayo. Doubtful reaction.

16. *Mule 2447.*—

*Injected* as above.

*Result.*—Distinct reaction.

*Tested* on the 23rd January by an injection of a mixture of the same virus with which it was immunised. Distinct reaction.

17. *Mule 2446.*—

*Injected* as above.

*Result.*—Distinct reaction.

*Tested* on the 23rd January with the three strains of mule virus, injected separately. No reaction.

18. *Mule 2462.*—

*Injected* as above.

*Result.*—Distinct reaction.

*Tested* on the 23rd January by an injection of 2 c.c. virus of the three strains of horses separately. Distinct reaction.

19. *Mule 2459.*—

*Injected* as above.

*Result.*—Slight but distinct reaction.

*Tested* on the 23rd January by an injection of 2 c.c. virus OTB horse 2418. No reaction.

20. *Mule 2465.*—

*Injected* as above.

*Result.*—Distinct reaction.

*Tested* on the 23rd January by injection of 2 c.c. virus OTBLPW of horse 2406. Slight but distinct reaction.

*Conclusion.*—The immunity obtained from the injection of the three strains of horse virus protected against a subsequent inoculation of mule virus of the same strain against which they were immunised, but the test with horse virus of the same strain produced reactions.

- 11b. Virus and serum as above, but dose of serum 300 c.c.
21. *Mule 2452.*—  
*Injected* on the 5th December with 300 c.c. serum and 2 c.c. virus mixture of horse virus O-T-B.  
*Result.*—Distinct reaction.  
*Tested* on the 23rd January with 2 c.c. virus of same mixture. Reaction, and death resulted on the 11th day from piroplasmosis.
22. *Mule 2459.*—  
*Injected* as above.  
*Result.*—Hardly any reaction.  
*Tested* on the 23rd January by separate injections of the three strains of horse virus. Slight but distinct reaction.
23. *Mule 2451.*—  
*Injected* as above.  
*Result.*—Slight reaction.  
*Tested* on the 23rd January by an injection of 2 c.c. virus OTB horse 2418; a distinct and typical reaction ensued.
24. *Mule 2453.*—  
*Injected* as above.  
*Result.*—Slight reaction.  
*Tested* on the 23rd January with 2 c.c. virus OTBLPW horse 2406. Distinct reaction.
25. *Mule 2458.*—  
*Injected* as above.  
*Result.*—Distinct reaction.  
*Tested* on the 4th January with virus horse 2407 Ord. strain; reaction. Retested on the 23rd January with 2 c.c. virus horse 2359 Bulawayo strain; no reaction.
26. *Mule 2460.*—  
*Injected* as above.  
*Result.*—Distinct reaction.  
*Tested* on the 23rd January with 2 c.c. virus horse 1869 Tzaneen strain; retarded reaction, not typical. Retested on the 23rd January with 2 c.c. virus horse 2407 Ord. strain; doubtful reaction.
27. *Mule 2457.* —  
*Injected* as above.  
*Result.*—Distinct reaction.  
*Tested* on the 3rd January with 2 c.c. virus horse 1959 Bulawayo strain; retarded reaction, not typical for horse-sickness. Retested on the 23rd January with 2 c.c. virus horse 1869 Tzaneen strain; no reaction.  
*Result.*—The immunisation with the three strains of horse virus did not protect against a subsequent inoculation of a mixture of the same virus, but protected when injected separately. It protected against virus OTB and OTBLPW, but in both instances reactions were noted. It protected against the constituents of the virus separately injected, although some doubtful reactions were noted.
- Résumé of Results.*—Of 14 animals immunised with a mixture of the three strains of mule virus two died of horse-sickness when tested with horse virus of the same strains;

some showed distinct and some showed doubtful reactions when injected with constituents of the same mixture. Of 13 animals immunised with a mixture of the three strains of horse virus, one died when tested with the same mixture. There were also distinct and doubtful reactions noted after injection of the mixture and constituents of the various kinds of virus.

*Conclusion.*—The immunisation by means of serum of mules and horses adequate to the three strains and the subcutaneous and simultaneous inoculation of corresponding virus protected, in the majority of cases, against a subsequent inoculation of the mixture or constituents of the said virus, but not completely, inasmuch as three deaths occurred and many reactions were noted. The death was due to the injection of the three strains of virus derived from horses. It follows, therefore, that the virus of horses of the same strain is most virulent. The practical conclusion is that the immunisation against the three strains in the way indicated, viz., by mixing adequate sera and injecting the same against adequate virus mixture, does not produce sufficient immunity.

EXPERIMENT NO. 4.—Serum mixture of mules (hyperimmunised with Ord. virus), of horses (hyperimmunised with Tzaneen virus), of horses (hyperimmunised with Bulawayo virus), injected subcutaneously and simultaneously with adequate virus derived from horses and mules.

(a) Dose of serum 300 c.c.; virus mixture of horse 2407 (Ord. strain), of horse 1869 (Tzaneen strain), of horse 2359 (Bulawayo strain), 3 c.c., injected subcutaneously and simultaneously.

1. *Mule 2484.*—

*Injected* on the 19th December, 1906, with 300 c.c. serum of above mixture and 3 c.c. horse virus O-T-B.

*Result.*—Distinct and typical reaction.

*Tested* on the 23rd January with 2 c.c. virus of same mixture.  
No reaction.

2. *Mule 2485.*—

*Injected* as above.

*Result.*—Reaction, with symptoms of dikkop, and the animal died on the 12th day.

3. *Mule 2486.*—

*Injected* as above.

*Result.*—Distinct reaction.

*Tested* on the 23rd January with 2 c.c. virus of same mixture.  
No reaction.

4. *Mule 2487.*—

*Injected* as above.

*Result.*—Distinct reaction.

