

SUMMARY OF RESULTS GIVEN BY VARIOUS TYPES ARRANGED ACCORDING TO DOSE.

Percentage of Reactions caused by injection of Virus in Varying Doses (as per Col. 1).

	Type 2891.	Type 2694.	Type 2415.	Type 2732.	Type 2539.	Type 1965.
	%	%	%	%	%	%
Subcutaneous injections of 2 c.c.	10	62	11	22	55	31
" " " 3 c.c.	—	30	25	—	—	—
" " " 5 c.c.	—	55	—	—	—	—
" " " 10 c.c.	—	100	—	—	—	—
" " " 20 c.c.	—	—	—	—	—	—
" " " 50 c.c.	—	—	—	—	—	—
Intrajugular injections of 2 c.c.	—	—	—	—	75	—
" " " 3 c.c.	—	—	—	—	—	—
" " " 5 c.c.	—	—	—	—	—	25
" " " 10 c.c.	—	—	—	—	—	33
" " " 50 c.c.	—	—	—	—	—	—
TOTAL	15	54	14	25	55	32

1. Arranged according to virulency, the various types show the following ascending order:—2415, 2891, 2732, 1965, 2694, and 2539.

2. The differences in the virulency is particularly marked; 2891 and 2415 correspond, and these two vira both originated from the Natal virus. Again with types 2539, 2694, and 2732, these all directly descended from type 2415, yet 2732 only causes 25 per cent. reactions, whereas 2539 and 2694 caused 55 per cent. and 54 per cent. respectively.

CONCLUSIONS.

1. The virus Tzaneen has in no instance been virulent for all mules injected; this virulency differs, so to say, from animal to animal; one particular animal seems to influence the virus in such a way that this virulency is either decreased or increased.

2. This reduction of virulency is by no means the result of the dose of virus or of method of injection; small and large doses, injected subcutaneously or intrajugularly, equally fail to produce reactions.

3. This difference in virulency is either due to the virus itself or to the injected animal, but considering that animals which resisted quantities of virus of one particular type, subcutaneously or intrajugularly, contract horse-sickness from a subsequent subcutaneous injection of a smaller quantity, it shows that it is not so much the resistance of the animal but the virulency of the virus of the given animal.

4. It is probably correct to conclude that both animal and virus must be in a certain relation to each other before a reaction can ensue.

G.—FEVER REACTIONS IN HORSES SIMULATING HORSE-SICKNESS.

In a publication of mine, which appeared in the "Deutsche Wochenschrift Thiermedizin" in 1907, I described a fever reaction in horses under the title of "Ephemeral fever in horses," resembling horse-sickness in character, but of a shorter duration and which did not end fatally.

It was proved at that time that a recovery from this ephemeral fever did not produce any immunity against horse-sickness. The fact that microscopical examination of blood taken from animals suffering from either horse-sickness or ephemeral fever gave negative results and that the virus of both fevers could be preserved for any length of time, suggested some connection between the two, although the immunity not being reciprocal, negated the identification for the time being.

During the experiments on horse-sickness a similar occurrence was noted again, and this was followed up for some time on numerous animals, the object being again to note whether the reaction had any connection with the disease.

At the same time a number of experiments were made by exposing horses during the night, when it was noticed that one of them showed a reaction, again typical for horse-sickness. The blood of this animal was also used for inoculation purposes, but did not create immunity; however, since the fact was noted that even virulent strains of horse-sickness virus did not give absolute immunity against each other, it was considered advisable to proceed further into the question, accepting the possibility that there may yet be a connection between the two.

EXPERIMENT NO. 1.

With an ephemeral fever virus known as the "Two-mule Tzaneen Virus."

1. Origin of this strain.

Mule 1995.—Injected on 4th August, 1909, with virus 1965, originating from horse 1087, known as the origin of virus Tzaneen.

Result.—Contracted horse-sickness and died.

Mule 1996.—Injected as above.

Result.—Contracted horse-sickness and recovered.

The reaction of 1996 was very mild, lasting from 4th to 9th day, almost atypical.

This animal was tapped on the 7th day after injection, and this blood was virulent, in as much as it produced horse-sickness in the mule 2034, which on 11th August was injected intrajugularly with 5 c.c. virus 1996 of the same date, and mule 2034 died of horse-sickness nine days after injection.

