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Investigations into the Transmission of Bluetongue in Sheep during the Season 1931/1932.

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In addition to the work on horsesickness described in the preceding paper, we carried out a number of experiments with bluetongue in sheep along similar lines. The work on bluetongue was mainly undertaken to make use of the time, when the horsesickness work could not be undertaken.

From the epizootological point of view, horsesickness and bluetongue are very closely related to one another, and what has been said about horsesickness—it being non-contagious by ordinary means of contact, the seasonal and also to a certain extent the geographical distribution, the dependence on the amount of rain, the occurrence in marshy vleis and absence on hill tops, the danger of grazing at night, during the late afternoon or the early hours of the morning, the protection afforded by stables—applies practically as well to horsesickness as to bluetongue and does not need to be discussed at length.

It seems to be very probable from the information at hand, that horsesickness and bluetongue, if transmitted at all through insects, are carried by the same or by closely related species. As in the case of horsesickness we suspected, for reasons discussed with the results of the mosquito survey, *Aedes* species as the most probable transmitting agents, and this genus only was used in our experiments.

According to references in the literature up to now, no transmission experiments have been carried out with bluetongue.

I. EXPERIMENTAL TECHNIQUE.

The methods adopted in our work on bluetongue were in general very similar to those used for horsesickness, and have been described in full in the second paper of this series. We can limit ourselves therefore to a brief summary.

The mosquitos were obtained by catching the adults and larvae or pupae in the field and keeping the latter in the laboratory until the imagines hatched out. The larvae originated from natural breeding places, or during the drier part of the season from artificially flooded places. Specimens hatched in the laboratory from larvae or pupae obtained from the field were more suitable than those caught as adults, the latter generally showing a much higher mortality.

The mosquitoes were kept, before use, in large mosquito netting enclosed cages covered on four sides by hessian kept wet by allowing water to percolate through it from above, in order to secure the necessary degree of humidity. When not needed directly they were fed on a 10 per cent. aqueous solution of sugar, otherwise pure water only was provided.

The infected specimens were stored in our warm room, in which the temperature was maintained at an average of 24° to 25° C. From time to time, however, the temperature varied between 20° and 30° C. as the heating had to be controlled by hand.

In the first series of experiments the mosquitoes were all stored in jam jars, the lids being replaced by mosquito netting, 10 specimens in each. In order to secure the necessary humidity the jars were kept on wet cotton wool in slightly larger jars provided with lids having a few holes in them. The mosquitoes were fed on sugar water, a small piece of cotton wool soaked in a 10 per cent. solution being placed on the net covering the jar. This method was quite satisfactory, as was the case with horsesickness, when the mosquitoes were used for injection and only small numbers needed.

For the experiment in which the feeding of larger numbers was intended, our specially designed cages were employed. These were kept on shelves, being surrounded on four sides by wet hessian, thus ensuring the desired humid conditions inside the cages. For some species, especially *A. caballus*, it was found advisable to further increase the humidity by covering the floors of the cages with wet filter paper. In the case of the small mosquito groups the jars were always employed, as the cages, which were limited in number, had to be reserved for the larger groups.

The method employed for the injection of mosquitoes consisted firstly of stunning the insects by hitting the test tubes in which they were contained against the palm of the hand. The stunned specimens were then dropped on to a small quantity of normal sheep serum contained in a mortar and ground up to form a fine emulsion by means of a pestel. A further quantity of serum was then added and the whole injected subcutaneously into the sheep on the inner aspect of one thigh.

For feeding purposes the small wire cages covered with mosquito netting, described in the technical section, were used in all cases. The liberating of mosquitoes in fly-proof stables containing sheep was not attempted, as no good results were expected.

The wool was carefully clipped as close as possible to the skin from an area on the back of the sheep corresponding with one surface

of the cage, and the cage, containing the mosquitoes, was then placed in position on this area. Six pieces of tape attached to the wool around the clipped area, two on each of the long sides and one on each of the short ends, tied together over the cage served to hold it in position. To ensure a sufficient degree of humidity inside the cages a piece of damp cotton wool was placed on top of the cage and held in position by means of the tapes. Without this precaution a considerable mortality could occur. Up to four cages could be placed on one sheep in this manner, two on either side of the mid line.

As our stable for mosquito feeding, with special arrangements for obtaining a humid atmosphere, was mostly in use for horsesickness experiments, the feeding on sheep had to be conducted in the ordinary sheep stables. On very dry nights the cotton wool occasionally dried up, resulting in a more or less high mortality amongst the mosquitoes, but under normal conditions the water contained in the cotton wool was sufficient to last out the night.

By this method of mosquito feeding it was not necessary to isolate individual sheep, as the cages are not interfered with by other sheep which may be present in the same box. Furthermore, the sheep used were apparently in no way irritated by the presence of the cage(s), as no attempt was made to rub it (or them) off, or, at any rate in our experiments no sheep succeeded in dislodging a cage.

The feeding results on the whole were not as good as was the case in the horsesickness experiments. The percentage of mosquitoes which engorged themselves, and furthermore, the amount of blood taken up by the individuals was definitely less, whereas the mortality amongst the unfed specimens was certainly higher. A number of reasons may be advanced to account for this fact. The pressure exerted on the cage, on which depended its contact with the skin, was not so great as in the case of the cages used on horses, so that slight movements of the skin tended to disturb feeding mosquitoes. The regulation of humidity was less effective and the mosquitoes had ample opportunity of taking up water. Short wool covering the clipped surface, which was never shaved, mechanically interfered with the insertion of the proboscis and the wool fat present probably also played a part in preventing good feeding.

However, our results were sufficiently satisfactory to permit of our disregarding these minor difficulties.

II. STRAINS OF VIRUS USED IN THE EXPERIMENTS AND EXPERIMENTAL ANIMALS.

In our experiments three different strains of bluetongue virus were used, the ordinary vaccine strain, and two strains recovered from fresh spontaneous cases.

1. *Bluetongue Vaccine Virus.*—In the first series of experiments described hereafter the virus wherewith we produced the experimental cases of bluetongue for feeding mosquitoes on, consisted of the laboratory vaccine strain. This strain had originally been obtained from a natural case of bluetongue which had occurred on the station in a lamb in February, 1927. Blood from this lamb had been

injected into sheep from which, by successive subinoculations into other sheep at or immediately after the reaction period, the virus strain had passed through 48 generations. These subinoculations were carried out over the period 5.2.27 to March, 1932, the last sheep being 31930 and 31554, the preserved blood of which was used in our experiments.

On reviewing the history of this virus strain certain facts are apparent which would account for some of our failures or apparent failures to transmit the disease through the agency of mosquitoes. In the first place only two deaths, which could be definitely attributed to blue tongue, were caused. Of the two sheep injected with blood from the original lamb which died of bluetongue, one died on the fourth day after injection after having shown no temperature reaction whatsoever, the other showed a typical though not marked reaction commncing on the sixth day and returning to normal on the eleventh day, the maximum temperature registered being 105.2° F. This latter sheep was used for subinoculations into two further sheep, death resulting in both cases with typical symptoms of bluetongue. The first of these two sheep showed a temperature reaction commencing on the third day with a maximum of 106.2° on the seventh day, and death on the ninth day. In the second case the reaction lasted from the seventh to the twelfth days, with a maximum of 105.1°. The temperature then remained in the neighbourhood of 102° F., with death on the seventeenth day. No further deaths were recorded during the whole course of the subinoculations, except five which occurred between the tenth and twenty-second generations, and for which some other explanation, having no relation to bluetongue, could be given.

In all, about 113 sheep were used up to the production of the present vaccine strain. Of these, 86 per cent. showed temperature reactions, 5 per cent. showed very doubtful reactions, whereas about 5 showed no evidences of reactions. Of the 86 per cent. showing reactions 20 per cent. were looked upon as slight reactions, where the temperature remained below 105° and was as a rule not maintained for longer than half a day, or fluctuated markedly between 103° and 105° for a few days. However, the scrutiny of the temperature charts of the sheep used in the production of the virus has revealed the fact that a slight or doubtful reaction does not necessarily indicate the intensity of the reaction following upon a subinoculation of blood from such a case. Marked reactions frequently result from subinoculations from slight cases and the converse also holds good. One further point of interest is the production of clinical symptoms. As pointed out above, only two deaths from bluetongue resulted from subinoculations from the original lamb, both of which occurred in the second generation. Up to the eleventh generation slight mucoid or muco-purulent discharges from the nostrils, dyspnoea, and swelling and reddening of the buccal mucous membrane generally accompanied by reddening of the coronets, were observed. Thereafter, no clinical symptoms were recorded, the virus having become so attenuated as to produce only temperature reactions.

During the course of our work temperature reactions were the only guide as to the success or otherwise of the transmission experiments. In this connection it must be pointed out that only temperatures exceeding 104° F., and maintained for periods of one or more days, and furthermore, making their appearance within the limits of the vaccine strain incubation period, which as revealed during the production of the vaccine varied between three and twelve days, were considered to be positive. There exists, therefore, some doubt as to the possible number of positive cases produced. All the experimental animals were, however, subjected to a further subinoculation of vaccine in order to test their immunity and, although such subinoculations may not always succeed or an animal may have acquired a partial immunity not sufficient to absolutely protect it, this test was looked upon as the final proof of the original transmission of the disease.

2. *Ixopo Virus.*—A sample of blood obtained from a case of bluetongue in sheep on the farm of a Mr. Harrek in the Ixopo district of Natal was received from the Government Veterinary Officer of Ixopo on 4.4.32 and this constituted the virus strain used in our experiments and designated Ixopo virus. Unfortunately no information could be obtained as to the extent and severity of the outbreak, but it was ascertained that sheep had actually died from bluetongue on the farm in question. Bluetongue is known to occur yearly in this locality, so that there can be little doubt but that we were dealing with blood from a naturally contracted case.

3. *Kameelfontein Virus.*—On the 5th April, 1932, the Extension Officer of the Pretoria District sent in blood from three lambs which died of bluetongue belonging to Mr. Opperman of the farm Kameelfontein in this district. Although not a very extensive outbreak, this owner lost several other sheep from typical bluetongue. Yearly inoculation against bluetongue is carried out on this farm, but this was not done this year on account of the season having been very dry and there being little chance of bluetongue in the farmer's opinion. As mentioned previously the blood received was taken from young lambs, so that even if relapses of bluetongue do occur there is little chance of our having obtained anything but a natural field strain of the virus, as this was the first season that these lambs had been exposed to infection.

4. *Experimental Animals.*—The sheep used in our experiments are periodically obtained by the Laboratory for experimental purposes and for the production of the bluetongue vaccine from known bluetongue free areas. They are bought from a few well known reliable farmers in the Phillipstown District of the Cape Province, which is a Karroo area. No guarantee of their susceptibility to bluetongue is obtained, but agreements are made that so far as possible the sheep must be obtained only from the Phillipstown District. A further precaution that is taken is to buy only two-tooth sheep in order that they will have had as little chance as possible of having been exposed to natural infection. It is the absolute exception to encounter an immune sheep, so that for practical purposes it may be concluded that all the sheep used are susceptible to bluetongue.

III. EXPERIMENTS WITH THE ORDINARY VACCINE STRAIN OF BLUETONGUE VIRUS.

At the commencement of our experiments we only had at our disposal the laboratory strain used for the preparation of the bluetongue vaccine which, as previously stated, had been isolated from a natural case five years before, and had been passed through nearly 50 generations in the laboratory by means of direct successive blood inoculations. This was therefore not the most suitable strain and we could anticipate difficulties in our transmission experiments.

We began the experiments, as in the case of horsesickness, with the injection of infected mosquitoes after intervals varying between ½ and 19 days, dating from the infective meal. In most cases an interval of from 5 to 15 days was chosen. In the event of a positive case being obtained by this method it was our intention to attempt the further transmission of the infection by the feeding of the same species of mosquitoes on susceptible sheep.

The experiments here under review in this section were commenced in November, 1931, and concluded in March, 1932, when we obtained a positive case and at the same time also received material from natural " field " cases. The work was interrupted for more than a month by our attempts to transmit further what we took to be a successful case caused by the original vaccine strain of the virus, but which eventually turned out to be a doubtful temperature reaction.

For these experiments we used six different species of mosquitoes, viz., *Aedes caballus*, *A. dentatus*, *A. hirsutus*, *A. lineatopennis*, *A. punctothoracis* and *A. vittatus*. *Aedes caballus*, *A. lineatopennis* and *A. hirsutus* were regarded as the most probable vectors for reasons outlined above. *Aedes dentatus* and *A. vittatus* were looked upon as of secondary importance, whereas *A. punctothoracis* was not considered to be of any practical significance.

In all, 12 experiments were made in which about 250 mosquitoes were injected into 10 sheep. After an observation period of three weeks or more, these sheep were all injected with 1 to 2 c.c. of bluetongue vaccine (hereafter referred to as B.T.V.) in order to test their susceptibility or immunity. Whenever it was considered necessary blood from the experimental sheep showing a febrile reaction was injected into one or two susceptible animals in order to verify the original reaction.

In the following pages the various experiments themselves will be described and thereafter a discussion of the results of all the experiments together will be given at the end of each section.

(*a*) Virus Sheep.

Six sheep were used in this series of experiments whereon the mosquitoes were fed. They were injected with the following B.T.V. material: 47th generation (batch 809), 48th generation (sheep 31930) or with blood from one of our own vaccine virus sheep (49th generation).

Virus sheep 1 (=sheep 32230). Injected on 14th November, 1931, with 1 c.c. B.T.V. 31930.

Result.—Temperature normal up to 18th November a.m.; p.m. 105.4°; the following morning 103.8°. Temperature on 20th, 22nd, 23rd, 25th and 27th November between 104° and 104.6°. The temperature reaction was thus not very marked.

With blood from virus sheep 1, two other sheep, viz. 32338 and 31651, were injected. The first sheep showed a typical temperature reaction with 106.9° as maximum. In the case of the second sheep the reaction was weak, the temperature not exceeding 104.7°.

Virus sheep 2 (=sheep 32331) infected in experiment No. 1 (for temperature reaction see experiment 1).

Virus sheep 3 (=sheep 32338) injected on 26th November with 1 c.c. blood from virus sheep 1.