*Tested* on the 23rd January with 2 c.c. virus of same mixture.  
No reaction.

5. *Mule 2488.*—

*Injected* as above.

*Result.*—Distinct reaction.

*Tested* on the 23rd January with same mixture. No reaction.



*Conclusion.*—The immunity given to five mules by a mixture of three strains of horse virus injected in the dose of 3 c.c. protected against a subsequent inoculation of virus of the same strain.

(b) Dose of serum 300 c.c. virus mixture of mule 2287 (Ord. strain), of mule 2268 (Tzaneen strain), and mule 1954 (Bulawayo strain).

6. *Mule 2489.*—

*Injected* on the 19th December, as indicated above.

*Result.*—Distinct and typical reaction.

*Tested* on the 23rd January with same mixture; a slight reaction.

7. *Mule 2490.*—

*Injected* as above.

*Result.*—Distinct reaction.

*Tested* on the 23rd January with 2 c.c. of same mixture; a severe and high reaction.

8. *Mule 2491.*—

*Injected* as above.

*Result.*—Reaction.

*Tested* on the 23rd January with same mixture of virus; no reaction.

9. *Mule 2492.*—

*Injected* as above.

*Result.*—Slight and typical reaction.

*Tested* on the 23rd January with same mixture; typical reaction and signs of dikkop.

10. *Mule 2493.*—

*Injected* as above.

*Result.*—Typical reaction.

*Tested* on 23rd January with same mixture; slight reaction.

*Result.*—The five mules immunised by the mixture of the three strains protected against a subsequent inoculation of the same three strains, but reactions were noted in four cases, and in one with signs of dikkop, showing that the protection was not complete.

*Résumé.*—The immunity obtained by the inoculation of the three strains of horse virus protects better against itself than the immunity obtained from the three strains of mule virus protects against the same three strains of mule virus.

*Conclusion.*—Immunity obtained from the three strains of horse virus is stronger than the immunity obtained from the three strains of mule virus.

EXPERIMENT No. 5.—Serum mixture of horses immunised with virus O, of horses immunised with virus T, and of horses immunised with virus B, injected in the dose of 200 c.c. and the corresponding virus 3 c.c. subcutaneously and simultaneously.

(a) Virus mixture of mules 2287 O, 2268 T, and 1954 B, 24th December, 1906.

1. *Mule 2500.*—

*Injected* with 200 c.c. serum and 3 c.c. virus, as indicated above.

*Result.*—Slight but distinct reaction.

- Tested* on the 23rd January with 2 c.c. virus same mixture; slight reaction.
2. *Mule 2501.*—  
*Injected* as above.  
*Result.*—Distinct reaction, and signs of dikkop on the 15th day.  
*Tested* on the 23rd January by the same virus, injected separately. No reaction.
3. *Mule 2502.*—  
*Injected* as above.  
*Result.*—Distinct reaction, with lesions of dikkop on the 16th day.  
*Tested* on the 23rd January with 2 c.c. virus of same mixture. Slight reaction.  
*Conclusion.*—The reduction of the dose of serum to 200 c.c. caused a more distinct immunisation reaction, which protected against the subsequent inoculation of the same virus, but still accompanied with a slight reaction.
- (b) Virus mixture of horses 2407 O, 1869 T, and 2359 B.
4. *Mule 2503.*—  
*Injected* on the 24th December with 200 c.c. serum mixture and 3 c.c. virus mixture.  
*Result.*—Distinct reaction.  
*Tested* on the 23rd January with 2 c.c. virus of same mixture. Doubtful reaction.
5. *Mule 2504.*—  
*Injected* as above.  
*Result.*—Distinct reaction.  
*Tested* on the 23rd January with same virus mixture; slight reaction.
6. *Mule 2505.*—  
*Injected* as above.  
*Result.*—Distinct reaction, with signs of dikkop. The animal died on the 24th day after inoculation, with lesions of horse-sickness.  
*Result.*—Of three animals treated in the way indicated above one died as a result of the inoculation, and two reacted to the inoculation, and when tested on their immunity they proved refractory. There was a slight reaction, but somewhat doubtful as to its nature.  
*Conclusion.*—The reduction of the dose of serum to 200 c.c. permits a more severe immunisation reaction. The immunity obtained is the same as that obtained with a stronger dose of serum.

EXPERIMENT NO. 6.—With serum mixture of mules hyperimmunised with O virus, of horses hyperimmunised with O virus, of horses with T virus, and horses with B virus, injected subcutaneously and simultaneously with 2 c.c. virus OTB horse 2418 (a horse injected with the three strains of virus.)

1. *Mule 2506.*—  
*Injected* on the 24th December, 1906, with 300 c.c. serum of above mixture and 2 c.c. virus OTB.  
*Result.*—Slight reaction.

*Tested* on the 23rd January by separate injections of 2 c.c. virus of horses 2407, 1869 and 2359. Slight reaction, not quite typical for horse-sickness.

2. *Mule 2507.*—

*Injected* as above.

*Result.*—Slight reaction.

*Tested* on the 23rd January with a mixture of the three-horse vira. There was a slight reaction.

*Result.*—The immunity obtained through the injection of the virus OTB protected against a subsequent inoculation of the constituents of the same virus when injected either separately or mixed. A slight reaction was noticed after the test.

*Conclusion.*—The immunity obtained from OTB virus protected against its constituents.

EXPERIMENT No. 7.—Serum mixture of horses O-T-B. Dose of serum 300 c.c.

(a) Virus 2418 OTB (*vide* previous Experiment).

1. *Mule 2513.*—

*Injected* on the 12th January, 1907, with 300 c.c. serum of above mixture and 2 c.c. virus 2418.

*Result.*—Reaction.