Virus 1996 was utilised for immunisation in practice when it was noticed that of the animals injected, not all of them reacted with a simultaneous injection of serum; this was more particularly the case in Natal.

2. First generation.

Mule 2691.—Injected on 12th March, 1907, with 2 c.c. intrajugularly with virus mule 1996 (returned from Natal); and

Mule 2692.—Injected on 12th March, 1907, with 10 c.c. intrajugularly with virus mule 1996 (returned from Natal).

Results.—In both instances an irregular reaction followed, but not typical for horse-sickness. During these reactions the animals were tapped on several occasions. They were then submitted to a test with virus mule 2415, Tzaneen, fourth generation, when both contracted typical horse-sickness reactions and recovered; 2692 showing also dikkop.

With the blood collected from mule 2691 on the 24th March (twelve days after injection) and from 2692 on the 26th March (fourteen days after injection), a mixture of equal quantities was made.

Mule 3285.—Injected on 26th February, 1908, with 4 c.c. subcutaneously of this mixture.

Result.—Reaction followed from the 6th to the 9th day, reaching 104 on the 6th day and 103·6 on the two following days. The animal was tapped on the 8th day, and the virus called the “*Two-mule Tzaneen Virus.*”

Tested on the 20th April, 1908, with 2 c.c. virus mule 2415, Tzaneen, fourth generation.

Result.—Slight reaction with dikkop on 11th day, and recovered.

“*A.*” *Injections with mule virus 3285, dated 5th March, 1908 (Two-mule Tzaneen virus).*

3. Second generation.

“*A.*” *Horse 3404.*—Injected on the 10th March, 1908, with 2 c.c. subcutaneously with virus mule 3285.

Result.—Reaction from the 6th to the 10th day; reaching 106 in the evening of the 8th day. Pulse averaged 46 during this reaction.

Horse 3404 was in an experiment later with virus 3265 (exposure)* on the 30th March, 1908, and on 15th April with virus 3266 (four-mule† Tzaneen virus). Finally on the 27th April it was injected simultaneously and subcutaneously with serum and virus 2415, when it contracted horse-sickness and died.

“*B.*” *Mule 3279.*—Injected on the 10th of March subcutaneously with 2 c.c. virus.

Result.—Reaction from the 3rd to the 10th day, reaching 104·6 on 5th and 6th day, when the animal was tapped (16th and 17th March). On 30th March injected with virus 3265 (exposure virus), and 11th April with virus 3266 (four-mule Tzaneen virus).

Tested on 20th April with 2 c.c. virus mule 2415, Tzaneen, fourth generation.

Result.—Reaction again followed from the 6th to the 11th day.

“*C.*” *Horse 3331.*—This animal was previously used on 10th March for virus 3390 (four-mule Tzaneen virus) without reaction. Injected subcutaneously on the 30th March, 1908, with 2 c.c. virus mule 3285.

Result.—No reaction.

Injected on the 15th April with virus 3261 (exposure). Finally submitted to simultaneous injection of serum and virus 2415.

Result.—Contracted horse-sickness and died.

“*D.*” *Horse 3250.*—Injected subcutaneously on the 30th March, 1908, with a mixture of 10 c.c. virus mule 3285, and 100 c.c. serum CD.

Result.—Reaction from 5th to 9th day, reaching 106 on evening of 7th day. The animal was bled on the 8th day.

Injected on the 15th April with virus 3266 (four-mule Tzaneen virus).

Result.—No reaction.

Injected on the 7th May subcutaneously with virus 2926 (donkey foal), Simpson III.

Result.—No reaction.

Injected on the 21st May with 2 c.c. same virus, intrajugularly.

Result.—No reaction.

Injected on 3rd June with 2 c.c. same virus (2926), intrajugularly.

Result.—Horse-sickness reaction developed, of which the animal died.

* See experiment No. 4.

† See experiment No. 2.

4. Third generation.

"E." *Horse* 3328.—Injected on the 20th March, 1908, subcutaneously with 5 c.c. virus mule 3279.

Result.—Reaction from 4th to 8th day, with an evening temperature of 105·2 on 5th day and a pulse of 84 and 88 on 5th and 6th days.