Result.—On 2nd December the temperature began to rise and the febrile reaction lasted for 5 days. On 2nd December the temperature registered 103.8 and 105.6° (morning and afternoon), on the 3rd 105.5 and 104.8°, the 4th 106.5 and 106.9°, the 5th 106.2 and 105.2°, the 6th (a.m.) 104.2° and the 7th 104° and 103.5°. This was looked upon as a medium reaction.

Virus sheep 4 (=sheep 31635). Injected on 27th January, 1932, with 2 c.c. B.T.V. 31930.

Result.—Temperature normal up to the morning of 1st February. Afternoon temperature 104.2°; 2nd (a.m.) 103.3°; 3rd 105.0° and 104.9°; 4th 105.8° and 106.2°; 5th 105.3° and 104°; and 6th and 7th between 104.0° and 105.0°. This was quite a good reaction, lasting 7 days and showing a maximum of 106.2°.

Virus sheep 5 (=sheep 31582). Injected on 18th February with 2 c.c. B.T.V., batch 809, reported by the Government Veterinary Officer, Barberton, as having caused mortality amongst sheep immunized with it under field conditions. In a number of sheep injected with the material at the laboratory the course of the infection was, however, identical to that produced by our ordinary vaccine virus.

Result.—The temperature remained below 104° up to 22nd February. On the 23rd it rose to 104 and 104.4°; the 24th 104 and 104.6°; 25th 105.7 and 106.4; 26th 105.4 and 104.7; 27th 105.0 and 105.3; 28th (a.m.) 105.2°; 29th 106 and 106.3; 1st March 105.0 and 105.4°; 2nd 104.0 a.m. and p.m. and on the 3rd 103.2 and 104.1. Thereafter the temperature remained below 103.6. This temperature reaction was very marked, the maximum being 106.4 and the duration about 10 days.

Virus sheep 6 (=32120). Injected at the same time as virus sheep 5 with the same material.

Result.—The temperature reaction in virus sheep 5 was normal (below 104) up to 2nd February. On the 3rd it rose to 104.4 and 105.6; 4th 105.6 and 107.2; 5th 106.0 and 104.0; 6th 103.8 and 104.4; 7th (a.m.) 105.2. From 8th February onwards the temperature remained normal. This reaction was of medium intensity, lasting 5 days, with a maximum temperature of 107.2°.

(*b*) Experiments with *Aedes caballus*.

Aedes caballus was used in six experiments in which a total of 124 specimens were injected into susceptible sheep after intervals of from 18 hours to 16 and 17 days.

The virus sheep Nos. 1, 2, 3 and 6 were used for feeding the mosquitoes on.

The mosquito groups consisted of:

Group 1.—Fed on the afternoon of 19th November, 1931, from virus sheep 1, the second day of fever. Temperature 103.8 and 103.2. Of these mosquitoes, hatched from larvae, 19 specimens engorged. Used for experiments 1 and 2.

Group 2.—Remainder of group 1. Fed on 20th November from same virus sheep on third day of fever. Temperature 104.2. 20 Specimens engorged. Used for experiments 2 and 5.

Group 3.—The unengorged mosquitoes of group 2, fed on same virus sheep between 20th November (afternoon) and the following morning. Third day of fever. Temperature 104.2 and 103.8. 23 Specimens engorged. Used for experiments 2 and 5.

Group 4.—Fed on virus sheep 2 on 25th November (afternoon). First day of fever. Temperature about 105.2. 11 Specimens (caught as adults) engorged. Used for experiment 3.

Group 5.—The same material as above fed on virus sheep 2 during the following night. Second day of fever. Temperature 105.2 and 104.8. 36 Specimens engorged. Used for experiment 3.

Group 7.—Fed during the night of 3rd December, 1931, on virus sheep 3. Second day of fever. Temperature 104.8 and 106.5. 51 Specimens (caught as adults) engorged. Used for experiment 6.

Group 8.—Fed on the same virus sheep during the following night. Third day of fever. Temperature 106.9 and 106.2. 29 Specimens (caught as adults) engorged. Used for experiment 6.

Group 14.—Fed on virus sheep 6 during night of 25th February, 1932. Third day of fever. Temperature 106.4 and 105.4. 75 Specimens (hatched from larvae) engorged. Used for experiment 4.

EXPERIMENT 1 (B.T. 1). 5 *Aedes caballus*.
Injection. Interval 18 *hours. Sheep* 32331.

On 20th November, 1931, this sheep was injected subcutaneously with an emulsion of five specimens of *A. caballus* (group 1), which had fed the previous afternoon on an infected animal.

Reaction.—On 24th November (4 days p.i.) the sheep showed the first rise of temperature, up to 105°, and on the next day 105.2. On the 26th a slight fall occurred, and on the 27th the temperature registered 103.2 in the morning. The same day it rose to 108° only to commence falling the following day again (106.2 and 105.3), and from 1st December, the temperature regained normal where it remained.

Immunity test.—On 11th December, three weeks after the injection of the mosquitoes, the sheep was tested for immunity by the injection of 1 c.c. B.T.V. subcutaneously. During the following three weeks (the day of injection excluded) the temperature reached 104° for only one afternoon. The super-infection was thus negative and the sheep immune.

Subinoculations.—On 25th November, two sheep, Nos. 31722 and 32346, were each injected subcutaneously with 1 c.c. blood from sheep 32331. The former sheep showed a typical reaction with 107° as maximum, whereas in the latter the reaction was very weak or even doubtful.

The result of the main experiment was therefore *positive*, confirmed by immunity test and subinoculation into other sheep.

EXPERIMENT 2 (B.T. 2). 20 *Aedes caballus*.
Injection. Interval 5 *days. Sheep* 32335.

Into this sheep were injected subcutaneosuly 20 *A. caballus* which had fed approximately 5 days previously on an infected sheep. Seven specimens of group 1 were injected on 24th November, the same number of group 2 the following day, and on the day after 6 of group 3.

Reaction.—On 2nd December, 8 days after the injection of the first group of mosquitoes a rise of temperature occurred, 104.3° being registered, which, however, was not maintained, the temperature regaining normal the following day.

Immunity test.—On 15th December, three weeks after the commencement of this experiment, the immunity of the sheep was tested by the subcutaneous injection of 1 c.c. B.T.V. The temperature rose 9 days later, reaching its maximum, 106°, the same day. It remained above 104° for four days. This reaction may be considered as moderate.

Result.—There may have been a slight immunity developed in this case but the experiment would appear to be *negative*.

EXPERIMENT 3 (B.T. 4). 30 *Aedes caballus*.
Injection. Interval 5 *days. Sheep* 32303.

Into this sheep 30 specimens of *A. caballus* were injected after the same interval as in the preceding experiment, 8 specimens of group 4 on 30th November and 22 of group 5 the following day.

Reaction.—On 8th December, 7 days after the injection of the last group of mosquitoes, a weak, but quite definite, febrile reaction commenced, which, however, lasted only four days. On 8th December the temperature registered 102.8 and 104.5°; 9th 104 and 105°; 10th 103.3 and 105.9; 11th 103.6 and 104.0. The temperature then returned to normal, remaining thereafter under 103.8°.

Immunity test.—On 23rd December, 22 days after the last injection of mosquitoes, the immunity test consisting of 1 c.c. B.T.V. subcutaneously, was applied. The temperature rose to 104.7 on 28th December and two days later reached 107. The whole reaction lasted 4 days, and was quite typical.

Subinoculation.—Blood from this sheep, taken on 10th December during the short febrile reaction, was injected on that date into sheep 32149. During an observation period of three weeks this sheep never showed a temperature surpassing 104° for longer than half a day. The sheep was tested for immunity three weeks after the injection by the subcutaneous inoculation of B.T.V. A marked febrile reaction resulted, lasting 9 days and showing a maximum of 108°.

The *result* of this experiment must be regarded as *doubtful*, probably as negative. There was a certain febrile reaction, just at the time when it could be expected, but it was not very marked. The immunity test was positive, the sheep therefore not having acquired any immunity. Furthermore, a subinoculation of blood into a susceptible sheep was negative.

EXPERIMENT 4 (B.T. 14a). 40 *Aedes caballus.*

Injection. Interval 7 days. Sheep 31713.

This sheep was injected subcutaneously on 4th March, 1932, with 79 mosquitoes belonging to six different species of *Aedes*, viz., 40 *A. caballus*, 3 *A. dentatus*, 2 *A. hirsutus*, 30 *A. lineatopennis*, 1 *A. punctothoracis* and 3 *A vittatus.* All these mosquitoes had been fed about 7 days previously on an infected sheep. The 40 *A. caballus* which alone concern us here belonged to group 14.

Reaction.—The day following the injection a slight rise in temperature, to 104.2°, took place, but thereafter the temperature remained constant, varying between 101.8 and 103.8°.

Immunity test.—On 5th April, one month after the injection of mosquitos this sheep was injected with 2 c.c. B.T.V. Four days later the temperature rose, passing 104 and reaching 107.4° on the 7th day p.i. The temperature reaction was marked and lasted seven days.

Result.—As shown by the immunity test the sheep had remained fully susceptible and the experiment was thus negative.

EXPERIMENT 5 (B.T. 5). 14 *Aedes caballus.*

Injection. Interval 15 *days. Sheep* 32333.

14 Mosquitoes, which had fed approximately 15 days previously from an infected sheep during the third day of its fever reaction were injected into the above sheep as follows:—9 Specimens of group 2 on 5th December, 1931, and two days later 5 of group 3.

Reaction.—During an observation period of three weeks the animal showed no typical temperature reaction, except that the day following the second injection the temperature rose to 104.2° and later, on the 6th, the 12th and the 15th days after the first injection temperatures of 104.3, 106.4 and 104.2° were recorded. These temperatures were, however, not maintained for periods of longer than ½ day in each case, and were therefore considered as of no consequence.

Immunity test.—On 28th December, three weeks following the last injection of mosquitoes the sheep was injected with 1 c.c. B.T.V. Four days later the temperature reached 105.6°, and after a further period of two days 106.4°, was recorded which constituted the maximum. The reaction persisted for five days and though not marked was quite typical.

Subinoculation.—1 c.c. blood from this sheep taken one day after the short rise of temperature to 106.4°, was injected into sheep 31236 on 18th December. During an observation period of 27 days the temperature did not exceed 103.4° This sheep was then subinoculated with 1 c.c. B.T.V. and seven days

later the temperature rose to 106.2°. The whole reaction lasted 2-2½ days and was not very marked. There existed therefore the possibility of a certain amount of immunity having been developed.

Result.—In our opinion the experiment must be regarded as *negative*, as the sheep had shown no typical reaction and later proved to be susceptible to bluetongue. Furthermore, a subinoculation of blood into another sheep failed.

EXPERIMENT 6 (B.T. 10). 15 *Aedes caballus.*

Injection. Interval 16-17 *days. Sheep* 32340.

In this experiment 15 *A. caballus*, 9 of group 7 and 6 of group 8, were injected on 26th December, 1931. These mosquitoes had fed 16-17 days previously on an infected animal during the second and third days of fever.

Reactions.—Exacerbations to 104° occurred during the first week following the injection, but thereafter 103.4° was the maximum temperature recorded in the course of the observation period of 25 days.

Immunity test.—On 15th January, 1932, the sheep was inoculated with 1 c.c. B.T.V., the injection being followed by a marked reaction, 106°, constituting the maximum, was reached on the 7th day p.i., and on the 11th day the temperature fell to 102°. The temperature remained above 104° for four days.

Result.—This experiment was therefore *negative.*

(*c*) EXPERIMENTS WITH *Aedes lineatopennis.*

With *A. lineatopennis* four experiments were conducted and 112 specimens injected into susceptible sheep.

Virus sheep Nos. 1, 4, 5 and 6 were used, and the mosquito groups consisted of:

Group 1.—Fed on virus sheep 1 on 20th November (afternoon). Third day of fever. Temperature 104.2°. Four specimens (caught as adults) engorged. Used for experiment 7.

Group 2.—Fed on the same virus sheep during the following night. Third day of fever. Temperature 104.2 and 103.8°. 26 Specimens (caught as adults) engorged. Used for experiments 7 and 9.

Group 5.—Fed on virus sheep 5 during the night of 4th to 5th February. Second to third day of fever. Temperature 107.2 and 106.0°. 90 Specimens (caught as adults) engorged. Used for experiment 10.

Group 6.—Fed at same time on virus sheep 4. Third day of fever. Temperature 106.2 and 105.3°. 42 Specimens (caught as adults) engorged. Used for experiment 10.

Group 7.—Fed during the following night on same virus sheep. Fourth day of fever. Temperature 104 and 105°. 40 Specimens (caught as adults) engorged. Used for experiment 10.

Group 8.—Fed on virus sheep 5 during the same night. Third to fourth day of fever. Temperature 104 and 103.8°. 32 Specimens (caught as adults) engorged. Used for experiment 10.

Group 9.—Fed on virus sheep 6 during the night 25th to 26th February. Third day of fever. Temperature 106.4 and 105.4°. 42 Specimens (reared from larvae) engorged. Used for experiment 8.

EXPERIMENT 7 (B.T. 3). 20 *Aedes lineatopennis.*

Injection. Interval 5 *days. Sheep* 31848.

20 *A. lineatopennis* were injected into this sheep about five days after their infective meal. They were comprised of 3 of group 1, injected 25th November, 1931, and 17 of group 2 injected the following day. They had fed on an infected sheep during the third day of fever, its temperature of 104.2° at the time, however, having been very low.

Reaction.—During an observation period of three weeks the sheep showed no typical febrile reaction. The temperature passed 104° shortly after the injection and again four days after the second injection, but in each case was maintained for only ½ day.

Immunity test.—On 17th December the sheep was inoculated with 1 c.c. B.T.V. The temperature commenced rising four days later, the fever lasting five days with a maximum of 105.6°. The reaction was thus very mild.

Result.—The sheep being susceptible, the experiment must be regarded as *negative.*

EXPERIMENT 8 (B.T. 14b). 30 *Aedes lineatopennis.*

Injection. Interval 7 days. Sheep 31713.