*Tested* on the 20th February with 2 c.c. virus mule 2287 Ord. strain; reaction. Retested on the 13th March with 2 c.c. virus mule 1954 Bulawayo strain; no reaction.

2. *Mule 2514.*—

*Injected* as above.

*Result.*—Distinct reaction with signs of dikkop on the 9th day.

*Tested* on the 21st February with 2 c.c. virus mule 2268 Tzaneen; typical reaction with dikkop on the 10th day. Retested on the 13th March with 2 c.c. virus mule 1954 Bulawayo; again a typical reaction.

3. *Mule 2515.*—

*Injected* as above, and died on the 12th day from horse-sickness.

4. *Mule 2516.*—

*Injected* as above.

*Result.*—Typical reaction.

*Tested* on the 21st February with 2 c.c. virus mule 1954 Bulawayo; typical reaction. Retested on the 13th March with virus 2268 Tzaneen; no reaction.

5. *Mule 2517.*—

*Injected* as above.

*Result.*—Reaction, and death from dikkop on the 13th day.

6. *Mule 2518.*—

*Injected* as above.

*Result.*—Typical reaction.

*Tested* on the 21st February with 2 c.c. virus mule 2287 Ord., horse 1869 Tzaneen, and mule 1954 Bulawayo; typical and distinct reaction. Retested on the 13th March with 2 c.c. virus 2287 Ord. strain; no reaction.

*Conclusion.*—Of six mules immunised with OTB virus 2418 and serum mixture of horses, two died. The remainder

obtained an immunity, which was, however, not complete, inasmuch as the subsequent inoculation of the various strains again produced reactions, mule 2514 even developing dikkop for a second time.

(b) Virus 2406 OTBLPW [*vide* Experiment No. 3 (11)].

7. *Mule 2519.*—

*Injected* on the 12th January, 1907, with 300 c.c. serum and 2 c.c. virus 2406.

*Result.*—Reaction.

*Tested* on the 21st February with 2 c.c. virus 2287 Ord.; reaction. Retested on the 13th March with 2 c.c. virus 2268 Tzaneen; no reaction.

8. *Mule 2520.*—

*Injected* as above.

*Result.*—Reaction.

*Tested* on the 21st February with 2 c.c. virus 2268 Tzaneen; slight reaction. Retested on the 13th March with 2 c.c. virus 1954 Bulawayo; no reaction.

9. *Mule 2521.*—

*Injected* as above.

*Result.*—Died of horse-sickness on the 11th day.

10. *Mule 2522.*—

*Injected* as above.

*Result.*—Reaction.

*Tested* on the 21st February with 2 c.c. virus 1954 Bulawayo; reaction, lesions of dikkop; animal died on the 12th day, and on post-mortem examination showed lesions of horse-sickness and piroplasmosis.

11. *Mule 2523.*—

*Injected* as above.

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

*Tested* on the 21st February with 2 c.c. virus 2287 O, 2268 T, and 1954 B; reaction. Retested on the 13th March with 2 c.c. virus 1954 B; no reaction.

12. *Mule 2524.*—

*Injected* as above.

*Result.*—Reaction.

*Tested* on the 21st February with 2 c.c. virus OTB 2418; slight reaction. Retested on the 13th March with 2287 Ordinary; no reaction.

*Result.*—Of six animals immunised with virus OTBLPW one died due to immunisation. When tested, one died with lesions of dikkop, and all showed reactions when tested with one of the constituents of the virus.

*Conclusion.*—The immunisation with a serum mixture composed of serum of horses hyperimmunised O, hyperimmunised T, and hyperimmunised B, and of virus of a polyvalent nature containing the constituents adequate to the serum, does not protect completely against the same constituents when injected separately or mixed. It therefore cannot be expected that the polyvalent immunity will be obtained in the way indicated. This fact is probably due

to an inadequate fitting of the serum mixture to the polyvalent virus whereby a surplus of one or the other serum occurs, overcompensating the adequate strain of the polyvalent virus, so that it does not leave any impression on the system of the animal, and therefore causes no immunity.

EXPERIMENT No. 8.—With serum OTB, viz., of horses hyperimmunised with virus origin 2418 OTB.

1. *Mule 2581.*—

*Injected* on the 25th January, 1907, with 300 c.c. serum OTB and 2 c.c. virus 2418 simultaneously and subcutaneously.

*Result.*—Distinct reaction.

*Tested* on the 28th March with 2 c.c. virus 1954 Bulawayo; a slight but retarded reaction. Retested on the 23rd April with 2 c.c. virus 2628 Ordinary; no reaction. Retested on the 7th May with 2 c.c. virus mixture O-T-B of horse 2709, horse 2148, and horse 2168; distinct reaction.

2. *Mule 2588.*—

*Injected* as above.

*Result.*—Slight reaction.

*Tested* on the 4th April with 2 c.c. virus 1954 Bulawayo; no reaction. Retested on the 23rd April with 2 c.c. virus horse 2709 O; no reaction. Retested on the 7th May with virus mixture O-T-B of horses 2709, 2148, and 2168; slight reaction.

3. *Mule 2589.*—

*Injected* as above.

*Result.*—Hardly any reaction.

*Tested* on the 20th March with 2 c.c. virus 2418 OTB; no reaction. Retested on the 4th April with virus mule 2628 Ordinary, mule 2268 Tzaneen, and mule 1954 Bulawayo, injected separately; no reaction. Retested on the 23rd April with virus horses 2709, 2148, and 2298, viz., O-T-B separately; a slight atypical reaction.

4. *Mule 2590.*—

*Injected* as above.

*Result.*—Very slight reaction.

*Tested* on the 6th March with 2 c.c. virus 2418 OTB; no reaction. Retested on the 28th March with 2 c.c. virus 2287 Ordinary; no reaction. Retested on the 23rd April with 2 c.c. virus 2298 Bulawayo; an atypical reaction. Retested on the 7th May by an injection of virus O 2709, virus T 2148, and virus B 2168; no reaction.