Tested on the 6th April with 2 c.c. virus 2891, Tzaneen.

Result.—Developed horse-sickness with dikkop and recovered.

"F." *Horse* 3373.—Injected on the 20th March, 1908, with 5 c.c. subcutaneously virus horse 3404 (19th March, 1908).

Result.—Reaction from 4th to 8th day, with an evening temperature of 105·6 on 5th day and 105·4 on 6th day, and a pulse of 62 and 76 respectively.

Injected on the 30th March with 2 c.c. virus 2891, Tzaneen, subcutaneously.

Result.—Contracted horse-sickness and died.

"G." *Mule* 3422 (Immune to virus 2884, CD).—Injected on the 30th of March, 1908, with 2 c.c. virus horse 3404, subcutaneously. Rise of temperature from 6th to 11th days with maximum temperature of 103·2 on evening of the 7th day.

"H." *Mule* 3423 (Immune to virus 2884, CD).—Injected as above. Sharp rise from 5th to 10th days; highest temperature 103·8 on evening of 6th day.

"I." *Horse* 3239.—Injected on the 30th March, 1908, subcutaneously with 10 c.c. virus horse 3404, and 100 c.c. serum C.D.

Result.—Reaction from 4th to 10th days, highest temperature 105·6 on evening of the 6th day with a pulse of 70.

Injected on the 15th April with virus 3266 (four-mule Tzaneen virus).

Result.—No reaction.

Injected in August, 1908, simultaneously with serum and virus Tzaneen.

Result.—Reaction and recovered.

"J." *Mule* 3278.—This animal was previously injected on 10th March, 1908, with virus 3390 (four-mule Tzaneen virus).

Result.—Slight reaction.

Injected on the 6th April, 1908, with 2 c.c. virus horse 3404, subcutaneously, dated 19th March, 1908 (two-mule Tzaneen virus).

Result.—No reaction.

Tested on the 18th April with 2 c.c. virus mule Tzaneen, fourth generation.

Result.—Slight reaction.

"K." *Horse* 3464.—Infused on 6th April with 3000 c.c. virus horse 3250.

Result.—On evening of 7th day temperature noted of 103, but this was the only indication of a disturbance.

Tested on the 22nd April with virus mule 3359, Tzaneen.

Result.—Contracted horse-sickness and recovered.

5. Fourth generation.

"L." *Horse* 3252.—Injected on the 11th April subcutaneously with 2 c.c. virus horse 3239 (7th April, 1908).

Result.—Reaction from the 5th to the 15th day with an average evening temperature of 104.

Tested on the 29th of May with 2 c.c. virus mule 3398, Tzaneen.

Result.—Developed horse-sickness and died.

“M.” *Horse foal* 1993 (immune to virus 2884, CD).—Injected on the 11th April subcutaneously with 2 c.c. virus horse 3239 (7th April, 1908).

Result.—Slight reaction from 5th to the 12th day; maximum temperature 102·6 on evening of the 7th and 9th days.

“N.” *Horse foal* 1997 (immune to virus 2884, CD).—Injected on the 11th April as above.

Result.—Slight reaction from the 2nd to the 13th days; highest temperature 103·6 on evening of 9th day.

“O.” *Horse* 3470.—Injected on the 11th April, 1908, subcutaneously with 100 c.c. virus horse 3239 (7th April, 1808).

Result.—Reaction started the following day and lasted to the 9th day; average temperature 103.

Tested in July simultaneously and subcutaneously with serum and virus 3398, Tzaneen.

Result.—Contracted horse-sickness and recovered.

RESULTS.

Virus 1996, Tzaneen, second generation, used on the station produced horse-sickness. Virus 1996, Tzaneen, second generation, issued to Natal and subsequently returned when injected into two mules, gave atypical reaction. These two mules were tested later with Tzaneen, fourth generation virus, and both showed reactions and recovered. Blood collected at the time of the atypical reaction from these two mules was mixed, and the mixture is referred to as the two-mule Tzaneen virus. This mixture serves as the origin of the virus used.

First generation (of ephemeral fever virus).

Of 2 mules and 1 horse injected with two-mule Tzaneen virus, 1 mule and 1 horse showed reactions. When tested later with Tzaneen, fourth generation, all showed horse-sickness reactions and 1 horse and 1 mule died.