Into this sheep 30 *A. lineatopennis* of group 9 were injected on 4th March, 1932, together with 49 specimens of five other *Aedes* species.

The course of the experiment has already been described under experiment 4, and there the result was shown to be *negative.*

EXPERIMENT 9 (B.T. 6). 2 *Aedes lineatopennis.*

Injection. Interval 16 *days. Sheep* 31683.

On 12th December, 1931, two *A. lineatopennis* of group 2 which had had an infective feed 16 days previously were injected into this sheep.

Reaction.—No febrile reaction occurred throughout the observation period of more than five weeks, although 13 days p.i. the temperature exceeded 104° for a period of half a day.

On 14th January, 1932, this sheep was used in experiment 17 (*Aedes vittatus* as a control, but with negative results.

The *immunity test* was conducted on 11th February by the subcutaneous inoculation of 1 c.c. B.T.V. The resultant febrile reaction, with an incubation period of 6 days, a duration of 5 days and with maximum of 106.4°, was typical.

Result.—The experimeut must, therefore, be regarded as *negative.*

EXPERIMENT 10 (B.T. 13). 60 *Aedes lineatopennis.*

Injection. Interval 17-19 *days. Sheep* 31703.

In this experiment 60 *A. lineatopennis* of groups 5 and 8 were injected on 22nd February, 1932. The mosquitoes had fed on the second to fourth days of the fever reaction on virus sheep 5 and 6, i.e. between February 4th p.m. and 6th a.m., thus 17 to 19 days before.

Reaction.—On the 2nd, 3rd and 8th days following the injection temperatures between 104 and 104.4° were recorded, otherwise the temperature remained normal. On 3rd March, the 10th day, p.i. a *definite fever reaction* set in which lasted until 10th March, i.e. 8 days. The maximum was 106.2° and on three days the temperature remained above 105°. Although of medium intensity this reaction was nevertheless quite definite. [For exact temperatures of virus sheep 8 (p. 20).]

Immunity test.—This sheep was tested for immunity on 5th April, 43 days after the injection of mosquitoes, by the subcutaneous injection of 2 c.c. B.T.V. During an observation period of three weeks the temperature remained normal, varying between 101.8 and 103.4°. A *complete immunity* had therefore been acquired.

Subinoculation.—On 10th March, two sheep, 31556 and 32269, were each subinoculated with 2 c.c. blood from the above-mentioned animal, taken the day after the height of the febrile reaction. Both these sheep showed typical bluetongue reactions. In the first case the temperature commenced rising on the fifth day, p.i. and returned to normal on the 13th day, exceeding 104° on seven days with 106° as maximum. The second sheep reacted on the sixth day, the temperature remaining above 104° for six consecutive days with a maximum of 106°. On 10th April, 31 days after the injection of blood from the experimental animal, the immunity of these two sheep was tested by the inoculation of 2 c.c. B.T.V. The temperatures remained normal throughout the observation period of three weeks, varying between 101.4 and 103.4 in the first case and 102.3 and 104° in the latter animal.

Result.—This experiment is therefore definitely *positive.* The injection of the infected mosquitoes was followed, after an incubation period of 10 days, by a typical bluetongue reaction and the sheep later proved to be immune.

Subinoculations of blood into two susceptible sheep produced typical temperature reactions, these sheep subsequently developing complete immunity to bluetongue.

(*d*) Experiments with other Aedes Species.

Apart from *A. caballus* and *A. lineatopennis*, small numbers of four other *Aedes* species were used, viz., *A. vittatus*, *A. dentatus*, *A. hirsutus* and *A. punctothoracis*.

Virus sheep Nos. 3 and 6 and mosquito groups:

A. vittatus, *group* 1.—Fed on virus sheep 3 during the night, 4th to 5th December, 1931. Third day of fever. Temperature 106.9-106.2°. Seven specimens (reared from larvae) engorged. Used for experiment 11.

A. vittatus, *group* 3.—Fed on virus sheep 6 during the night, 25th to 26th February, 1932. Third day of fever. Temperature 106.4-105.7°. Three specimens (reared from larvae) engorged. Used for experiment 12.

A. dentatus, *group* 3.—Fed on virus sheep 6 together with *A. vittatus*, group 3. Four specimens (reared from larvae) engorged. Used for experiment 12.

A. hirsutus, *group* 4.—Fed together with *A. vittatus*, group 3. 3 specimens (reared from larvae) engorged. Used for experiment 12.

A. punctothoracis, *group* 2.—Fed together with the preceding group.—1 specimen (reared from larvae) engorged. Used for experiment 12.

Experiment 11 (B.T. 7). 6 *Aedes vittatus*.

Injection. Interval 5 *days. Sheep* 32308.

On 10th December, 1931, 6 *A. vittatus* of group 1 were injected into this sheep. The mosquitoes had fed five days previously on an infected sheep during the third day of fever. Another injection of mosquitoes into his sheep was made seven days later, but this will be referred to under experiment 16 in the following chapter.

Reaction.—The temperature remained normal for 19 days, when a sudden but short rise to 105.6° took place. This was followed by a period of three weeks in which the temperature remained normal. During the following week fluctuations to 104 and 104.8° occurred.

Immunity test.—On 27th January, after an observation period of about seven weeks, the sheep was inoculated with 2 c.c. B.T.V. Seven days later the temperature rose to 105°, the reaction being mild but typical, lasting 4½ days, with 105.8° as maximum.

Result.—This experiment was negative. No typical reaction had occurred following the injection of mosquitoes and the sheep had remained susceptible.

Experiment 12 (B.T. 14*c*). *Aedes vittatus*, *A. hirsutus* and *A. punctothoracis*.

Injection. Interval 7 *days. Sheep* 31713.

Into this sheep six different species of mosquitoes were injected, which included *A. caballus* and *A. lineatopennis* mentioned under their respective heads, and 3 *A. vittatus*, group 3, 3 *A. dentatus*, group 3, 2 A. *hirsutus*, group 4 and 1 *A. punctothoracis*, group 2.

A full description of this experiment has been given under experiment 4 (*Aedes caballus*).

The result, as mentioned therein, was *negative*.

(*e*) Results of the Injection of Mosquitoes infected with Bluetongue Vaccine Virus.

In 12 experiments we injected into susceptible sheep 251 mosquitoes which had fed on sheep experimentally infected with a

vaccine strain of bluetongue virus from ½ to 19 days previously. These mosquitoes were of the following species:—

Aedes cabalus, 124 specimens.
Aedes dentatus, 3 specimens.
Aedes hirsutus, 2 specimens.
Aedes lineatopennis, 112 specimens.
Aedes punctothoracis, 1 specimen.
Aedes vittatus, 9 specimens.

In the case of two of these experiments the transmission was successful.

In the first of these two experiments (experiment 1) 5 *Aedes caballus* were injected ½ day after having fed on an infected sheep. The febrile reaction in the experimental animal was very marked, the maximum temperature being as high as 108°.

This result indicated that 5 mosquitoes are capable of taking up sufficient virus to reproduce the disease.

This was certainly remarkable in view of the fact that the reaction in the virus sheep from which the mosquitoes had fed had been very weak. In fact, had such a reaction occurred in our experimental sheep, we would have been inclined to regard it as doubtful or even negative.

The second positive experiment (experiment 10) is of more importance. It served to demonstrate that *the bluetongue virus is capable of persisting in* Aedes lineatopennis *in a fully virulent form for periods of at least* 17 *days*.

60 Specimens were injected subcutaneously, and after an incubation period of 10 days a typical fever reaction resulted, which lasted for 8 days. When injected almost 1½ months later with the same strain of virus the sheep proved to be completely immune. Two other sheep, subinoculated with blood from this animal taken during its reaction, showed typical reactions, themselves later acquiring complete immunity.

The other experiments were all negative with the exception of one (viz.: experiment 3), which may be looked upon as doubtful. In this case a somewhat atypical reaction occurred which could possibly have been due to a mild infection with bluetongue, but when tested for immunity the sheep proved to be normally susceptible.

The negative experiments and the one doubtful case embody the injection of 186 mosquitoes at varying intervals after the infective meal as follows:—

5 *to* 7 *days after feeding.*	15 *to* 17 *days after feeding.*
90 *Aedes caballus.*	29 *Aedes caballus.*
3 *Aedes dentatus.*	2 *Aedes lineatopennis.*
2 *Aedes hirsutus.*	
50 *Aedes lineatopennis.*	
1 *Aedes punctothoracis.*	
9 *Aedes dentatus.*	

We will here limit the discussion to the experiments with *Aedes caballus* and *A. lineatopennis* as the number of specimens of the other species used was very small.

We obtained one positive result with 112 *Aedes lineatopennis* injected and only negative results with *A. caballus*, with the exception of experiment 1, when 5 *A. caballus* injected half a day after feeding produced a positive reaction. This case, however, we do not regard as being of much importance in view of the actual transmission as not sufficient time was allowed for the development of the virus in the insect host. The number of experiments conducted is certainly not sufficient to justify the conclusion that one species is a probable transmitter and the other not.

The question presents itself, What can the reason be for the negative results? The chance of the virus developing in the mosquito may be quite small. This may be an important factor. It will be recalled that the virus which we used had been passed through a large number of generations from sheep to sheep by subinoculation in the laboratory. By these successive subinoculations it had been transformed into a vaccine virus, a much attenuated strain, and thus one profound biological alteration had taken place. Another alteration, a change, possibly a reduction, in its developmental capacity in the insect host would, therefore, not be at all surprising.

Another possibility is that the different virus sheep may not have been equally suitable for infecting the mosquitoes. We used 6 sheep in these tests. On virus sheep 4 and 5 the *A. lineatopennis*, which transmitted the infection, were fed. On the others the following mosquitoes fed:—

Sheep 1, 34 *A. caballus*,	22 *A. lineatopennis*.	Result negative.
Sheep 2, 30 *A. caballus*,	nil.	Result doubtful.
Sheep 3, 15 *A. caballus*,	nil.	Result negative.
Sheep 6, 40 *A. caballus*,	30 *A. lineatopennis*.	Result negative.

In virus sheep 1 the reaction was a very mild one, and only exceptionally would we utilize such a case for feeding mosquitoes on. However, on this same sheep those mosquitoes fed which, injected the following day, produced a marked reaction in experiment 1. The other mosquitoes which fed from virus sheep 1 must therefore also have taken up sufficient virus.

Sheep 2 gave the doubtful case.

In the case of sheep 3 only 15 specimens were used.

Sheep 6 is, however, quite remarkable in that 79 mosquitoes comprising 40 *A. caballus* and 30 *A. lineatopennis*, which fed from it, were injected 7 days later without result, notwithstanding the fact that the febrile reaction was very marked.

It is, therefore, not unreasonable to suppose that the virus in one sheep is more capable of developing in mosquitoes than is that in another sheep. On the other hand, as the same virus strain was used to infect all the animals, the varying results experienced may have been due to a general reduction in the developmental capacity of the strain in mosquitoes. Apart from this only a *certain period* in the duration of the febrile reaction may be suitable for the infection of the mosquitos. We were unfortunately not able to carry out a sufficient number of experiments to definitely ascertain this point.

IV. EXPERIMENTS WITH VIRUS COLLECTED FROM A DOUBTFUL CASE OF BLUETONGUE PRODUCED BY THE INJECTION OF MOSQUITOES FED ON B.T.V. VIRUS.

In the preceeding section we discussed (experiment 3) a doubtful case of bluetongue produced by the injection of *A. caballus* 5 days after feeding. The temperature had suggested a mild reaction of bluetongue, and we hoped that this strain, which we considered had displayed some slight aptitude for developing in the insect, would regain its natural virulence in time by further passage through mosquitoes. It must be mentioned that the result of the immunity test did not become known until after the following experiments had been conducted.

In this series of experiments the same procedure was adopted, viz., the subcutaneous injection of emulsified mosquitoes at intervals of 5 to 20 days after the infective meal.

We used 57 specimens of mosquitoes belonging to three species of *Aedes*, viz., *A. dentatus*, *A. hirsutus* and *A. vittatus*, as we had no others at our disposal at that time.

For the feeding of the mosquitoes the following sheep was used:—

Virus sheep 7 (sheep 32303). This sheep was the same as that used in experiment 3 (*Aedes caballus*), and the course of the temperature reaction has been fully described under that experiment.

A. Experiments with *Aedes dentatus*.

Only one experiment, with 9 specimens, was made with this species.

Virus sheep 7.

Mosquito group 2.—Fed on virus sheep 7 during the night of 10th to 11th December. Third day of fever. Temperature 105.9-103.6°. 14 specimens (reared from larvae) engorged. Used for experiment 13.

Experiment 13 (B.T. 8). 9 *Aedes dentatus*.

Injection. Interval 5-6 *days. Sheep* 32046.

On 17th December, 1931, 9 *A. dentatus* of group 2 were injected into the above sheep which, 5-6 days previous, had fed on an infected sheep during the third day of its febrile reaction.

Reaction.—For the first 11 days the temperature remained normal. On 29th December it rose from 103.6° (a.m.) to 105.2°, falling the following morning to 103°, but rising again that afternoon to 106°. Thereafter it remained normal for one week only, to rise again to 105° on 8th January, where it remained for only half a day. This was followed by three days of normal temperature, when a further rise occurred. On 12th January, 104.2 and 105.0° were recorded; on the 13th 104.8 and 104.9°, and on the 14th 104.2 and 102.6°. Thereafter no further abnormal temperatures were registered, although the sheep was kept under observation for the following three months to ascertain whether these sudden temperature elevations were regular or periodic occurrences.

On 21st March the *immunity test* was applied, 2 c.c. B.T.V. being injected. This injection was followed by a reaction six days later, the temperature remaining elevated (above 104°) for 5½ days, with a maximum of 106.8°. The sheep had therefore acquired no immunity.

Subinoculation.—On 31st December, the day following the second rise in temperature, a subinoculation of 1 c.c. blood from this sheep was made into sheep 31979. Four days later a rise occurred, 106° being reached, followed

on the next day by temperatures of 105.2 and 103.4°. It then returned to normal, but on the 13th day, p.i. there was another short rise to 105.2°. The sheep was kept under observation for over 11 weeks, during which period the temperature, although slightly irregular, only three times exceeded 104 for half a day in each case. On 21st March this sheep was inoculated with the same virus as that employed in the case of the original sheep. The ensuing reaction was very marked, persisting for 6½ days with a maximum of 106.8°.