5. *Mule 2591.*—

*Injected* as above.

*Result.*—Reaction.

*Tested* on the 28th March with virus of mule 2268 Tzaneen; no reaction. Retested on the 23rd April with 2 c.c. virus horse 2148 Tzaneen; reaction. Retested on the 7th May with a mixture of virus O-T-B horses 2709, 2148, and 2168; no reaction.

*Conclusion.*—The immunisation with serum OTB and corresponding virus OTB protected against a subsequent inoculation of any constituent of the virus separately or collectively injected. In the case of 2581, however, there was a distinct reaction to the injection of horse virus mixture. In the other instances slight and atypical reactions were noted, and it may be doubtful whether they are due to the test injection.

EXPERIMENT No. 9.—With serum OTBLPW, viz., of horses hyper-immunised with virus origin horse 2406 OTBLPW.

1. *Mule 2592.*—

*Injected* on the 25th January with 300 c.c. serum OTBLPW and 2 c.c. virus horse 2406 subcutaneously and simultaneously.

*Result.*—Slight reaction.

*Tested* on the 4th April with 2 c.c. virus mule 2268 Tzaneen strain; no reaction. Retested on the 23rd April with 2 c.c. virus horse 2148 Tzaneen; also no reaction. Retested on the 7th May with mixture of virus O-T-B of horses 2709, 2148, and 2168; no reaction.

2. *Mule 2594.*—

*Injected* as above.

*Result.*—Slight reaction.

*Tested* on the 4th April with 2 c.c. virus of mule 2628 Ordinary; no reaction. Retested on the 23rd April with 2 c.c. virus of horse 2148 Tzaneen; no reaction. Retested on the 7th May with mixture of horse virus 2709 Ordinary strain, 2148 Tzaneen, and 2168 Bulawayo strain; no reaction.

3. *Mule 2595.*—

*Injected* as above.

*Result.*—Slight reaction.

*Tested* on the 28th March with 2 c.c. virus of mule 2287 Ordinary strain; reaction. Retested on the 23rd April with 2 c.c. virus of 2298 Bulawayo strain; reaction. Retested on the 7th May with mixture of horse virus 2709, 2148, and 2168; reaction.

4. *Mule 2596.*—

*Injected* as above.

*Result.*—Slight reaction.

*Tested* on the 20th March with 2 c.c. virus of 2406 OTBLPW; no reaction. Retested on the 4th April with mixture of mule 2628 O, 2268 T, and 1954 B strain; no reaction. Retested on the 23rd April with 2 c.c. horse virus of 2709, 2148 and 2298, injected separately; slight and short reaction.

*Result.*—The immunisation of four mules with serum OTBLPW and corresponding virus protected against a subsequent inoculation of any constituents of this virus injected separately or collectively. The two first animals showed no reaction to the test; the third animal had a reaction after every injection, and the fourth animal showed an atypical reaction.

TABULATED RÉSUMÉ OF TESTS OF PREVIOUS EXPERIMENTS.  
POLYVALENT SERUM AND POLYVALENT VIRUS.

Mule.	IMMUNISATION.				I. TEST.			II. TEST.			III. TEST.		
	Se- rum.	Virus.		Result.	Virus.		Result.	Virus.		Result.	Virus.		Result.
		Orig.	Orig.		Gen.	Orig.		Gen.	Orig.		Gen.	Orig.	
2388	Horses O-T-B	M. { O T } H. { B } 38 1 4		R	Tzn.	1	—	Tzn.	1	?	Ord.	38	—
2381	"	"	"	R	Bul.	4	?	Bul.	4	—	Tzn.	1	?
2377	"	"	"	?	Ord.	38	R	Ord.	38	—	"	1	—
2378	"	M. { O T } 38 1		R	M. { O T } 38 1		R	Tzn.	1	—	Ord.	38	—
2385	"	"	"	?	"	"	—	"	12	R	"	38	—
2386	"	"	"	—	"	"	R	{ O T } 38 1	—	—	"	62	—
2384	"	"	"	R	"	"	—	"	12	RD†	—	—	—
2387	"	"	"	R	"	"	?	Tzn.	12	R	Ord.	38	R
2380	"	"	"	?	"	"	—	{ O T } 38 1	?	?	"	38	?
2467	Mule O + Horses O-T-B	M. { O T } B 38 1 1		R	M. { O T } B 38 1 1		?	—	—	—	—	—	—
2466	"	"	"	R	H. { O T } B 38 1 1		R†	—	—	—	—	—	—
2472	"	"	"	R	M. { O T } B "		?	—	—	—	—	—	—
2474	"	"	"	R	H. { O T } B "		R†	—	—	—	—	—	—
2468	"	"	"	R	OTB	5	—	—	—	—	—	—	—
2471	"	"	"	R	"	5	R	—	—	—	—	—	—
2475	"	"	"	R	—	—	—	—	—	—	—	—	—
2473	"	"	"	R	M. { O T } B 38 1 1		R	—	—	—	—	—	—
2461	"	"	"	R	"	"	—	—	—	—	—	—	—
2464	"	"	"	R	OTB	5	—	—	—	—	—	—	—
2470	"	"	"	R	OTB LPW	1	—	—	—	—	—	—	—
2450	"	"	"	R	Ord.	38	R	Bul.	1	—	—	—	—
2448	"	"	"	R	Tzn.	1	?	Ord.	38	R	—	—	—
2469	"	"	"	R	Bul.	1	R	Tzn.	1	?	—	—	—
2449	"	H. { O T } B 38 1 3		R	M. { O T } B 38 1 1		?	—	—	—	—	—	—
2447	"	"	"	R	H. { O T } B 38 1 3		R	—	—	—	—	—	—

R—Reaction.