Second generation.

Of 3 mules and 4 horses injected with two-mule Tzaneen virus, first generation, 2 mules and 4 horses gave reactions.

One mule and 4 horses were tested later and all showed reactions, and 2 horses died of horse-sickness.

Third generation.

Of 3 horses and 2 horse foals injected with two-mule Tzaneen virus, second generation, 2 horses gave reactions, the 2 horse foals slight reactions and the other horse a very slight reaction. (This last animal had been infused with 3000 c.c. of the mixture, and when tested later with Tzaneen, sixth generation, showed horse-sickness reaction and recovered.) The other 2 horses were also tested with Tzaneen, sixth generation, and both showed horse-sickness reactions, 1 died.

CONCLUSIONS.

The injection of two-mule Tzaneen virus strain produced fever reactions. No mortality occurred as a result of this injection, notwithstanding the high fever and pulse.

No immunity was given by this reaction against subsequent injections of horse-sickness virus. The quantity of virus injected had no influence on the severity of the clinical symptoms.

The immunity obtained from the two-mule virus protected against subsequent inoculation of the four-mule virus (see also later).

Animals immune against horse-sickness when injected with two or four mule Tzaneen virus showed reactions.

Animals injected with a mixture of two-mule Tzaneen virus and horse-sickness serum showed reactions.

Animals which had reactions from the two-mule Tzaneen virus, contracted horse-sickness, and the majority died when injected with horse-sickness virus.

EXPERIMENT NO. 2.

With an ephemeral fever virus known as the "Four-mule Tzaneen Virus."

The virus of mule 1996, which was utilised in Natal, produced a reaction in two mules, accompanied with lesions of dikkop. They were tapped and the blood forwarded to our station. Several other experiments were made, the principal animal of which was mule 2415 (compare article the "Variability of a given strain of horse-sickness virus").

The blood obtained on 2nd January, 1907, from mule 2415 was injected into mule 2539. Reaction began after three days and was over on the 11th day. It was typical in character for horse-sickness, but rather short in duration. The animal recovered (for tests, *vide* article quoted above).

Mule 2539 was tapped on the 6th and 8th days after injection. Blood of this reaction taken on the 8th day was virulent for mule 2495 (4th February, 1907) (subcutaneously 2 c.c.), and again for horse 3248 (17th February, 1908), subcutaneously 50 c.c. (*vide* article referred to). The blood of mule 2539 taken on the 6th day was used for injection of the following two animals: Mules 2693 and 2694, and blood of the 8th day for injection of mules 2669, 2695, and 2696.

"A." *Mule 2669.*—Injected on the 27th February, 1907, subcutaneously with 2 c.c. virus mule 2539, Tzaneen, fifth generation (30th January, 1907).

Result.—An indication of a reaction, but nothing definite. Animal was bled on the 11th day.

Tested on the 20th March with 5 c.c. virus mule 2415, Tzaneen, fourth generation, and had a typical horse-sickness reaction and recovered.

"B." *Mule 2693.*—Injected on the 20th March subcutaneously with 2 c.c. virus mule 2539 (28th January, 1907).

Result.—No distinct reaction. Animal bled on the 8th day.

Tested on the 4th of April with virus 2629, Tzaneen, fifth generation (14th February, 1907).

Result.—Typical horse-sickness reaction resulted and recovered.

"C." *Mule 2694.*—Injected on the 20th March, 1907, subcutaneously with 2 c.c. virus mule 2539 (28th January, 1907).

Result.—No definite reaction, but some irregularity in the temperature was noted, and animal was bled on the 8th day.

Tested on the 4th April with virus mule 2415.

Result.—Developed horse-sickness reaction with dikkop on the 14th day and recovered.

"D." *Mule 2695.*—Injected on the 20th March, 1907, subcutaneously with 2 c.c. virus mule 2539 (30th January, 1907).

Result.—No definite reaction, but an irregular slight disturbance in the temperature curve. Animal was tapped on the 8th day.

Tested on the 4th April with virus 2629, Tzaneen.

Result.—Reaction and recovered.

“ E.” *Mule* 2696.—Injected on the 20th March, 1907, subcutaneously with 2 c.c. virus mule 2539 (30th January, 1907).