Result.—It is rather difficult to form any definite conclusion in this case and we prefer to regard the result as *doubtful*. The original sheep, which, prior to the mosquito injection had registered a very constant temperature, showed a somewhat doubtful reaction after an incubation period of 13 days. Furthermore, in the sheep subinoculated from it a slight temperature reaction occurred but in neither case was any immunity developed.

B. Experiments with *Aedes Hirsutus*.

Aedes hirsutus was made use of in two experiments wherein 26 specimens were injected 5 and 16 days after the infective feed.

Virus sheep 7 and mosquito group:

Group 3.—Fed on virus sheep 7 during the night of 10th to 11th December. Third day of fever. Temperature 105.9 and 103.6°. 29 specimens (reared from larvae) engorged. Used for experiments 14 and 15.

Experiment 14 (B.T. 9). 20 *Aedes hirsutus*.
Injection. Interval 5-6 *days. Sheep* 32118.

On 17th December, 1931, 20 *Aedes hirsutus* of group 3 were injected. These mosquitoes had fed 5-6 days before on an infected sheep during the third day of a febrile reaction.

Reaction.—A rise in temperature occurred on 25th December, the readings being 104.6, and a little later on 105.6, followed the next morning by 103.8°. Unfortunately morning temperatures only were taken (holidays). On 30th December and 13th January temperatures of 106 and 104.8°, respectively, were recorded, which were, however, only fluctuations. Further fluctuations of 106 and 106.4° occurred on 21st and 25th January, followed by normal temperatures for the remainder of the observation period of almost 10 weeks.

Immunity test.—On 5th April, 1932, 110 days after the injection of the mosquitoes, the immunity was tested by the injection of 2 c.c. B.T.V. A marked febrile reaction followed, commencing 6 days later and lasting for 5 days with a maximum temperature of 107°.

A *subinoculation* of 1 c.c. blood from this sheep was made into sheep 31764 on 28th December, 3 days after the slight initial temperature reaction referred to above. From 31st December to 9th January, the 4th to the 12th day following the inoculation, the sheep showed an extremely irregular temperature exceeding 104° on 5 days (on 2 days afternoon temperatures were not recorded), whereon 106°, 106.7°, 105.2°, 104.8° and 105° were registered. These elevations were in each case followed on that or the following day by drops in temperature to 103° or lower. The temperature remained more regular during the ensuing four weeks exceeding 104° for half a day on only one occasion. On 10th February, the immunity test was applied, 1 c.c. B.T.V. being injected. After an incubation period of 6 days a short but definite reaction occurred which lasted for 2½ days, 106.8° being the maximum temperature.

The results of this experiment are not altogether clear. A slight temperature reaction followed the injection of mosquitoes, but the immunity test clearly demonstrated that no immunity had been acquired. Another sheep, subinoculated with blood from this animal, showed a very irregular temperature over a period of 8 days, running up as high as 106.7° on one occasion. This reaction was not typical for bluetongue, but similar reactions are met with from time to time in subinoculations of the vaccine virus strain. The reaction following the immunity test of the second sheep was mild, lasting for only 2½ days, seeming to indicate that some slight immunity had been developed.

The transmission in this experiment must, therefore, be regarded as *doubtful*, in all probability negative.

EXPERIMENT 15 (B.T. 11). 6 *Aedes hirsutus*.

Injection. Interval 16 *days. Sheep* 32298.

This sheep was injected with 6 *Aedes hirsutus* of the same group as those used in the preceding experiment, but 16 days after the infective meal.

Reaction.—The temperature remained normal for 12 days, varying between 101.5 and 104°. On 10th January, a mild temperature reaction commenced, lasting three days, and accompanied by discharges from the nostrils. Temperatures of 106 and 103.2° were recorded on 10th January, on the 11th 105.2 and 105°, on the 12th 103.4 and 105.2° and on the 13th 103.2 and 102.6°. During this reaction an occasional rise in temperature up to 106.2° was noted. The animal was kept under observation for the following 11 weeks, during which time it showed a very regular temperature, only twice, for periods of half a day at a time, reaching 104°.

Immunity test.—On 5th April, 99 days after the injection of mosquitoes the immunity was tested by the injection of 2 c.c. B.T.V. Six days later a very marked febrile reaction followed, lasting 7 days, with a maximum temperature of 108°.

Subinoculation.—Blood from this sheep taken on 11th and 14th January, during and shortly after the reaction referred to above, was injected into sheep 32349 on the latter date. Throughout the course of an observation period of more than 12 weeks no definite febrile reaction occurred. On the 4th, 6th and 7th days, p.i. slight rises in temperature to 104 and 104.4° occurred, and on the 20th day, p.i. 105° was exceeded for half a day, otherwise it remained normal. 88 days after the injection (i.e. on 11th April) the sheep was tested for immunity by the subcutaneous injection of 1 c.c. B.T.V. The reaction which followed was very marked lasting 8 days with a maximum temperature of 106.7°.

On 27th April a further sheep (No. 34738) was injected with preserved blood taken on 11th January from the original sheep (No. 32298), but in this case 10 c.c. were injected intrajugularly in order to increase the chances of infection. No definite reaction, however, occurred. Temperatures of 104° were exceeded several times for not longer than half a day on each occasion, but this had also been the case before the sheep was infected. On 28th May the animal was tested for immunity by the injection of 1 c.c. B.T.V. A typical reaction lasting 4 days with temperatures up to 106.4° followed after an incubation period of 4 days.

Result.—This experiment has to be regarded as *negative*. A mild temperature reaction was shown, but when tested for immunity the sheep proved to be normally susceptible and also subinoculations of blood, taken during the febrile reaction, into 2 susceptible sheep failed.

C. EXPERIMENTS WITH *Aedes Vittatus*.

With this species two experiments were conducted wherein 22 specimens were injected 5 and 20 days after feeding on an infected sheep.

Virus sheep 7 and mosquito group:

Group 2.—Fed on virus sheep 7 during the night of 10th to 11th December. Third day of reaction. Temperature 105.9° and 103.6°. 40 specimens (reared from larvae) engorged. Used for experiments 16 and 17.

EXPERIMENT 16 (B.T. 7). 14 *Aedes vittatus*.

Injection. Interval 5 *days. Sheep* 32308.

This sheep had been used once before, viz., in experiment 11, where it had been injected with 6 *A. vittatus* group 1.

On 17th December it was injected with 14 *A. vittatus* of group 2 which had fed 5-6 days before on sheep 7 during the third day of its febrile reaction.

Reaction.—No typical reaction resulted. Once only, 13 days after the injection of mosquitoes, the temperature exceeded 105° for half a day.

Immunity test.—On 27th January after an observation period of 20 days the sheep was injected with 2 c.c. B.T.V. The resultant reaction was quite typical, lasting 4-5 days, with a maximum temperature of 105.8°.

The *result* of this experiment was definitely *negative*.

EXPERIMENT 17 (B.T. 12). 8 *Aedes vittatus*.

Injection. Interval 20 *days. Sheep* 32288.

For this experiment 8 *Aedes vittatus* of the same group as in the preceding experiment were used on 31st December, 1931, but the injection was made 20 days after their initial feeding.

Reaction.—Four days following the injection of the mosquitoes (i.e. on 4th January) the temperature rose rapidly to 106°, where it remained throughout the day. On the 5th 105.8 and 107° were recorded, on the 6th 106.7 and 105°, the 7th 104 and 103.7°, and on the 8th 104.4 and 104°. It then returned to normal and remained below 104° for the following 18 days. This temperature reaction was certainly very marked, and it closely resembled the usual bluetongue reactions. However, the incubation period of four days was very short, especially when we take into consideration the fact that only a very small quantity of virus could have been injected with the crushed mosquitoes. Following upon the temperature reaction slight clinical symptoms were manifested consisting of distinct warmth of the feet and coronets, slight discharges from both nostrils and frequent licking of the lips.

Immunity test.—On 27th January the sheep was tested for immunity by the injection of 2 c.c. B.T.V. subcutaneously. Seven days later the temperature rose to 105.4°, remaining at 105 during the following day, but falling the day after below 104°. Two fluctuations to 105° occurred on the 12th and 20th day, p.i. but they lasted only half a day each. The temperature reaction following upon the immunity test injection was very weak, lasting only 1½ days, with a maximum of not even 106°.

Subinoculation.—On 7th January a sheep, 31696, was subinoculated subcutaneously with 1 c.c. blood from the preceding sheep taken two days previously. No typical febrile reaction followed this injection. Twice only, viz., 4 and 11 days p.i., short rises of temperature to 106 and 105.2° respectively, occurred. Five weeks after the subinoculation of blood, i.e. on 10th February, 1 c.c. B.T.V. was injected. This was followed by a marked temperature reaction lasting 8-9 days with a maximum of 106.6°.

Another sheep, 32299, was subinoculated with 1 c.c. of the same material as sheep 31696, on 14th January. No temperature reaction resulted with the exception of a few slight fluctuations up to 104°. On 11th April, twelve weeks after this subinoculation, 1 c.c. B.T.V. was injected, which resulted in a pronounced temperature reaction lasting 4 days with 108.8° as maximum.

Together with the above sheep a third, viz. 31683, was subinoculated with the same material. This sheep had been used in a negative experiment (see experiment 9, *A. lineatopennis*). Again no typical reaction ensued. 16 days, p.i. a short rise in temperature to 105° took place. On 10th February this sheep was inoculated with 1 c.c. B.T.V., which was followed by a typical reaction of 5½ days' duration, showing a maximum temperature of 106.4°.

On 27th April we injected yet another sheep, No. 34690, with the same preserved material as in the preceding cases. In this case, however, 10 c.c. was injected intrajugularly. For 21 days the temperature remained normal (not exceeding 104°). Then a mild reaction followed, the 19th 105.6, the 20th 103.8 and 104.8, and the 21st 103.4 and 104.8 being recorded, whereafter the temperature dropped to normal again. On 28th May the sheep was tested for immunity by injection of 1 c.c. B.T.V. After an incubation period of 7 days a clear reaction, lasting 6 days, with 107.4 as a maximum, followed.

The *result* of this experiment has to be regarded as *doubtful*. Four days after the injection of the mosquitoes a distinct temperature reaction lasting 3 days with 107° as maximum of temperature appeared, followed by slight clinical symptoms. Except for the short incubation period, the reaction resembled a typical bluetongue reaction. Besides, the temperature reaction following upon the immunity test was very weak, lasting only 1½ days. Blood taken during the end of the febrile reaction failed, however, to transmit the disease to 4 susceptible sheep.

D. Results of the Injection of Mosquitoes Fed on a Doubtful Case produced by B.T.V. which had been subjected to Passage through Mosquitoes.

On a sheep showing a mild bluetongue-like reaction which had been produced by the injection of a number of *A. caballus* fed five days previously on a true experimental case of bluetongue (B.T.V.), we fed a number of *A. dentatus*, *A. hirsutus* and *A. vittatus*. These were injected into susceptible sheep at intervals of from 5 to 20 days following the feed.

The results of these experiments were as follows:—

Injection of 9 *A. dentatus* 5-6 days after feeding (experiment 13) was followed 13 days later by a temperature reaction lasting 2 days with 106° as maximum. A subinoculation of blood into another sheep produced a similar short reaction four days after the injection. Both these sheep when tested for immunity about three months later proved to be susceptible. The result was looked upon as doubtful.

20 *Aedes hirsutus* (experiment 14) were injected after the same interval, resulting in a short febrile reaction 8 days later in which the temperature exceeded 105°. This sheep was found to be still normally susceptible almost 4 months later. A sheep subinoculated with 1 c.c. blood from this case showed an extremely irregular temperature, which rose as high as 106·7°, from the 4th to the 12th day following injection. The immunity test, conducted one month later, was followed by a very mild reaction of only 2½ days' duration. This case must also be regarded as somewhat doubtful.

Another lot of *A. hirsutus*, consisting of 6 specimens, was injected after a period of 16 days (experiment 15). 12 Days later a temperature reaction commenced closely resembling a mild bluetongue reaction, 106° being the maximum temperature recorded. Three months later, however, this sheep was found to be normally susceptible. A sheep, injected with 1 c.c. blood taken during the course of this reaction failed to react, although it was fully susceptible as demonstrated at a later date. A further sheep was injected later on with 10 c.c. blood given intrajugularly and this also failed to show any typical fever reaction. This experiment also appears to be doubtful.

A further experiment (experiment 16) wherein 14 *Aedes vittatus* were injected five days after feeding, gave negative results.

8 *A. vittatus* of the same group were injected into another sheep after a longer interval, viz., 20 days (experiment 17). A very distinct temperature reaction combined with slight clinical symptoms made its appearance 4 days later, lasting 4-5 days and showing a maximum temperature of 107°. This reaction very closely resembled that of a typical bluetongue reaction. The immunity test injection produced a very weak reaction persisting for only 2 days, the maximum temperature not exceeding 105.4°. An immunity appears to have been developed, not sufficient, however, to completely prevent a reaction, but nevertheless sufficiently strong to considerably modify it. Three sheep were injected with 1 c.c. blood each, from this sheep taken during the febrile reaction. None of these sheep reacted typically

and all three were later proved to be susceptible to injection of known virulent blood. Furthermore, a fourth sheep was injected with 10 c.c. blood intrajugularly and still no typical temperature reaction ensued. From the results obtained by the injection of mosquitoes in the first instance, we must regard this experiment as doubtful.

The results obtained in these experiments are certainly of interest. We started off with a sheep showing a mild temperature reaction not followed by immunity, and in most of the subsequent cases, produced by injections of mosquitoes fed from this sheep, marked reactions resulted, some of which were indistinguishable from true vaccine strain bluetongue reactions. These, however, were not followed by immuity or at any rate the immuity conferred was extremely weak. Subinoculations of blood from these cases into susceptible sheep did not succeed at all, or, where slight febrile reactions resulted, no immunity was acquired.