?—Doubtful.

R†—Reaction and died.

RD†—Reaction with Dikkop and died.

## TABULATED RÉSUMÉ OF TESTS, ETC.—(Continued).

## POLYVALENT SERUM AND POLYVALENT 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.	
2446	Mule O + Horses O.T.B	H.	$\left. \begin{array}{l} O \\ T \\ B \end{array} \right\} \begin{array}{l} 38 \\ 1 \\ 3 \end{array}$	R	M.	$\left. \begin{array}{l} O \\ T \\ B \end{array} \right\} \begin{array}{l} 38 \\ 1 \\ 1 \end{array}$	—	—	—	—	—	—	—
2462	"	"	"	R	H.	$\left. \begin{array}{l} O \\ T \\ B \end{array} \right\} \begin{array}{l} 38 \\ 1 \\ 1 \end{array}$	R	—	—	—	—	—	—
2459	"	"	"	R	OTB	5	—	—	—	—	—	—	—
2465	"	"	"	R	OTB LPW	1	R	—	—	—	—	—	—
2452	"	"	$\left. \begin{array}{l} 38 \\ 1 \\ 1 \end{array} \right\}$	R	H.	$\left. \begin{array}{l} O \\ T \\ B \end{array} \right\} \begin{array}{l} 38 \\ 1 \\ 1 \end{array}$	R†	—	—	—	—	—	—
2459	"	"	"	?	"	"	R	—	—	—	—	—	—
2451	"	"	"	R	OTB	5	R	—	—	—	—	—	—
2453	"	"	"	R	OTB LPW	1	R	—	—	—	—	—	—
2458	"	"	"	R	Ord.	38	R	Bul.	1	—	—	—	—
2460	"	"	"	R	Tzn.	1	?	Bul.	38	?	—	—	—
2457	"	"	"	R	Bul.	3	?	Tzn.	1	—	—	—	—
2484	Mule O + Horses T+B	H.	$\left. \begin{array}{l} O \\ T \\ B \end{array} \right\} \begin{array}{l} 38 \\ 1 \\ 1 \end{array}$	R	H.	$\left. \begin{array}{l} O \\ T \\ B \end{array} \right\} \begin{array}{l} 38 \\ 1 \\ 1 \end{array}$	—	—	—	—	—	—	—
2485	"	"	"	RD†	—	—	—	—	—	—	—	—	—
2486	"	"	"	R	H.	$\left. \begin{array}{l} O \\ T \\ B \end{array} \right\} \begin{array}{l} 38 \\ 1 \\ 1 \end{array}$	—	—	—	—	—	—	—
2487	"	"	"	R	"	"	—	—	—	—	—	—	—
2488	"	"	"	R	"	"	—	—	—	—	—	—	—
2489	"	M.	$\left. \begin{array}{l} O \\ T \\ B \end{array} \right\} \begin{array}{l} 38 \\ 1 \\ 1 \end{array}$	R	M.	$\left. \begin{array}{l} O \\ T \\ B \end{array} \right\} \begin{array}{l} 38 \\ 1 \\ 1 \end{array}$	R	—	—	—	—	—	—
2490	"	"	"	R	"	"	R	—	—	—	—	—	—
2491	"	"	"	R	"	"	—	—	—	—	—	—	—
2492	"	"	"	R	"	"	RD	—	—	—	—	—	—
2493	"	"	"	R	"	"	R	—	—	—	—	—	—
2500	Horses O.T.B	M.	$\left. \begin{array}{l} O \\ T \\ B \end{array} \right\} \begin{array}{l} 38 \\ 1 \\ 1 \end{array}$	R	M.	$\left. \begin{array}{l} O \\ T \\ B \end{array} \right\} \begin{array}{l} 38 \\ 1 \\ 1 \end{array}$	R	—	—	—	—	—	—
2501	"	"	"	RD	"	"	—	—	—	—	—	—	—
2502	"	"	"	RD	"	"	R	—	—	—	—	—	—
2503	"	H.	$\left. \begin{array}{l} O \\ T \\ B \end{array} \right\} \begin{array}{l} 38 \\ 1 \\ 1 \end{array}$	R	H.	$\left. \begin{array}{l} O \\ T \\ B \end{array} \right\} \begin{array}{l} 38 \\ 1 \\ 1 \end{array}$	?	—	—	—	—	—	—
2504	"	"	"	R	"	"	R	—	—	—	—	—	—
2505	"	"	"	RD†	—	—	—	—	—	—	—	—	—