Result.—Slight irregular temperature reaction, in no way typical for horse-sickness. Animal was tapped on the 8th day.

Tested on the 4th of April with virus mule 2415, Tzaneen.

Result.—Reaction and recovered.

Experiments with a mixture of virus of mules 2669 (10th March, 1908), 2693 (28th March, 1908), 2694 (28th March, 1908), and 2695 (28th March, 1908) in equal quantities; of this mixture one mule was injected, viz. :—

“ F.” *Mule* 3390.—Injected on the 26th February, 1908, subcutaneously with 8 c.c. of above mixture.

Result.—Reaction from 6th to 11th days, resembling a horse-sickness reaction in character with maximum temperature of 103·6 on 8th day and a pulse record of 60 on the 8th, 9th, and 10th days. The animal was tapped on the 8th, 9th, and 11th days, and this blood (3390) is called the four-mule Tzaneen virus.

Mule 3390 was tested on the 20th of April with 2 c.c. virus mule 2415, Tzaneen, fourth generation.

Result.—Developed a typical horse-sickness reaction and recovered.

Second generation.

“ G.” *Horse* 3331.—Injected on the 10th of March, 1908, subcutaneously with 2 c.c. virus mule 3390 (6th March, 1908).

Result.—Irregular temperature disturbance, but not of a definite character. In an experiment with serum and virus 2415 on the 23rd of April; contracted horse-sickness and died.

“ H.” *Mule* 3278.—Injected on the 10th of March, 1908, subcutaneously with 2 c.c. virus mule 3390 (6th March, 1908).

Result.—Slight reaction on the 4th, 5th, and 6th days. The animal was bled on the 6th day.

Injected on the 6th of April with virus 3404, two-mule Tzaneen.

Result.—No reaction.

Tested on the 18th of April with virus mule 2415.

Result.—Slight reaction.

Third generation.

“ I.” *Horse* 3266.—Injected on the 30th of March subcutaneously with 2 c.c. virus mule 3278 (16th March, 1908).

Result.—Reaction from 7th to 11th days with maximum temperature of 104 on 3rd day. Bled on the 8th and 9th days.

Injected with virus 3239 (two-mule Tzaneen virus).

No result.

Finally used in an experiment with serum and virus, Tzaneen.

Result.—Contracted horse-sickness and died.

Fourth generation.

J.” *Horse* 3274.—Injected on the 11th of April subcutaneously with 2 c.c. virus horse 3266 (8th April, 1908).

Result.—Reaction from 4th to 9th days with a maximum temperature of 105 and pulse 54. Bled on the 7th day.

Tested on the 7th July with virus 3406, Tzaneen, seventh generation.

Result.—Contracted horse-sickness and died.

"K." *Horse* 3460.—Injected on the 11th of April subcutaneously with 100 c.c. virus horse 3266 (8th April, 1908).

Result.—Irregular temperature from the day after injection for a week.

Injected on the 21st of July with virus 3619, Tzaneen, twentieth generation, three days later injected with serum.

Result.—Contracted horse-sickness and died.

"L." *Horse foal* 1766 (immune to virus 2884, CD).—Injected on the 11th of April subcutaneously with 2 c.c. virus horse 3266 (8th April, 1908).

Result.—Reaction from 4th to 9th days.

"M." *Horse foal* 1991 (immune to virus 2884, CD).—Injected on the 11th of April, as above.

Result.—Reaction from the 2nd to the 10th days; highest temperature of 104.2 on evening of 5th day.

RESULTS.

The reaction obtained from an inoculation of four-mule Tzaneen virus corresponds in character to that obtained in the previous experiments with the two-mule Tzaneen virus, and since the origin of these two vira is the same, we have a reason to expect this to be the case.

Since immunity obtained from the four-mule virus protected against the inoculation of the two-mule virus (see previous experiments) further support is given to our conception.

Further since the immunity obtained from the two-mule virus protects against the four-mule virus, we have to accept that these two vira are the same.

EXPERIMENT NO. 3.

With a mixture of Tzaneen two-mule virus and four-mule virus.

"A." *Horse* 3383.—Injected on the 11th of April subcutaneously with 100 c.c. of a mixture of 3266 (four-mule virus) and 3239 (two-mule virus).