The temperature reactions noted may not of course have been connected with bluetongue in any way and may have been purely accidental or coincidental, but in many cases where the sheep were kept under observation for several months the temperatures remained remarkably constant. Furthermore, in most cases the reactions commenced within the limits of the known bluetongue incubation period; in other words, just when they were to be expected. In this connection one must not lose sight of the fact, that immunity does not invariably follow bluetongue, there being notable exceptions recorded, and moreover, that subinoculations with normally virulent virus are not successful in producing results in all cases.

We have regarded these experiments as negative or at most doubtful, which appears to be the only rational conclusion. Had we regarded them as positive, *A. hirsutus* and *A. vittatus* would naturally be incriminated as transmitters, which, with the results so far obtained, cannot be assumed.

V. EXPERIMENTS WITH VACCINE VIRUS AFTER ONE DEFINITE PASSAGE THROUGH MOSQUITOES.

In the first section we described an experiment (experiment 10) in which a true case of bluetongue was obtained by the injection of a large number of *Aedes lineatopennis* 17 to 19 days after the infective meal. This case was verified by subinoculations of blood from it into two susceptible sheep and subsequent immunity tests. This appeared to be an excellent case wherewith to carry on the experiments and endeavour to obtain results in the more natural manner, viz., by the actual feeding of mosquitoes.

At that time, about the middle of March, large numbers of *A. caballus* and a fair number of *A. lineatopennis* were available from one or other of our experimental breeding places. *A. hirsutus* was not so plentiful, but could periodically be obtained in moderate numbers. These three species were therefore used in this series of experiments.

It was our intention to commence feeding the mosquitoes on susceptible sheep 14 to 15 days after the infective meal and once again

5 days later, if any specimens still survived. In the event of no typical reactions resulting the remaining mosquitoes would be injected.

In all, 9 experiments were conducted in which 93 mosquitoes fed on susceptible sheep after intervals of 14 to 20 days and 11 specimens were injected 22 to 36 days following the original feed on the reacting sheep.

A. Virus Sheep.

The mosquitoes were fed on the following three sheep:

Virus sheep 8=sheep 31703 from experiment 10 (into which *A. lineatopennis* were injected 17 to 18 days after feeding).

Result.—Temperature reaction: 3rd March, 103.2 and 105.8°; 4th 103.6 and 104.0°; 5th 103.4 and 104.2°; 6th (a.m.) 104°; 7th 104.9 and 104.6°; 8th 104.6 and 105.9°; 9th 106.2 and 105.2; 10th 104.5 and 104.6° and 11th March 103.2 and 103.5°. It is rather difficult to estimate in this case where the actual bluetongue reaction commenced, 3rd or 7th March may either be looked upon as the commencement.

Virus sheep 9=sheep 31556 from experiment 10 (injected on 10th March with blood from virus sheep 8).

Result.—The temperature remained normal until 14th March. On 15th 103 and 104.6°; 16th 103.6 and 103°; 17th 104.8 and 106°; 18th 105.3 and 105°; 19th 105 and 103.8°; 20th (a.m.) 103.8°; 21st 104.3 and 105.1°; 22nd 104.2 and 104.2°; and 23rd 103.3 and 104.1°. From the 24th onwards the temperature remained normal. An injection of B.T.V. on 10th April revealed the presence of complete immunity. In this case 15th March or perhaps 17th March may be regarded as the commencement of the reaction.

Virus sheep 10=sheep 32269 from experiment 10 (injected together with preceding sheep with blood from virus sheep 8).

Result.—Temperature normal until 14th March. On 15th 102.8 and 104°; 16th 103.2 and 105°; 17th 105.6 and 106°; 18th 105.6 and 105.1°; 19th 104.2 and 105.1°; 20th (a.m.) 104° and on 21st 105.8 and 105°. From the 22nd onwards the temperature remained normal except for two short elevations on the 24th and from the 26th to the 27th. When tested on 10th April this sheep showed complete immunity. The commencement of the reaction may, in this case, be regarded as 15th or 16th March.

B. Experiments with *Aedes caballus*.

A large number of *A. caballus* were fed on the three virus sheep, of which 36 engorged themselves from the original sheep and 410 from the two subinoculated cases. This number was very considerably reduced within the first week after feeding, the high mortality resulting from the deposition of large numbers of eggs.

In two experiments we succeeded in feeding 41 specimens on susceptible sheep after an interval of 14 to 15 days, 10 specimens in one experiment after 18-19 days, and 1 specimen remained for injection after an interval of over one month.

Experiment 18 was carried out with the mosquitoes fed on the original case, and experiments 19 to 21 with those infected on the two subinoculated sheep.

Virus sheep 8 to 10 and mosquito groups:

Group 16.—Fed on virus sheep 8 during the night of 9th to 10th March. Seventh (or third) day of fever. Temperature 105.2 and 104.5°. 36 specimens (caught as adults) engorged. Used for experiment 18.

Group 17.—Fed on virus sheep 10 during the night of 17th to 18th March. Third (or second) day of fever. Temperature 106 and 105.6°. 112 specimens (caught as adults or reared from larvae) engorged. Used for experiments 19-21.

Group 18.—Fed at the same time as group 17 on virus sheep 9. Third (or first to second) day of fever. Temperature 106 and 105.3°. 186 specimens (caught as adults or reared from larvae) engorged. Used for experiments 19-21.

Group 20.—Fed on virus sheep 10 on night of 18th to 19th March. Fourth (or third) day of fever. Temperature 105.1 and 104.2°. 112 specimens (caught as adults or reared from larvae) engorged. Used for experiments 19-21.

EXPERIMENT 18 (B.T. 15). 16 *Aedes caballus*.
Feeding. Interval 14-15 *days. Sheep* 32326.

On the night of 23rd to 24th March *A. caballus* group 16 was allowed to feed on this sheep and 12 engorged specimens were obtained. On the 24th the remainder were again placed on this sheep, of which 4 engorged themselves, making a total of 16 engorged specimens. These mosquitoes had fed 14 to 15 days previously on sheep 31703 from experiment 10 during the seventh or third day of the reaction (the actual commencement of the reaction is difficult to ascertain), this sheep having been injected with *A. lineatopennis*.

Reaction.—No typical febrile reaction followed the feeding of the mosquitoes, the temperature remaining normal except for 26th and 27th March, and 9th and 18th April, when it rose to 104°, and on one occasion to 105.2°.

On 19th April the *immunity test* was applied, 1 c.c. B.T.V. being injected. A marked reaction followed on the 4th day, which lasted 6 days and showed a maximum of 107.1°.

Result.—This experiment was therefore *negative* as no typical reaction followed the feeding of the mosquitoes, whereas the sheep proved to be normally susceptible.

EXPERIMENT 19 (B.T. 18). 25 *Aedes caballus*.
Feeding. Interval 14-15 *days. Sheep* 33528.

On 1st April a cage containing *A. caballus*, groups 17, 18 and 20 combined, was placed on the above sheep and during the night 25 specimens engorged themselves. These mosquitoes had fed 14-15 days previously between March 17th (p.m.), and the 19th (a.m.) on virus sheep 9 and 10.

Reaction.—On 7th April, 6 days after the feeding of the mosquitoes, the temperature rose to 106.2°, remained at 105.4 to 106.2° during the following day, and was still 106° the next morning. It then dropped back to normal, where it remained for 9 days. On 19th April a second rise took place, 105.8 and 103.2° being recorded on that day and 106 and 105° on the 20th with 103.6 and 104.8° on the day following. This was followed up to 3rd May by another period of normal temperatures, when 106.5 and 105.5° were registered. These temperatures were maintained, however, for only the one day. During the next 5 days the temperature was again normal.

Subinoculations.—On 8th April, during the first rise of temperature, sheep 34660 was subinoculated with 2 c.c. blood and on 21st April during the second temperature rise, once more with 2 c.c. blood taken on that date. The sheep was kept under observation up to 8th May, 31 days after the first and 18 days after the second inoculation. A temperature of 104° was only surpassed on the day of the first injection, on the 2 following days and on the 8th day, 104.7° being the highest temperature registered. Apart from this the temperature remained normal. On 9th May the immunity of the sheep was tested by the injection of 1 c.c. B.T.V. On 15th May, 6 days later, the temperature began to rise to 105.6°. It then dropped and 4 days later was normal again. The result of this subinoculation therefore was negative, no reaction occurring, the animal being susceptible.

With the same material as that used in the preceding case another animal, sheep 34663, was injected on 8th and 21st April. This sheep was kept under observation up to 12th May, 34 days after the first and 21 after the second inoculation. Only once, 7 days after the first injection, was a temperature higher than 104 (104.2°) registered for ½ day, otherwise the temperature was normal throughout the observation period. On 12th May 1 c.c. B.T.V. was injected in order to test the immunity. After an unmaintained rise of temperature (104.6°) on 17th May, an abrupt rise up to 107° occurred on 19th May. The temperature remained at 107° for another ½ day, then for 3 days at 105° and from 25th May onwards was normal again. This sheep was, therefore, fully susceptible, and no reaction having followed the subinoculations, this experiment must also be regarded as negative.

Immunity test.—On 9th May, 38 days after the feeding of the mosquitoes, and 29 days after the first temperature reaction, the original sheep of the main experiment was tested for immunity by injection of 1 c.c. B.T.V. On 15th May the temperature reached 104.2°, the next day 107°, but fell one day later back to normal (103°). Then another reaction, lasting about 5 days with 105° as maximum occurred.

Result.—The result of this experiment is somewhat difficult to interpret. Five days after the feeding of the mosquitoes a short but clear reaction (106°) was noticed, certainly occurring earlier than a mosquito-transmitted reaction would be expected. Ten days later another reaction appeared during which temperatures above 104 and 106° as maximum were noticed on 5 days. Subinoculations of blood taken during these exacerbations failed to produce a reaction in susceptible sheep. Furthermore, after the immunity test the sheep reacted, although not absolutely typical, at any rate clearly enough. The reaction after the feeding of the mosquitoes and that which followed the immunity test were of equal intensity. The experiment cannot be regarded as positive nor with certainty as negative, and we prefer therefore to regard it as *doubtful.*

EXPERIMENT 20 (B.T. 21). *Aedes caballus.*

Feeding. Interval 18-19 *days. Sheep* 33544.

On 5th April the remainder of the mosquitoes used in the preceding experiment, viz. groups 17, 18 and 20 were fed on sheep 33544 and 10 specimens engorged themselves during the night. In this case 18 to 19 days had elapsed since the inital feed on the virus sheep 9 and 10.

Reaction.—The temperature of this sheep remained normal for 11 days. On 18th April it rose from 102.6° to 105°, remained at 104.6° and 105.4° during the following day and fell back from 105 to 103.6° on the third day. Thereafter it remained normal except for a short rise to 104.8° on 25th April.

Subinoculations.—2 c.c. blood were injected into sheep 31841 on 20th April, the last day of the reaction. The sheep was kept under observation for 22 days. No reaction was noticed, the temperature remaining between 101.8 and 103.8°. On 12th May this sheep was tested for immunity by the injection of 1 c.c. B.T.V. Seven days later the temperature began to rise and reached 108.2° on 20th May. The reaction was very marked, the temperature remaining above 105° for 6 consecutive days. As no reaction had followed the original injection the subinoculation was negative.

Together with this animal sheep 34422 was injected with the same material. On the 2nd and 3rd day after the inoculation the temperature was somewhat elevated (104 and 105°), but thereafter normal up to 12th May, when the sheep was tested for immunity by injection of 1 c.c. B.T.V. Ten days later the sheep reacted, the fever lasting 6-7 days, and 107° being the highest temperature noticed. This subinoculation was also negative.

Immunity test.—On 9th May, 34 days after the feeding of the mosquitoes and 19 days after the fever reaction the immunity of sheep 33544 of the main experiment was tested by the injection of 1 c.c. B.T.V. Six days later the temperature reached 105° and on the next day rose further up to 106.8°. It remained in the vicinity of 106 for one more day, commenced falling the next day, at first slightly, and regained normal on 22nd May.

Result.—This experiment is still more difficult to interpret than the preceding. Eight days after the feeding of the mosquitoes a fever reaction commenced, showing its maximum 3 days later. It was not very severe, but was quite typical, occurring in a sheep with normally regular temperatures and at the time when a reaction from the feeding of the mosquitoes could be expected. A subinoculation of the blood, taken during the fever reaction, into two susceptible sheep failed to produce any result and furthermore, the immunity test of the original sheep was quite marked. We therefore prefer to regard this experiment as *doubtful* as well.

EXPERIMENT 21 (B.T. 24). *Aedes caballus.*

Injection. Interval 35-36 *days. Sheep* 34615.

Of the combined mosquito groups 17, 18 and 20, which had been used in experiments 19 and 20, only one specimen remained one month after the initial feed. We could not induce this specimen to feed again on another sheep, and

on 22nd April it was, therefore, injected after an interval of 35 to 36 days into sheep 34615.

Reaction.—The sheep was kept under observation for 20 days. During this period no reaction occurred, 103.4° being the highest temperature registered.

The *immunity test* was applied on 12th May through injection of 1 c.c. B.T.V. Five days later a temperature reaction began to appear which, however, was not typical and not very marked. It lasted about 6 days with temperatures varying between 103 and 105.2°.

Result.—We regard this experiment also as *negative*, as no reaction followed the injection of the mosquito. The fever following the subsequent virus injection was not marked and perhaps the sheep was slightly immune. We do not think, however, that through this the result of our experiment was largely influenced.

C. Experiments with *Aedes lineatopennis*.

On virus sheep No. 8, i.e. the original case, 44 *A. lineatopennis* were fed, and 92 specimens on the two subinoculated animals together. These constituted practically the end of our supply of this species as the season was almost over and *A. lineatopennis* was becoming comparatively rare. The mortality amongst this species, although considerable, was noticeably less than amongst *A. caballus* whereas they were both kept under identical conditions.

In three experiments we succeeded in feeding 39 specimens, 15 after an interval of 14 to 15 days, 8 after 14 to 20 days and 16 after 18 to 26 days. In a fourth experiment 10 specimens were injected after 22 days.

Experiments 22 and 23 were carried out with the mosquitoes fed on the original case; experiments 24 and 25 with those fed on the subinoculated sheep.