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



## TABULATED RESUMÉ OF TESTS, ETC.—(Continued).

## POLYVALENT SERUM AND POLYVALENT VIRUS.

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.	
2506	Mule O + Horses O-T-B	OTB	5	R	H. { O 38 T 1 B 1 }		?	—	—	—	—	—	—
2507	"	"	5	R	" "		R	—	—	—	—	—	—
2513	Horses O-T-B	"	5	R	Ord.	38	R	Bul.	1	—	—	—	—
2514	"	"	5	RD	Tzn.	1	RD	Bul.	1	R	—	—	—
2515	"	"	5	R†	—	—	—	—	—	—	—	—	—
2516	"	"	5	R	Bul.	1	R	Tzn.	1	—	—	—	—
2517	"	"	5	RD†	—	—	—	—	—	—	—	—	—
2518	"	"	5	R	{ M. O 38 H. T 1 M. B 1 }		R	Ord.	38	—	—	—	—
2519	"	{ OTB LPW }	1	R	Ord.	38	R	Tzn.	1	—	—	—	—
2520	"	"	1	R	Tzn.	1	R	Bul.	1	—	—	—	—
2521	"	"	1	R†	—	—	—	—	—	—	—	—	—
2522	"	"	1	R	Bul.	1	RD†	—	—	—	—	—	—
2523	"	"	1	RD	M. { O 38 T 1 B 1 }		R	Bul.	1	—	—	—	—
2524	"	"	1	R	OTB	5	R	Ord.	38	—	—	—	—
2581	OTB	OTB	5	R	Bul.	1	R	Ord.	38	—	H. { O 70 T 13 B 10 }		R
2588	"	"	5	R	"	1	—	Ord.	70	—	" "		R
2589	"	"	5	?	OTB	5	—	M. { O 38 T 1 B 1 }		—	" "	{ 70 13 11 }	?
2590	"	"	5	R	"	5	—	Ord.	38	—	Bul. 11		?
									4th test		H. { O 70 T 13 B 10 }		—
2591	"	"	5	R	Tzn.	1	—	Tzn.	13	R	" "	{ 70 13 10 }	—
2592	{ OTB LPW }	{ OTB LPW }	1	R	"	1	—	"	13	—	H. { O 70 T 13 B 10 }		—
2594	"	"	1	R	Ord.	38	—	"	13	—	" "	{ 70 13 10 }	—
2595	"	"	1	R	"	38	R	Bul.	11	R	" "	" "	R
2596	"	"	1	R	{ OTB LPW }	1	—	M. { O 38 T 1 B 1 }		—	" "	{ 70 13 11 }	R

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

## EXTRACTS FROM ABOVE TABULATED RÉSUMÉ.

*Deaths from Immunisation.*

- A.—3 mules, immunised with serum horses O-T-B and  
virus  $\left\{ \begin{array}{l} \text{M. } \left\{ \begin{array}{l} \text{O 38 gen.} \\ \text{T 1 gen.} \end{array} \right\} \\ \text{H. B 1 gen.} \end{array} \right\} : \dots \dots \dots \dots \dots \text{No deaths.}$
- 6 mules, immunised with same serum as above and  
virus M.  $\left\{ \begin{array}{l} \text{O 38 gen.} \\ \text{T 1 gen.} \end{array} \right\} : \dots \dots \dots \dots \dots \text{No deaths.}$
- 3 mules, immunised with same serum as above and  
virus M.  $\left\{ \begin{array}{l} \text{O 38 gen.} \\ \text{T 1 gen.} \\ \text{B 1 gen.} \end{array} \right\} : \dots \dots \dots \dots \dots \text{No deaths.}$
- 3 mules, immunised with same serum as above and  
virus H.  $\left\{ \begin{array}{l} \text{O 38 gen.} \\ \text{T 1 gen.} \\ \text{B 1 gen.} \end{array} \right\} \dots \dots \dots \dots \dots 1 \text{ death.}$
- 6 mules, immunised with serum as above and virus OTB,  
5th generation  $\dots \dots \dots \dots \dots 2 \text{ deaths.}$
- 6 mules, immunised with serum as above and virus  
OTBLPW, 1st generation  $\dots \dots \dots \dots \dots 1 \text{ death.}$
- B.—5 mules, immunised with serum  $\left\{ \begin{array}{l} \text{M.O} \\ \text{H.T} \\ \text{H.B} \end{array} \right\}$  and  
virus H.  $\left\{ \begin{array}{l} \text{O 38 gen.} \\ \text{T 1 gen.} \\ \text{B 1 gen.} \end{array} \right\} : \dots \dots \dots \dots \dots 1 \text{ death.}$
- 5 mules, immunised with serum as above and  
virus M.  $\left\{ \begin{array}{l} \text{O 38 gen.} \\ \text{T 1 gen.} \\ \text{B 1 gen.} \end{array} \right\} : \dots \dots \dots \dots \dots \text{No deaths.}$
- C.—14 mules, immunised with serum  $\left\{ \begin{array}{l} \text{M.O} \\ \text{H.O} \\ \text{H.T} \\ \text{H.B} \end{array} \right\}$  and  
virus M.  $\left\{ \begin{array}{l} \text{O 38 gen.} \\ \text{T 1 gen.} \\ \text{B 1 gen.} \end{array} \right\} : \dots \dots \dots \dots \dots \text{No deaths.}$
- 13 mules, immunised with serum as above and  
virus H.  $\left\{ \begin{array}{l} \text{O 38 gen.} \\ \text{T 1 gen.} \\ \text{B 1 gen.} \end{array} \right\} : \dots \dots \dots \dots \dots \text{No deaths.}$
- 2 mules, immunised with serum as above and virus OTB,  
5th generation  $\dots \dots \dots \dots \dots \text{No deaths.}$
- D.—5 mules, immunised with serum OTB and virus OTB, 5 gen. No deaths.
- E.—4 mules, immunised with serum and virus OTBLPW, 1 gen. No deaths.

*Tests.*

A.—3 mules, immunised serum horse O-T-B and virus  $\begin{matrix} \text{M.} \left\{ \begin{matrix} \text{O} \\ \text{T} \end{matrix} \right\} \\ \text{H.} \quad \text{B} \end{matrix}$ .

- 1 Mule, 1st and 2nd test with Tzaneen and 3rd test with Ord., 38th generation, had a doubtful reaction with 2nd test (Tzaneen, 1st generation).
- 1 Mule, 1st and 2nd tests with Bulawayo, 4th generation, and 3rd test with Tzaneen, 1st generation, had a doubtful reaction with 1st test (Bulawayo) and with 3rd test (Tzaneen).
- 1 Mule, 1st and 2nd tests with Ord., 38th generation, and 3rd test with Tzaneen, 1st generation, had a distinct reaction with the 1st test (Ord.)

6 Mules, immunised with serum horse O-T-B and virus mules  $\begin{matrix} \left\{ \begin{matrix} \text{T} \\ \text{O} \end{matrix} \right\}$ .