Result.—Reaction from 3rd to the 10th days, with a maximum temperature of 105 on the 6th day.

Injected with virus 3749, Tzaneen, twenty-first generation, succeeded by serum.

Result.—Contracted horse-sickness and died.

"B." *Horse* 3259.—Injected on the 15th of April subcutaneously with 4 c.c. of above mixture.

Result.—Reaction on 5th, 6th, and 7th days. Maximum temperature 105 on the 6th day.

Injected on the 19th June with virus donkey foal 2926, Simpson III.

Result.—Contracted horse-sickness and died.

"C." *Horse* 3338.—Injected on the 18th of April subcutaneously with 4 c.c. of above mixture.

Result.—Slight reaction on the 10th, 11th, and 12th days.

Injected on the 7th of May simultaneously with serum and virus, Tzaneen.

Result.—Reaction and recovered.

"D." *Horse* 3485.—Injected on the 18th of April, as above.

Result.—Temperature exacerbation on the 13th and 14th days. On 7th of May in an experiment with serum and virus 3285, Tzaneen, fifth generation,

Result.—Contracted horse-sickness and died.

“ E.” Horse 3445.—Injected on the 18th of April, as above.

Result.—No reaction; one temperature exacerbation on 13th day. In experiment on 15th June with serum and virus 3398, Tzaneen.

Result.—Contracted horse-sickness and died.

“ F.” Horse 3302.—Injected on the 18th of April, as above.

Result.—Irregular temperature with exacerbations on 8th, 9th, 12th, and 13th days.

Injected on the 29th of May with virus 3361, Tzaneen, succeeded by serum.

Result.—Reaction and recovered.

“ G.” Horse 3482.—Injected on the 18th of April, as above.

Result.—Reaction started immediately with maximum temperature of 104 on the 4th day, lasting to the 8th day. Maximum pulse 100 on 5th day, 74 on 6th day, and 64 on the 7th day.

Tested on the 7th of May simultaneously with serum and virus 3500, Tzaneen.

Result.—Contracted horse-sickness and died.

RESULTS.

Of eight horses injected with 100 c.c. of a mixture of two and four mule Tzaneen vira, indications of reactions were noted in seven cases.

When tested later with Tzaneen virus of a higher generation of a virulent horse-sickness strain, all contracted horse-sickness and six died.

EXPERIMENT NO. 4.

With an ephemeral virus, known as “ Exposure Virus.”

(Experiments with a horse which was exposed to natural infection of horse-sickness by letting it run during the night in a paddock at the Laboratory, Daspoort, just outside Pretoria.)

“ A.” Horse 3298 was exposed on the 15th of February in this way. This horse showed a temperature reaction resembling horse-sickness from the 5th to the 14th days, with a maximum temperature of 104·8 on the 9th day. Maximum record of pulse was 62 on the 9th day.

A microscopical examination on 9th and 12th days showed *Piroplasma equi* very rare. Examination on the 11th day gave negative results.

3298 was bled on 9th, 10th, 11th, and 12th days, and blood was used for experiments, enumerated later.

Tested on the 6th of April subcutaneously with 2 c.c. virus mule 2891, Tzaneen, fourth generation.

Result.—Contracted horse-sickness and died.

First generation.

“ B.” Horse 3265.—Injected on the 2nd of March, 1908, subcutaneously with 2 c.c. virus horse 3298 (26th February, 1908).

Result.—Reaction from 5th to 12th day with a maximum temperature of 105 on the 6th day, and a maximum pulse of 72 on the 7th day. Animal was tapped on the 6th, 8th, and 9th days.

Injected on the 15th of April with virus 3266, Tzaneen, four-mule.

Result.—No reaction.

Tested on the 4th of July with virus mule 3650, Tzaneen.

Result.—Contracted horse-sickness and died.

"C." *Horse* 3474.—Injected on the 6th of April, 1908, subcutaneously with 2 c.c. virus horse 3298 (26th February, 1908).

Result.—Hardly any reaction, only one exacerbation to 103 on the 7th day. Animal proved to be immune against horse-sickness in later experiments.

Second generation.

"D." *Horse* 3254.—Injected on the 20th of March subcutaneously with 2 c.c. virus horse 3265 (10th March, 1908).