Virus sheep: 8 to 10 and mosquito groups:

Group 11.—Fed on virus sheep 8 during the night of 9th to 10th March. Second (or third) day of fever. Temperature 105.2 and 104.5°. 44 specimens (caught as adults) engorged. Used for experiments 22 and 23.

Group 12.—Fed on virus sheep 10 during the night of 17th to 18th March, Third (or second) day of fever. Temperature 106 and 105.6°. 47 specimens (caught as adults or reared from larvae) engorged. Used for experiments 24 and 25.

Group 13.—Fed at the same time as gronp 12 on virus sheep 9. Third (or first to second) day of fever. Temperature 106 and 105.3°. 11 specimens (caught as adults or reared from larvae) engorged. Used for experiments 24 and 25.

Group 14.—Fed on the same virus sheep the following night. Fourth (or second to third) day of fever. Temperature 105 and 105°. 15 specimens (caught as adults or reared from larvae) engorged. Used for experiments 24 and 25.

Group 15.—Fed during the same night on virus sheep 10. Fourth or third day of fever. Temperature 105.1 and 104.2°. 19 specimens (caught as adults or reared from larvae) engorged. Used for experiments 24 and 25.

Experiment 22 (B.T. 16). 8 *Aedes lineatopennis*.

Feeding. Interval 14-20 *days. Sheep* 31694.

On 23rd March, *A. lineatopennis* (group 11) were placed on this sheep, two specimens feeding during the night. The following day the remainder were put on to this sheep, four more engorged specimens being obtained. On 29th March all the remaining mosquitoes of this group were again allowed to feed on this sheep, and during the night 2 out of the 12 engorged. Mosquitoes were therefore fed on this sheep 8 times, viz., 2 specimens after an interval of 14 days, 4 after 15 days and 2 specimens after 20 days. The mosquitoes had engorged themselves on a sheep during the second and third days of a febrile reaction.

Reaction.—Sheep 31694 was kept under observation for 28 days (21 days after the last mosquitoes had fed) during which period the temperature remained normal, the highest point reached being 103.4°.

Immunity test.—On 20th April the immunity was tested by the injection of 1 c.c. B.T.V. On the 6th day p.i. the temperature rose to 105, fell to 103.1° that afternoon, registered 102.6° and 107° the following day, then 105.2 and 106.4° and on the 9th day p.i. 103.8 and 104.9°. From the 10th day onwards it remained normal. Although a maximum temperature of 107° was recorded the reaction was not very marked, being of a remittant nature and remaining above 104° for a period of less than 48 hours.

Result.—Notwithstanding the somewhat atypical nature of the immunity test this experiment must be regarded as *negative.*

EXPERIMENT 23 (B.T. 17). 10 *Aedes lineatopennis.*
Injection. Interval 22 *days. Sheep* 31777.

On 31st March, 10 specimens (the remainder of *A. lineatopennis* group 11), which had been used in the preceding experiment, were injected into sheep 31777. The interval between the feeding of the mosquitoes on the virus sheep and their injection was 22 days.

Reaction.—Except for a slight rise the day after injection, the temperature of this sheep remained normal for 12 days. A very slight temperature reaction then commenced which lasted for two days, the temperature recorded on the afternoon of the 13th day being 102.4 and 104.6° and the following morning 103.4 and 104.5°. Throughout the following week the temperature, although fluctuating remained normal.

Immunity test.—On 21st April, 3 weeks after the injection of mosquitoes, the immunity was tested, 1 c.c. B.T.V. being injected. The reaction that followed was not typical. Seven days, p.i. the temperature rose to 105.6°, remained at 104.4 for 48 hours and thereafter returned to normal for 2 days, when another rise up to 104.8° lasting ½ day, followed. During the following week the temperature was again normal.

Result.—It is not possible to express a definite opinion as to the result of this experiment and we have to regard it as *doubtful.* The injection of the mosquitoes was not followed by a definite fever reaction, but on the other hand no typical reaction was obtained by the injection of true bluetongue virus, the same batch of virus which gave a very marked reaction in a large number of other sheep. The sheep may possibly have acquired an immunity prior to the commencement of this experiment.

EXPERIMENT 24 (B.T. 19). 15 *Aedes lineatopennis.*
Feeding. Interval 14-15 *days. Sheep* 32076.

On 1st April, 1932, the combined groups 12 to 15 of *A.lineatopennis* were place on sheep 32076, and during the night 15 out of the 21 specimens fed. These mosquitoes had fed between 17th and 19th March on virus sheep 9 and 10.

Result.—After an incubation period of 9 days the temperature rose to 104°. Thereafter, one day of normal temperature followed, and on 13th April 102.8 and 104.2° were recorded; the 14th 105.9 and 104.8° and on the 15th 104 and 103.4°. This concluded the fever reaction, but the temperature thereafter remained somewhat higher than during the incubation period, often reaching or surpassing 104°.

Subinoculation.—On 14th April, i.e. the highest point of the fever reaction, two sheep, 34484 and 34531, were injected subcutaneously with 2 c.c. blood each, taken on this day.

The first sheep did not show a typical reaction. In the course of an observation period of 25 days, the highest temperature recorded was 104.6°, 104° being reached or surpassed on the 2nd, 6th to 8th and 14th to 16th days. On the 15th to 16th day the temperature varied between 104.2 and 104.6°, but this was probably also not of the nature of a true reaction. The sheep was tested for immunity by injection of 1 c.c. B.T.V. on 9th May. Seven days later the temperature reached 106.2°, but fell the next day to 103.6°. The following morning another rise made its appearance, the temperature remaining at 106° for 24 hours and then falling back to normal. This subinoculation was therefore negative.

The second sheep, however, showed a very marked reaction after an incubation period of 5 days, which lasted for 7 days, the maximum temperature

attained being 106.6°. For 4 consecutive days the temperature varied between 104.8 and 106.6°, during which time it fell below 105° for only half a day (for full references of the temperature see under virus sheep 13). Slight but typical clinical symptoms were shown, discharges from the nostrils, slight stomatitis and warm feet being evident. Dullness with a general sick appearance was apparent for a number of days. From 26th April onwards the temperature was normal. On 12th May, 28 days after the injection of blood and 17 days after the end of the febrile reaction the sheep was tested for immunity by injection of 1 c.c. B.T.V. The sheep was kept under observation up to 7th June. No reaction followed this subinoculation, 104° being the highest temperature noticed. This subinoculation was therefore positive.

Immunity test.—On 3rd May, 32 days after feeding the mosquitoes and 18 days after the fever reaction, the immunity of this sheep was tested, 1 c.c. B.T.V. being injected. On 9th May the 6th day, p.i., the temperature rose up to 106.6°. The following two days the temperature remained above 106°, 107.6° being registered as maximum. On the third day (12th May) the temperature was still at 106° and the sheep was then killed for other experiments. The course of the fever reaction indicated that the sheep was normally susceptible to bluetongue.

The result of this experiment is somewhat remarkable. Twelve days after the feeding of the mosquitoes a slight fever reaction with a maximum temperature of 105.9° occurred. It may have been a slight attack of bluetongue fever and therefore 2 other animals were subinoculated with the blood. The original sheep was tested for immunity 18 days after this fever reaction and proved to be normally susceptible. The first subinoculated sheep did not show any reaction of importance and later also proved to be susceptible. In the second sheep, however, the inoculation was followed by a typical and marked reaction with slight, but typical clinical symptoms, and when tested for immunity 17 days after the reaction it proved to be totally immune. We maintain that this experiment must be regarded as *positive*, on account of the definite result in one of the subinoculation experiments, notwithstanding the fact that the sheep had not acquired any immunity. The fever reaction following the feeding of the mosquitoes was short and not very marked, and although being in all probability of a true bluetongue nature, as one of the subinoculations proved clearly, it was not strong enough to give the sheep an immunity sufficient to resist a subsequent inoculation of bluetongue virus.

EXPERIMENT 25 (B.T. 22). 16 *Aedes lineatopennis*.

Feeding. Interval 18-26 *days. Sheep* 33554.

On 5th April, 1932, the remainder of the *A. lineatopennis* (combined) groups 12-15, used in the preceding experiment, were allowed to feed on sheep 33554, and 14 specimens (out of 22) engorged themselves during the night. Seven days later the survivors of this group were given another opportunity of feeding during the night of 12th to 13th April and 2 out of 6 specimens engorged. These mosquitoes had fed originally on virus sheep 9-10, the interval in the case of the 14 specimens having been 18-19 days and in the remaining two 25 to 26 days.

Reaction.—The temperature of the sheep remained normal for the first 8 days, disregarding the second feeding of the mosquitoes. Then on 14th April it rose from 103.4 to 104.8°, the next day registering 104.2°. One day of normal temperature followed, after which a more marked reaction set in. On 17th April, 12 days after the first feeding of the mosquitoes, the temperature remained at 106° throughout the day. The following day 106° and 105.6° were recorded and on the third day of the reaction 104.2 and 104.6°. For two more days a slightly elevated temperature was noted (maximum 103.9°) and thereafter it returned to normal and showed a regular course, remaining for 17 days below 104° except for one sudden rise up to 105°, lasting, however, only ½ day.

Subinoculation.—On 18th April, the second day of the fever reaction, 2 c.c. blood taken on this day were injected into sheep 32302, which was kept under observation for 21 days. During this period only once, 9 days after the injection was a temperature above 104 (104.4°) registered for ½ day. On 9th May 1 c.c. B.T.V. was injected for testing the immunity. On 16th May, 7 days later, the temperature reached 107.1° and 107.6°. It began to drop the next day, but an elevated temperature was maintained for the following 6-7 days. The sheep was fully susceptible and the subinoculation negative.

Together with the preceding animal another sheep, 32317, was injected with the same material. It was also kept under observation for 21 days. Except for some fluctuations, up to 107° on 21st April, to 104.5° on the 25th and to 105° on the 27th, which however were maintained in each case for only ½ day, the temperature remained normal. On 9th May the sheep was tested for immunity by the injection of 1 c.c. B.T.V. This injection was also followed by a marked reaction. The temperature began to rise on 15th May, reached 107.4° on the 17th and remained between 104 and 106° for 4 more days. This subinoculation was, therefore, also negative.

Immunity test.—On 9th May, 34 days after the feeding of the mosquitoes and 20 days after the second and more marked reaction, the original sheep was injected with 1 c.c. B.T.V. A typical reaction followed. Six days later the temperature began to rise, 106.8°, the maximum, being reached the next day. A more or less elevated temperature (103-106.2°) was maintained for 6 more days.

The *result* of this experiment must be regarded as *doubtful* for the same reasons as some of the previous ones. Eight days after the feeding of the mosquitoes quite a typical fever reaction with temperatures up to 106° appeared. However, the same sheep tested for immunity 20 days later proved to be normally susceptible. Two other susceptible animals injected with blood of this sheep taken towards the end of the fever period, did not show any reaction after the injection.

D. Experiments with *Aedes hirsutus*.

Only one experiment with three specimens could be conducted with *A. hirsutus*.

Virus sheep 9.

Mosquito group 5.—Fed on virus sheep 9 during the night of 18th to 19th March. Fourth (or second to third) day of fever. Temperature 105 and 105°. 10 specimens (reared from larvae) engorged. Used for experiment 26.

Experiment 26 (B.T. 20). 3 *Aedes hirsutus*.

Feeding. Interval 17 *days. Sheep* 33533.

On 4th April, 1932, *A hirsutus* group 5 was fed on the above sheep and 3 specimens engorged themselves. These mosquitoes had fed 17 days previously on an infected sheep.

Reaction.—Throughout the observation period of 21 days the temperature remainder normal, 103.4° being the maximum recorded.

On 26th April the *immunity test* was applied, 1 c.c. B.T.V. being injected. The temperature rose 3 days later up to 107°, whereas the next day 105.2 and 104.6° were registered. Two days of normal temperature followed. On the 7th day the temperature rose again to 105.7 and 105°. After 2 further days of normal temperature 105 and 105.4° were recorded on the 10th day and 104.8 and 105° on the 11th, after which the temperature returned to normal, where it remained. In this case temperature reactions were noticed on the 3rd and 4th, the 7th and the 10th to 11th days. The first elevation of temperature was too early for a bluetongue reaction; the two others were not typical and very weak.

Result.—The feeding of mosquitoes was no followed by any temperature reaction at all, but notwithstanding that the experiment has to be regarded as *doubtful*, as the reaction following the immunity test was, if it is to be regarded as a reaction at all, very weak.

E. Discussion of the results obtained with the Vaccine Strain of Virus after one definite passage through Mosquitoes.

In March, 1932, a positive case of bluetongue was obtained by the injection of 60 *Aedes lineatopennis*, which had fed on an infected sheep 17-19 days previous (*vide* Experiment 10 of the first chapter). Blood of the mosquito-infected sheep was subinoculated in two other animals, and these also showed typical reactions. On

these three sheep 592 mosquitoes were fed. 446 *A. caballus*, 136 *A. lineatopennis* and 10 *A. hirsutus*. At the time of the reactions of these sheep, about the middle of March, *A. caballus* were numerous, a fair number of *A. linneatopennis* could be obtained, whereas only a few specimens of *A. hirsutus* were bred.

The mosquitoes were used in this series of experiments mainly for feeding experiments. After their feeding on one of the virus sheep the mosquitoes were kept on sugar water in cages or jars in our warm room. They were then first fed on susceptible sheep after an interval of 14 days, allowing thus sufficient time for a development of the virus in these insects. Part of the mosquitoes were later fed once again on other susceptible sheep and some were emulsified and injected subcutaneously into sheep. When fever reactions followed the feeding or injection of mosquitoes, blood was subinoculated into other sheep. All the sheep used were tested for immunity after a sufficient time had elapsed by subinoculation of 1 c.c. of the normal vaccine virus.

In all, 9 experiments were carried out, the results of which we will briefly summarize.

Aedes caballus, experiment 18, fed 16 specimens after an interval of 14-15 days. No reaction. Sheep normally susceptible. Result *negative*.

A. caballus, experiment 19, fed 25 specimens after an interval of 14-15 days. Short reaction after 6 days and 10 days later. Immunity test applied 29 days after the first reaction gave a somewhat a typical reaction of similar intensity. Subinoculations into 2 susceptible sheep were negative. Result of the experiment *doubtful*.