- 2 Mules, 1st test M.  $\left\{ \begin{matrix} \text{O 38 gen.} \\ \text{T 1 gen.} \end{matrix} \right\}$  2nd test with Tzaneen, 12th generation, and 3rd test with Ord., 38th generation; 1 had doubtful reaction with 1st and reactions with the 2nd and 3rd tests; 1 had reaction with the 2nd test (Tzaneen).
- 1 Mule, 1st test with M.  $\left\{ \begin{matrix} \text{O 38 gen.} \\ \text{T 1 gen.} \end{matrix} \right\}$  2nd test with Tzaneen, 1st generation, and 3rd test with Ord., 38th generation, had a reaction with the 1st test.
- 1 Mule, 1st and 2nd tests with M.  $\left\{ \begin{matrix} \text{O 38 gen.} \\ \text{T 1 gen.} \end{matrix} \right\}$  and 3rd test with Ord., 62nd generation, had a reaction with the 1st test only.
- 1 Mule, 1st and 2nd tests with M.  $\left\{ \begin{matrix} \text{O 38 gen.} \\ \text{T 1 gen.} \end{matrix} \right\}$  and 3rd test with Ord., 38th generation, had doubtful reactions with the 2nd and 3rd tests.
- 1 Mule, 1st and 2nd tests with M.  $\left\{ \begin{matrix} \text{O 38 gen.} \\ \text{T 1 gen.} \end{matrix} \right\}$  had no reaction with 1st test, but reaction, dikkop, and died on the 2nd test.

3 Mules, immunised with serum horses O-T-B and virus mules  $\begin{matrix} \left\{ \begin{matrix} \text{O} \\ \text{T} \\ \text{B} \end{matrix} \right\}$ .

Tested with M.  $\left\{ \begin{matrix} \text{O 38 gen.} \\ \text{T 1 gen.} \\ \text{B 1 gen.} \end{matrix} \right\}$ ; 2 reactions.

2 Mules, immunised with serum horses O-T-B and virus horse  $\begin{matrix} \left\{ \begin{matrix} \text{O} \\ \text{T} \\ \text{B} \end{matrix} \right\}$ .

Tested with H.  $\left\{ \begin{matrix} \text{O 38 gen.} \\ \text{T 1 gen.} \\ \text{B 1 gen.} \end{matrix} \right\}$ ; 1 distinct and 1 doubtful reaction.

4 Mules, immunised with serum horses O-T-B and virus OTB.

1 Mule, 1st test with Ord., 38th generation, gave a reaction, and 2nd test with Bulawayo, 1st generation, gave no reaction.

1 Mule, 1st test with Tzaneen, 1st generation, reaction and dikkop, and also a reaction with 2nd test Bulawayo, 1st generation.

1 Mule, 1st test Bulawayo, 1st generation, gave a reaction, and none with 2nd test, Tzaneen, 1st generation.

1 Mule, tested with  $\left\{ \begin{array}{l} \text{M.O 38 gen.} \\ \text{H.T 1 gen.} \\ \text{M.B 1 gen.} \end{array} \right\}$  and showed a reaction; 2nd test with Ord., 38th generation, no reaction.

5 Mules, immunised with serum horses O-T-B and virus OTBLPW.

1 Mule, 1st test with Ord., 38th generation, and 2nd test with Tzaneen, 1st generation, had reaction with the 1st test.

1 Mule, 1st test with Tzaneen, 1st generation, and 2nd test with Bulawayo, 1st generation, had reaction with the 1st test.

1 Mule, 1st test with M.  $\left\{ \begin{array}{l} \text{O 38 gen.} \\ \text{T 1 gen.} \\ \text{B 1 gen.} \end{array} \right\}$  and 2nd test with Bulawayo, 1st generation, reacted with the 1st test.

1 Mule, 1st test with OTB, 5th generation, and 2nd test with Ord., 38th generation, also reacted on the 1st test.

1 Mule, tested with Bulawayo, 1st generation, reacted and died.

B.—4 Mules, immunised with serum  $\left\{ \begin{array}{l} \text{M.O} \\ \text{H.T} \\ \text{H.B} \end{array} \right\}$  and virus horse  $\left\{ \begin{array}{l} \text{O} \\ \text{T} \\ \text{B} \end{array} \right\}$  and tested with the same virus, gave no reaction.

5 Mules, immunised with serum as above and virus mules  $\left\{ \begin{array}{l} \text{O} \\ \text{T} \\ \text{B} \end{array} \right\}$  and tested with the same virus, gave 3 reactions and 1 reaction and dikkop.

C.—14 Mules, immunised with serum  $\left\{ \begin{array}{l} \text{M.O} \\ \text{H.O} \\ \text{H.T} \\ \text{H.B} \end{array} \right\}$  and virus mules  $\left\{ \begin{array}{l} \text{O} \\ \text{T} \\ \text{B} \end{array} \right\}$ .

4 Mules, tested with same virus; 1 reaction and 2 doubtful reactions.

2 Mules, tested with virus horse  $\left\{ \begin{array}{l} \text{O 38 gen.} \\ \text{T 1 gen.} \\ \text{B 1 gen.} \end{array} \right\}$ , reacted and died.