Result.—Reaction from 6th to 10th days, with temperature disturbance up to the 14th day. Maximum temperature 106 on 7th day, and maximum pulse 54 on the 8th day.

Tested on the 6th of April with virus 3263, Simpson, third generation.

Result.—Contracted horse-sickness and recovered.

"E." *Horse* 3260.—Injected on the 20th of March subcutaneously with 10 c.c. virus horse 3265 (10th March, 1908).

Result.—Reaction from 5th to 10th days with a maximum temperature of 106 on the 7th day, and maximum pulse of 60 on same day.

Tested on the 6th of April with virus 3244, Simpson, fourth generation.

Result.—Contracted horse-sickness and died.

"F." *Horse* 3261.—Injected on the 20th of March intrajugularly with 2 c.c. virus horse 3265 (10th March, 1908).

Result.—Reaction from 5th to the 12th days with a maximum temperature of 105·8 on 7th day and maximum pulse 62 on 8th and 9th days. Animal was bled on the 6th and 7th days.

Injected on the 15th of April with 4 c.c. mixture of two and four mule virus.

Result.—Reaction from 5th to 9th days, with a maximum temperature of 104 on 6th and 7th days.

"G." *Mule* 3420 (immune to virus 2884, CD).—Injected on the 30th of March subcutaneously with 2 c.c. exposure virus horse 3265 (10th March, 1908).

Result.—Sharp rise from 7th to 11th days, with a maximum temperature of 102 on evening of the 9th day.

"H." *Mule* 3421 (immune to virus 2884, CD).—Injected on the 30th March, as above.

Result.—Rise of temperature from the 6th to the 12th days, with temperature of 102 on evening of the 7th and 9th days.

"I." *Horse foal* 2054 (immune to virus 2884, CD).—Injected on the 11th of April subcutaneously with 2 c.c. virus horse 3261 (27th March, 1908).

Result.—Irregular reaction from the 3rd to 12th days, highest temperature of 104 on evening of the 6th day.

"J." *Horse foal* 2314 (immune to virus 2884, CD).—Injected on the 11th of April, as above.

Result.—Irregular, slight reaction.

RESULTS.—Animals immune against horse-sickness showed reactions when injected with ephemeral fever virus.

"K." *Horse* 3259.—Injected on the 30th of March subcutaneously with 2 c.c. virus, horse 3265.

Result.—No reaction.

Injected on the 15th of April with a mixture of two and four mule virus.

Result.—Reaction from the 5th, 6th, and 7th days, with a maximum temperature of 105. Died later of horse-sickness.

“ L.” *Horse 3459*.—Injected on the 31st of March with a mixture of 200 c.c. serum CD and 10 c.c. virus 3265.

Result.—Reaction from 5th to 10th days, with a maximum temperature of 103.4 on the 8th day.

Injected on the 21st of May simultaneously and subcutaneously with serum and virus 3398, Tzaneen.

Result.—Contracted horse-sickness and died.

“ M.” *Horse 3485*.—Injected on the 6th of April subcutaneously with 2 c.c. virus 3265.

Result.—Reaction from 5th to 10th days, with a maximum temperature of 106 on the 7th day.

Injected on the 18th of April with two and four mule virus.

Result.—No reaction.

Injected on the 7th of May with serum and virus 3285, Tzaneen.

Result.—Contracted horse-sickness and died.

Third generation.

“ N.” *Horse 3302*.—Injected on the 6th of April subcutaneously with 100 c.c. virus horse 3261 (27th March, 1908).

Result.—Reaction from 4th to 9th days, with a maximum temperature of 105.2 on 5th day.

Injected on the 18th of April with two and four mule virus (*vide* Experiment No. 3, “ F ”).

Result.—Irregular temperature followed.

Injected later with horse-sickness virus and recovered.

“ O.” *Horse 3338*.—Injected on the 6th of April intrajugularly with 100 c.c. virus 3261 (27th March, 1908).

Result.—Reaction from 2nd to 9th days, with a maximum temperature of 104.6 on 5th day.

Injected on the 18th of April with two and four mule virus.

Result.—Slight reaction.

Injected later with horse-sickness virus and recovered.