A. caballus, experiment 20, fed 10 specimens (the same mosquitoes as in experiment 19) after an interval of 19 days. A fairly typical reaction of medium intensity occurred 11 days later. The immunity test applied 19 days after this reaction gave a marked reaction. Subinoculations into 2 susceptible sheep failed to produce any reaction. The result of the experiment was *doubtful*.

A. caballus, experiment 21, 1 specimen, which had been used in the two preceding experiments, was injected after 35-36 days into a sheep. No reaction. Sheep normally susceptible. Result *negative*.

A. lineatopennis, experiment 22, fed 8 specimens after an interval of 14-20 days. No reaction. Sheep susceptible, reaction of the immunity test, however, somewhat atypical. Result *negative*.

A. lineatopennis, experiment 23, 10 specimens, the remainder of the preceding experiment, injected after 22 days. No definite reaction. Reaction of the immunity test not typical. Result *doubtful*.

A. lineatopennis, experiment 24, 15 specimens fed after an interval of 14-15 days. After 12 days a temperature reaction, however, not very marked, occurred. When tested 18 days later for

immunity the sheep was normally susceptible. During the short febrile reaction blood was subinoculated into 2 sheep. One animal remained normal, the other however developed a typical and marked fever reaction accompanied by slight but typical clinical symptoms after an incubation period of 5 days. There seems to be no cause for doubt as to the bluetongue character of this reaction, and furthermore, when tested 17 days after the conclusion of the reaction, the sheep proved to be totally immune. Apart from the result of the immunity test this experiment seems to be *positive* on account of the definite result in one of the subinoculated animals.

A. lineatopennis, experiment 25, fed 16 specimens, the remainder of the mosquitoes used in the preceding experiment, after an interval of 18-26 days. Eight days later a fairly typical temperature reaction appeared. However, 20 days later the sheep proved to be normally susceptible when tested for immunity. Blood taken at the end of the first febrile reaction and inoculated into 2 normal sheep gave negative results. The result of the main experiment was *doubtful*.

A. hirsutus, experiment 26, fed 3 specimens after an interval of 17 days. No reaction. Immunity test very doubtful. Result of the experiment therefore *doubtful*.

In reviewing the results of these experiments the main feature appears to be the fact that most of them are not clear cut.

The most important experiment undoubtedly is No. 24 with *A. lineatopennis*, fed after an interval of 14-15 days on a sheep. In this experiment one of the subinoculated animals, sheep 34531, showed a bluetongue reaction, which allowed no doubt as to its nature, a marked and typical temperature reaction, slight but typical clinical symptoms (the vaccine strain used never shows marked symptoms under experimental conditions) and a total immunity after the end of the reaction. The original sheep, however, showed only a short and mild reaction which produced no immunity.

The same mosquitoes were fed on another sheep 4-11 days later (experiment 25) and once more, in this case after 8 days, quite a typical reaction occurred. Also, in this sheep no immunity resulted and the subinoculations of blood (taken after the temperature had commenced to drop) were also negative.

Similar results were obtained in the experiments with *A. caballus*.

In experiment 19 after feeding a batch of mosquitoes which had been infected 14-15 days previously, 5 days later a short but quite marked temperature reaction appeared. Subinoculations, however, were negative and the sheep later proved to be normally susceptible.

The same mosquitoes, fed on another sheep 4 days later (experiment 20), gave once more the same result, quite a good febrile reaction after 11 days, no immunity and negative subinoculations.

It must be pointed out that all the sheep used in these experiments normally showed a regular temperature, and some of them a very regular one. The animals in this series were observed for at least a fortnight before use and those with somewhat irregular temperatures were discharged. The reactions therefore certainly were not normal fluctuations of the temperature.

Experiments with 16 *A. caballus* fed after 14-15 days (experiment 18), 1 specimen injected after 35-36 days (experiment 21), and 8 *A. lineatopennis* fed after 14-20 days (experiment 22) were negative. Experiments with 10 *A. lineatopennis* (No. 23) injected after 22 days and with 3 *A. hirsutus* (experiment 26) after 17 days were doubtful, although in all probability also negative. These negative or doubtful results were all obtained with mosquitoes fed on virus sheep 8 (infected by injection of mosquitoes), whereas the mosquitoes giving a positive or doubtful, but quite typical reactions, had infected themselves on virus sheep 9 and 10 (infected by subinoculation of blood from virus sheep 8).

It is, therefore, not impossible that part of our failures in these experiments was due to a slight degree of virulence of the virus. It is a known fact, that the virulence of the virus for sheep decreases very rapidly under laboratory conditions. We had the same experience, e.g. when injecting blood from spontaneous clinical cases into sheep in the laboratory, the course of the disease being mild and closely simulating that produced by the vaccine strain. This appears to be mainly due to the better conditions under which sheep are kept in stables than is the case under field conditions.

The small amount of virus injected into a sheep by an infected mosquito may just have been sufficient to produce a fever reaction, but it was insufficient to produce an immunity capable of resisting the comparatively massive doses normally used when injecting virus by means of the syringe.

Furthermore, the virus of this strain may have been present in the blood of mosquito-infected sheep in sufficient quantity to produce infections by subinoculation for only a relatively short time. Once present in sufficient amount in the blood, the virus would produce normal immunity as in sheep 34531 (cf. also the results of mosquito feeding on virus sheep 8, infected through mosquito injection and on virus sheep 9-10, infected through subinoculations).

These are certainly possibilities whereby our results could be explained but they do not entirely satisfy us.

Concluding this discussion we believe that we *have succeeded in transmitting a bluetongue infection by means of allowing infected specimens of* Aedes lineotopennis *to feed on susceptible sheep. The experiments, however, were not sufficiently clear cut and they do not permit of any definite conclusion about the natural transmission of bluetongue to be arrived at.*

On the other hand, the experiments certainly do not exclude *A. lineatopennis* and *A. caballus* as possible vectors.

VI. EXPERIMENTS WITH THE VACCINE VIRUS AFTER TWO (TRUE OR SUPPOSED) PASSAGES THROUGH MOSQUITOES.

In the preceding chapter we have described some experiments with a strain of the ordinary vaccine virus after a survival of 17-19 days in mosquitoes. After feeding the mosquitoes infected with this strain more or less marked fever reactions appeared in some cases, which, however, were not followed by immunity. Only in the case of one sheep infected by subinoculation of blood was a complete immunity acquired. On three of these sheep, mosquitoes were fed during the fever reaction and after an interval of at least 14 days, these were again fed on susceptible animals.

During the interval these mosquitoes were kept on sugar water in our warm room, which was only heated at night at that time as the day temperature was sufficiently high for virus development.

At the time of these experiments, viz., towards the end of the summer (7th–22nd April), *Aedes caballus* were still obtainable in sufficient numbers from one of our experimental breeding places. A fair number of *A. lineatopennis* was also present, but we lost the group of this species later through an unfortunate accident.

A. Virus Sheep.

The following three sheep, infected in experiments described in the preceding chapter, were used for infecting mosquitoes. The reactions shown by these animals were of a true bluetongue nature or at any rate practically indistinguishable from such reactions.

Virus sheep 11=sheep 33528 from experiment 19, reacting after feeding of *Aedes caballus*.

Result.—This sheep showed a series of febrile reactions. We are here concerned with only the first of them. The temperature on 7th April, 6 days after the feeding of the mosquitoes, was 102.6-106.2°, the 8th 105.4 and 106°, the 9th 106 and 103.2° and the 10th (a.m.) 104.6°. Tested later by injection of B.T.V. this sheep was susceptible.

Virus sheep 12=sheep 33554 from experiment 25, reacting after feeding of *A. lineatopennis*.

Result.—The mosquitoes were fed on 5th April, and 12 days later a short reaction occurred. The temperature on 17th April was 106 and 106°, the 18th 106 and 105.6°, the 19th 104.2 and 104.6°. Tested later by injection of B.T.V. the sheep had not acquired an immunity.

Virus sheep 13=sheep 34531 from experiment 24. This sheep was injected on 14th April, 1932, with blood of sheep 32076 which had shown a slight fever reaction after the feeding of infected *A. lineatopennis*.

The temperature was normal up to 18th April, and on the 19th 103.4 and 104.8°, the 20th 103.8 and 105.4, the 21st 105.6 and 105.7°, the 22nd 104.8 and 106.6°, the 23rd 105.5 and 106.1°, the 24th (a.m.) 105.6°, the 25th 103.9 and 104.8°. The temperature then went back to normal. When tested later by injection of B.T.V. this sheep proved to be totally immune.

B. Experiments with *Aedes caballus*.

On each of the three virus sheep one group of *A. caballus* was fed. In all, 382 specimens engorged themselves. The mortality amongst the mosquitoes in this case was considerable; especially was this the case amongst the first group which were caught as adults. They died *en masse* during the period of oviposition 4 days after they fed. The other two groups retained their vitality much longer.

With these mosquitoes 5 experiments (Nos. 27-31) were carried out and 136 specimens were fed again on susceptible sheep after an interval of 14-21 days.

Experiment 27 was carried out with mosquitoes fed on virus sheep 11; experiments 28 and 29 with specimens from virus sheep 12 and experiments 30 and 31 from virus sheep 13.

Virus sheep 11-13 and mosquito groups:

Group 21.—Fed during the night of 7th to 8th April, 1932, on virus sheep 11. First day of fever. Temperature 106.2-105.4°. 118 specimens (caught as adults) engorged. Used for experiment 27.

Group 28.—Fed during the night of 18th to 19th April on virus sheep 12. Second to third day of fever. Temperature 105.6-104.2°. 118 specimens (reared from larvae) engorged. Used for experiments 28 and 29.

Group 34.—Fed during the night of 22nd to 23rd April on virus sheep 13. Fourth day of fever. Temperature 106.6-105.5°. 121 specimens (reared from larvae) engorged. Used for experiments 30 and 31.

EXPERIMENT 27 (B.T. 25). 2 *Aedes caballus*.
Feeding. Interval 15 *days. Sheep* 34084.

During the night of 22nd to 23rd April, 1932, 2 (out of 3) *A. caballus* of group 21 were fed on this sheep. They had infected themselves 15 days before on virus sheep 11 (of experiment 19) during the first day of a febrile reaction.

Reaction.—The sheep was kept under observation for 21 days. During this period no reaction occurred, no temperature above 104° being recorded.

Immunity test.—On 14th May the sheep was inoculated with 1 c.c. B.T.V. On 19th May a short rise to 106° occurred, the temperature dropping to normal, however, the same day. On 21st May another, and this time marked, reaction began lasting 4-5 days with 107° as maximum.

Result.—This experiment was negative. After the feeding of 2 specimens no temperature reaction followed, whereas the sheep was normally susceptible.

EXPERIMENT 28 (B.T. 33). 41 *Aedes caballus*.
Feeding. Interval 15 *days. Sheep* 34550.

On 3rd May, 1932, *A. caballus* group 28 was put on sheep 34550 and 41 (out of 77) specimens engorged themselves during the night. These had fed 15 days before on virus sheep 12 (of experiment 25) during the second to third day of fever.

Reaction.—Up to 15th April, i.e. 12 days following the feed the temperature remained normal. A short rise to 105.6° lasting, however, less than 24 hours, then appeared. Thereafter another week of normal temperature followed. On 24th May, 104.6° was recorded, the temperature remained between 103.8 and 104.6° for 3 days and then dropped to normal.

Immunity test.—On 28th May, 25 days after the feeding of the mosquitoes the sheep was injected with 1 c.c. B.T.V. The following day the temperature started to rise again, 105.4 being recorded on 30th May. The temperature dropped one day later, but directly commenced rising again and fluctuated between 103.4 and 105.4° from 31st May to 4th June. On 6th May it was normal again but the following day a more typical reaction set in, lasting 5 days with 107° as the highest point of the reaction. On 17th June the sheep was discharged from observation.

Result.—It is difficult to form a definite opinion about the result of this experiment, and we have to regard it as *doubtful*. Twenty days after the feeding of the mosquitoes a temperature reaction set in; 4 days later the sheep was injected with B.T.V. The temperature remained irregular, but 10 days later a more typical reaction commenced which would appear to have been the reaction following the virus injection. On scrutinizing the temperature chart, however, the possibility of all these reactions commencing on 24th May, prior to the immunity test, being connected with one another presents itself. Furthermore, the inoculation period of what is considered to be that of the normal bluetongue reaction of the immunity test was longer than normal, viz., 10 days.

EXPERIMENT 29 (B.T. 40). 36 *Aedes caballus*.

Feeding. Internal 21 *days. Sheep* 34409.

During the night of 9th to 10th May the same mosquitoes as used in the preceding experiment were put on to sheep 34409. 49 specimens still remained and of these 36 engorged themselves. The interval was now 21 days.

Reaction.—On 17th May a short rise of temperature up to 104.8°, lasting, however, only ½ day, occurred. 10 days of normal temperature followed. The temperature then became somewhat irregular, varying between 102.8 and 104.6°.

Immunity test.—On 10th June, a month after the feeding of the mosquitoes, 1 c.c. B.T.V. was injected. Directly after this injection the temperature rose to 107°, but had regained normal by the next morning. After 8 days a marked reaction set in lasting about 6 days with temperatures of up to 106.8° and remissions of 102°.

Result.—This experiment is to be regarded as *negative*. No typical reaction followed the feeding of the mosquitoes, whereas the sheep proved to be susceptible.

EXPERIMENT 30 (B.T. 37). 40 *Aedes caballus*.

Feeding. Interval 14 *days. Sheep* 34175.

On 6th May, *A. caballus* (group 34) was put on to this sheep, and during the following night 40 (out of 61) specimens engorged themselves. These mosquitoes had fed 14 days before on virus sheep 13 (sheep 34531 from experiment 25) during the 4th day of fever.

Reaction.—During the first 12 days a normal and regular temperature was maintained (102.4-103.8°). It then began to rise, and on 22nd June 105 and 105.4° were reached. 23rd June 105.4 and 102.1° were recorded, the 24th (a.m.) 104.8°, the 25th 104.6 and 105°, the 26th 104.2 and 105°, the 27th 105 and 104.3° and the 28th (a.m.) 105.2°.