3 Mules, tested with OTB, 5th generation; only 1 gave a reaction.

1 Mule, tested with OTBLPW, 1st generation, gave no reaction.

1 Mule, 1st test with Ord., 38th generation, and 2nd test with Bulawayo, 1st generation, reacted with 1st test.

- 1 Mule, 1st test with Tzaneen, 1st generation, and 2nd test with Ord., 38th generation, had doubtful reaction with 1st and distinct reaction with 2nd test.
- 1 Mule, 1st test with Bulawayo, 1st generation, and 2nd test with Tzaneen, 1st generation; distinct reaction to the 1st and doubtful reaction to the 2nd test.
- 1 Mule was not tested.
- 13 Mules, immunised with serum as above and virus horses  $\left\{ \begin{array}{l} O \\ T \\ B \end{array} \right\}$ .
- 4 Mules, tested with same virus, gave 3 reactions and 1 death.
- 2 Mules, tested with virus mules  $\left\{ \begin{array}{l} O \text{ 38 gen.} \\ T \text{ 1 gen.} \\ B \text{ 1 gen.} \end{array} \right\}$ ; 1 gave a doubtful reaction.
- 2 Mules, tested with OTB, 5th generation; 1 gave a reaction.
- 2 Mules, tested with OTBLPW, 1st generation, gave 2 reactions.
- 1 Mule, 1st test with Ord., 38th generation, and 2nd test with Bulawayo, 1st generation; reacted to the 1st test.
- 1 Mule, 1st test with Tzaneen, 1st generation, and 2nd test with Ord., 38th generation, gave 2 doubtful reactions.
- 1 Mule, 1st test with Bulawayo, 3rd generation, and 2nd test with Tzaneen, 1st generation, gave a doubtful reaction to the 1st test.
- 2 Mules, immunised with serum as above and virus OTB and tested with horses  $\left\{ \begin{array}{l} O \text{ 38 gen.} \\ T \text{ 1 gen.} \\ B \text{ 1 gen.} \end{array} \right\}$ ; 1 gave a doubtful and the other a distinct reaction.

D.—5 Mules, immunised with serum OTB and virus OTB.

- 1 Mule, 1st test with Bulawayo, 1st generation, 2nd test with Ord., 38th generation, and 3rd test with horses  $\left\{ \begin{array}{l} O \text{ 70 gen.} \\ T \text{ 13 gen.} \\ B \text{ 10 gen.} \end{array} \right\}$ , gave reaction with 3rd test only.
- 1 Mule, 1st test with Bulawayo, 1st generation, 2nd test with Ord., 70th generation, and 3rd test with horse  $\left\{ \begin{array}{l} O \text{ 70 gen.} \\ T \text{ 13 gen.} \\ B \text{ 10 gen.} \end{array} \right\}$ , gave reaction to the 3rd test only.
- 1 Mule, 1st test with OTB, 5th generation, 2nd test with mules  $\left\{ \begin{array}{l} O \text{ 38 gen.} \\ T \text{ 1 gen.} \\ B \text{ 1 gen.} \end{array} \right\}$  and 3rd test with horses  $\left\{ \begin{array}{l} O \text{ 70 gen.} \\ T \text{ 13 gen.} \\ B \text{ 11 gen.} \end{array} \right\}$ , gave a doubtful reaction to the 3rd test.

- 1 Mule, 1st test with OTB, 5th generation, 2nd test with Ord., 38th generation, 3rd test with Bulawayo, 11th generation, and 4th test with horses  $\left\{ \begin{array}{l} \text{O 70 gen.} \\ \text{T 13 gen.} \\ \text{B 10 gen.} \end{array} \right\}$ , showed only a doubtful reaction on the 3rd test.
- 1 Mule, 1st test with Tzaneen, 1st generation, 2nd test with Tzaneen, 13th generation, and 3rd test with horses  $\left\{ \begin{array}{l} \text{O 70 gen.} \\ \text{T 13 gen.} \\ \text{B 10 gen.} \end{array} \right\}$ , had a reaction to the 2nd test.

E.—4 Mules, immunised with serum OTBLPW and virus OTBLPW.

- 1 Mule, 1st test with Tzaneen, 1st generation, 2nd test with Tzaneen, 13th generation, and 3rd test with horses  $\left\{ \begin{array}{l} \text{O 70 gen.} \\ \text{T 13 gen.} \\ \text{B 10 gen.} \end{array} \right\}$ , gave no reaction.
- 1 Mule, 1st test with Ord., 38th generation, 2nd test with Tzaneen, 13th generation, and 3rd test with horses  $\left\{ \begin{array}{l} \text{O 70 gen.} \\ \text{T 13 gen.} \\ \text{B 10 gen.} \end{array} \right\}$ , gave no reaction.
- 1 Mule, 1st test with Ord., 38th generation, 2nd test with Bulawayo, 11th generation, and 3rd test with horses  $\left\{ \begin{array}{l} \text{O 70 gen.} \\ \text{T 13 gen.} \\ \text{B 10 gen.} \end{array} \right\}$ , gave 3 distinct reactions.
- 1 Mule, 1st test with OTBLPW, 1st generation, 2nd test with mule  $\left\{ \begin{array}{l} \text{O 38 gen.} \\ \text{T 1 gen.} \\ \text{B 1 gen.} \end{array} \right\}$ , and 3rd test with horses  $\left\{ \begin{array}{l} \text{O 70 gen.} \\ \text{T 13 gen.} \\ \text{B 11 gen.} \end{array} \right\}$ , reacted only with the 3rd test.

*Conclusion.*—The immunisation against a polyvalent virus with an adequate serum produces immunity which protects against any of its constituents. The immunity is strong enough to prevent a reaction in the majority of cases, and strong enough to pass the reacting animals through a mild disease. Compared with the results obtained by the injection of a mixture of sera and their adequate vira, the immunity of the polyvalent virus and serum protects better against the constituents of the said polyvalent virus.

I must state here that the reactions noted after the injection may not always be due to the injection, but may be of a coincidental nature, or the reaction may not necessarily be a horse-sickness reaction. At the same time, we have no other means of controlling horse-sickness reactions except by the thermometer, inasmuch as slight reactions are not accompanied with characteristic symptoms.

For practical purposes, however, I consider that the immunity obtained by a polyvalent virus will, in practice, protect better than that obtained by an individual strain of virus.