“ P.” *Horse 3445*.—Injected on the 6th of April subcutaneously with 2 c.c. virus 3261 (27th March, 1908).

Result.—Reaction from 4th to the 10th days.

Injected on the 18th of April with two and four mule virus.

Result.—No reaction. Later, recovered from a horse-sickness experiment.

“ Q.” *Horse 3482*.—Injected on the 6th of April intrajugularly with 2 c.c. virus 3261 (27th March, 1908).

Result.—No reaction.

Injected on the 18th of April with two and four mule virus.

Result.—Reaction.

“ R.” *Horse 3331*.—Injected previously with mule 3390, Tzaneen, four-mule virus.

Result.—No definite reaction.

Injected on the 15th of April subcutaneously with 2 c.c. virus 3261 (27th March, 1908).

Result.—No reaction. Died later of horse-sickness.

RESULTS.—Animals injected with a mixture of this ephemeral fever virus and horse-sickness serum showed reactions.

The ephemeral fever virus did not completely protect against a later injection. Of two and four mule virus, some reactions were noted.

The immunisation of animals with exposure virus and two and four mule virus did not protect against horse-sickness.

EXPERIMENT NO. 5.

With exposure virus and two-mule Tzaneen virus.

“A.” Horse 3441.—Injected on the 6th of April subcutaneously with a mixture of 4 c.c. virus 3265 and 3404.

Result.—Reaction from 4th to the 11th days, with a maximum temperature of 106 on 6th day, and pulse record of 54.

Tested on the 20th of April simultaneously with serum and virus mule 2891, Tzaneen, fourth generation.

Result.—Contracted horse-sickness and died.

EXPERIMENT NO. 6.

With exposure virus, two-mule and four-mule Tzaneen virus.

“A.” Horse 3334 (previously injected with CD virus).—Injected on the 15th of April intrajugularly with 6 c.c. mixture of 3266 (four-mule virus), 3239 (two-mule virus), and 3261 (exposure).

Result.—No reaction. Proved to be immune against horse-sickness in subsequent experiment.

“B.” Horse 3400.—Injected on the 15th of April, as above.

Result.—Reaction from the 2nd to the 10th days, maximum temperature 105·8 to 106 from 4th to the 8th days, and maximum pulse of 66 on the 8th day.

Injected on the 15th of June with serum and virus 3398, Tzaneen.

Result.—Reaction and recovered.

“C.” Horse 3468.—Injected on the 15th of April, as above.

Result.—Hardly any reaction. Killed later.

“D.” Horse 3471.—Injected on the 15th of April, as above.

Result.—Reaction from 4th to the 10th days. On the 27th of April in an experiment with serum and virus 2415, Tzaneen, reaction and recovered.

“E.” Horse 3479.—Injected on the 15th of April, as above.

Result.—Reaction from 4th to the 7th days, with a maximum temperature of 103·8 on the 4th day.

Injected on the 14th of August with 3 c.c. virus 3659, succeeded by serum.

Result.—Contracted horse-sickness and died.

“F.” Horse 3481.—Injected on the 15th of April, as above.

Result.—Slight temperature disturbance, with a maximum temperature of 102·4 on the 6th day.

Injected on the 21st May simultaneously with serum and virus 3263, Simpson, third generation.

Result.—Contracted horse-sickness and died.

RESULTS.—The inoculation with a mixture of exposure and two-mule Tzaneen virus did not protect against subsequent injections of horse-sickness virus.

Of six animals injected with a mixture of (*a*) exposure, (*b*) two-mule, and (*c*) four-mule virus, two died and one was immune.

A combination of these vira did not protect against horse-sickness virus.

SUMMARY OF CONCLUSIONS.

The injection of two-mule Tzaneen virus protected against four-mule Tzaneen virus, but not completely against the exposure virus.

The two-mule Tzaneen virus, the four-mule Tzaneen virus, and the exposure virus when injected separately or as a mixture produced reactions and an acceleration of the pulse, and no animals died.

Since the two and four mule virus did not completely protect against the ephemeral fever virus, we have to conclude that these, although distinct, are to a certain extent related.

Neither of the three ephemeral fever vira protected against a horse-sickness vira, and therefore it has to be concluded that there is no connection between these ephemeral fevers and horse-sickness.