Immunity test.—The same day, 28th May, the sheep was injected subcutaneously with 1 c.c. B.T.V. That afternoon the temperature rose to 106.4, a temperature which was maintained until the following morning. It then dropped to normal and began to rise again on 5th June, 8 days after the injection, reaching 106.8°, the highest point, on 9th June. Four days later the reaction came to an end.

The *result* of the experiment is to be regarded as *doubtful*. 13-16 days after the feeding of 40 infected mosquitoes a marked fever reaction set in. At its highest point, owing to an unfortunate error, the sheep was tested for immunity and 8-9 days later a similar reaction occurred. It is possible in any case, that the first reaction was due to a bluetongue infection transmitted by the mosquitoes which, however, did not confer or had not then conferred to the sheep a sufficient degree of immunity to resist without any reaction the subsequent virus injection.

EXPERIMENT 31 (B.T. 41). 17 *Aedes caballus*.

Feeding. Interval 20 *days. Sheep* 34423.

On 12th May the remaining specimens of *A. caballus* (group 34), which had already been used in the preceding experiment, were put on to sheep 34423 and during the night 17 (out of 25) specimens fed.

Reaction.—On 14th May, two days after the feeding, a temperature of 106.2° was recorded, but the following day it commenced to drop, and remained normal for 9 days, after which a somewhat irregular course was noticed, temperatures of 104-104.8° being noticed on 24th, 29th, 31st May, 1st and 4th June.

Immunity test.—On 8th June, 27 days after the feeding of the mosquitoes, the sheep was injected subcutaneously with 1 c.c. B.T.V. 15th June, 7 days later, the temperature began to rise. The reaction which was of medium intensity, lasted 3-4 days, the highest temperature recorded being 106.4°.

The *result* of this experiment was *negative*. After the feeding of the mosquitoes no typical reaction occurred, whereas the sheep proved to be susceptible.

C. Experiments with *Aedes lineatopennis*.

When these experiments were undertaken in April *Aedes lineatopennis* was very difficult to obtain. Only one group could be fed on one of the virus sheep and this was again fed 15 days later on a susceptible sheep. Shortly afterwards this group was accidently lost.

Virus sheep 11 was used and *mosquito group* 16.—Fed during the night of 7th to 8th April on virus sheep 11. First day of fever. Temperature 106.2-105.4°. 75 specimens (caught as adults) engorged. Used for experiment 32.

Experiment 32 (B.T. 26). *Aedes lineatopennis*.
Feeding. Interval 15 *days. Sheep* 34797.

On 22nd April, 1932, *A. lineatopennis* group 16 was put on to sheep 34797 and during the night 10 (out of 20) specimens fed. They had their initial feed 15 days previously on sheep 33528 during the first day of a febrile reaction.

No real *reaction* followed the feeding of these mosquitoes during an observation period of 23 days. Only once, 4 days after the infecting feed of the mosquitoes, was a temperature in excess of 104° noticed (104.4°). This, however, was maintained for less than 24 hours.

Immunity test.—On 16th May the sheep was injected subcutaneously with 1 c.c. B.T.V. 24th May, 8 days later, the temperature began to rise, reaching 106.6°, the maximum of the reaction, 2 days later. The reaction lasted 3-4 days, and although not very marked, was quite typical.

The *result* of this experiment was *negative*, no typical reaction following the feeding of the mosquitoes on a sheep which, when tested for immunity, proved to be susceptible.

D. Results of Experiments with the Vaccine Virus after two (supposed) Passages through Mosquitoes.

In April, 1932, we obtained fever reactions in sheep after the feeding of mosquitoes which had infected themselves on a sheep showing a bluetongue reaction after the injection of mosquitoes or on two other animals injected with blood of the first sheep. These fever reactions resembled more or less true bluetongue reactions, but they did not produce any immunity. Only one sheep, subinoculated with blood taken from one of these animals during the fever period, showed a typical reaction combined with slight clinical symptoms and later a complete immunity.

On three of these sheep, two original and one of the subinoculated cases, 382 *Aedes caballus* and 75 *A. linneatopennis* were fed. These mosquitoes were kept as usual on sugar water in jars or cages in our warm room after the feed. Following an interval of 14 days or longer, the mosquitoes were fed on new susceptible sheep.

In all, 6 experiments were conducted, 5 with *A. caballus* and 1 with *A. lineatopennis*. In the experiments with *A. caballus* all three virus sheep were used; in the case of the latter species only one sheep, viz., one of the original cases.

Aedes caballus, experiment 27, was carried out with mosquitoes fed on virus sheep 11, one of the original cases. 2 specimens were fed after an interval of 15 days with *negative* results.

For *Aedes caballus*, experiment 28, mosquitoes from virus sheep 12 were used, also one of the original cases showing no subsequent immunity. 41 specimens fed after 15 days. The result was *doubtful*, as a few days before the sheep was injected for testing the

immunity a fever reaction set in, continuing through the incubation period of the immunity reaction, which, besides that, showed a normal course.

A. caballus, experiment 29, was carried out with the same mosquito group, of which 36 specimens fed for the second time after an interval of 21 days. In this case, however, the result was without doubt *negative;* no reaction followed the feeding of the mosquitoes, whereas the sheep was normally susceptible.

A. caballus, experiment 30, was made with mosquitoes which had fed on the subinoculated sheep showing a true bluetongue reaction followed by complete immunity. 40 specimens fed after an interval of 14 days. 13-16 days later a marked reaction set in. Before this reaction was closed unfortunately the immunity test was applied and this showed the normal course. The result therefore was *doubtful*.

A. caballus, experiment 31, was made with the same group of mosquitoes (17 specimens) after an interval of 20 days. The result was *negative*.

A. linneatopennis, experiment 32, with 10 specimens after an interval of 15 days. Result *negative*.

In all these experiments 5 susceptible sheep were bitten 139 times by infected *A. caballus* after intervals ranging between 14 and 21 days and by 10 *A. lineatopennis* after 15 days. Four of these experiments were certainly negative, whereas in two the results were doubtful. Especially in one of these cases (experiment 30), in which 40 *A. caballus* were fed after 14 days, there is a definite possibility that the experiment was really positive.

It must be remembered that these mosquitoes were fed on three virus sheep, of which only one was definitely positive. With mosquitoes fed on this sheep, experiment 30 was conducted. No further discussion of the results seems to be necessary and we may refer to the preceding chapter.

VII. EXPERIMENTS WITH A STRAIN FROM IXOPO.

At the beginning of April, 1932, through the courtesy of the Government Veterinary Officer, we received a quantity of preserved blood from a natural case of bluetonge from Ixopo (Natal). Our hope that with this material clinical cases of bluetongue could be produced and that in future we would not have to rely any more on only more or less typical temperature reactions as produced by the vaccine strain of bluetongue was, however, not realized. Two sheep were injected with this material, but both showed only very slight reactions lasting less than 48 hours in each case and showing no clinical symptoms. The virulence, therefore, was less than that of the ordinary vaccine strain. Mosquitoes were fed on these two sheep but thereafter the strain was abandoned.

At the time of the short febrile reaction in these sheep *Aedes caballus* was the only mosquito species at our disposal in large numbers. In all, 413 specimens engorged themselves. They were

kept in our warm room in the usual way after this feeding, but their numbers diminished rapidly owing to an extraordinary high mortality amongst them.

Apart from this species a few *A. lineatopennis* were fed and also some *A. durbanensis*, which appeared during that time in one of our experimental breeding places.

The experiments were conducted in the usual manner, the infected mosquitos being refed on susceptible sheep after an interval of at least 14 days.

A. Virus Sheep.

The following two sheep were injected with the Ixopo strain:

Virus sheep 14=sheep 31553. Injected on 4th April, 1932, subcutaneously with 2 c.c. preserved blood of a natural case.

Result.—The temperature remained normal or nearly normal up to 10th April. On the 11th (a.m.) it was 105°, on the 12th during the morning 107° and during the afternoon 104°. It then remained between 103 and 104.6° for 6 days and later on between 102 and 104°. The actual febrile reaction was, therefore, very short, lasting less than 48 hours.

Virus sheep 15=sheep 31570. Injected together with the preceding sheep with the same material.

Result.—The temperature remained normal up to 10th April, when 103.8° was recorded during the morning. On the 11th it registered 107 and 106.8°, the 12th 105.8 and 103.4° and from the next day onwards normal temperatures were maintained, ranging between 102 and 104.6°. In this case as well the reaction, although definite, was very short, less than 48 hours.

B. Experiments with *Aedes caballus*.

On both virus sheep a batch of *A. caballus* was fed during the nights following 11th and 12th April. Owing to the very short duration of the reaction, the last two batches were actually fed after the temperature had already returned to normal.

During tht first night 121 specimens were fed, of which 17 survived at the end of 15 days. Of 252 fed during the second night 23 survived. 24 days later only 6 out of the four batches were left. The mortality was extremely high in this case.

The mosquitoes were fed on three susceptible sheep; the two batches fed on the virus sheep during the first night were refed on the first of these three animals after an interval of 15 days, the last two groups were refed at the same time on the second sheep and the remaining specimens of the lots together were fed on the last animal at the end of 24 days. After 45-46 days 2 specimens were still alive and they were injected into a sheep.

The two virus sheep 14 and 15 were used and the following mosquito groups:

Group 22.—Fed on virus sheep 15 during the night of 11th to 12th April. First to second day of fever. Temperature 106.8-105.8°. 95 specimens (reared from larvae) engorged. Used for experiments 33, 35 and 36.

Group 23.—Fed on virus sheep 14 during the same night. First day of fever. Temperature 107.8°. 26 specimens (reared from larvae) engorged. Used for experiments 33, 35 and 36.

Group 24.—Fed on the same virus sheep during the following night. First day after the end of the febrile reaction. Temperature 104-104.2°. 150 specimens (reared from larvae) engorged. Used for experiments 34-36.

Group 25.—Fed on virus sheep 15 during the same night. First day after the end of the febrile reaction. Temperature 103.4-102.6°. 142 specimens (reared from larvae) engorged. Used for experiments 34-36.

EXPERIMENT 33 (B.T. 28). 13 *Aedes caballus*.

Feeding. Interval 15 *days. Sheep* 34758.

On 26th April, 1932, the remaining specimens of the combined groups 22 and 23 were put on to sheep 34758 and 13 (out of 17) specimens engorged themselves during the ensuing night. These mosquitoes had fed on an infected sheep 15 days previously at the peak of a short febrile reaction.

Reaction.—The temperature of this sheep during the week preceding the feeding of the mosquitoes had never exceeded 103°. 7 days after the feeding of the mosquitoes, however, a temperature of 103.4° was noticed, the following morning 103.8° was recorded and for the following 5 days temperatures higher than 103° were frequently seen. Thereafter the temperature became somewhat irregular with unmaintained rises to 104.2 and 104.4° on 19th and 27th May, the day before the application of the immunity test.

Immunity test.—On 28th May, 32 days after the feeding of the mosquitoes, 1 c.c. blood of virus sheep 14, taken on 12th April, was injected subcutaneously. The temperature commenced rising immediately either owing to the virus injection or as a continuation of the slight rise noticed on the previous day, and on 30th May, 105.2° was reached. An evening remission to 102.6° occurred on this date, but on the 31st the temperature commenced rising again and the reaction continued, reaching 105.6, its highest point, on 4th June. The reaction came to an end shortly after this.

Result.—We regard this experiment as *negative*, notwithstanding the fact that something like a febrile reaction commenced just before the application of the immunity test. The reaction following the immunity test was of medium intensity, but a more marked fever could not be expected with this strain owing to its low virulence.

EXPERIMENT 34 (B.T. 30). 14 *Aedes caballus*.

Feeding. Interval 15 *days. Sheep* 34606.

On 27th April, 1932, the surviving specimens of *A. caballus* group 24 and 25 were put on to sheep 34606 and during the following night 14 (out of 23) specimens engorged themselves. These mosquitoes had fed 15 days previously on the day following the febrile reactions of the two sheep infected with the Ixopo strain.

Reaction.—During the first 8 days the temperature remained normal (102-103.4°). On 6th May, it rose to 104.8°, returned to normal the next day, but showed a further slight elevation (104 and 104.2°) on the following two days. Thereafter it remained practically normal up to 27th May, showing only two slight elevations (104 and 103.8°), lasting in each case ½ day.

Immunity test.—On 28th May the sheep was injected subcutaneously with 1 c.c. blood of sheep 31553, one of our virus sheep. Four days later, on 1st June, a sudden rise to 105.6° occurred, which lasted, however, for only ½ day. A further rise occurred 2 days later and the temperature remained between 105 and 106.8° for 3 days. It then dropped again, but remained more elevated than usual (104-105.4°) for the next ten days.

The *result* of this experiment was *negative*. The reaction occurring 9 days after the feeding of the mosquitoes was not sufficiently definite to be of any importance. The immunity test was of medium intensity but clear enough.

EXPERIMENT 35 (B.T. 35). 4 *Aedes caballus*.

Feeding. Interval 23-24 *days. Sheep* 34390.

On 6th May, 1932, the remaining mosquitoes of the two preceding experiments were used again and 4 out of 6 specimens engorged themselves. They had fed 23-24 days before on two infected sheep during the top point of the febrile reactions and during the day after the end of this reaction.

Reaction.—To start with the temperature remained normal for the first 14 days (101.6-103.2°). A short rise to 104.6° then occurred, whereafter another period of normal temperatures followed.

Immunity test.—On 28th May, 22 days after the feeding of the mosquitoes, the sheep was injected with 1 c.c. blood of sheep 31533, the same material as used in the preceding tests. Five days later a definite rise in temperature occurred, 106.8° being reached 2 days later. The fever period lasted 4-5 days, the reaction being typical and quite marked.

The *result* of this experiment was *negative*. No reaction followed the feeding of the mosquitoes, and the sheep was normally susceptible.

EXPERIMENT 36 (B.T. 40). 2 *Aedes caballus*.
Injection. *Interval* 45-46 *days*. *Sheep* 34360.

On 27th May 2 specimens of the combined groups 22-25 were still alive and they were injected into sheep 34360 on that day. They had had their original feed on a virus sheep 45-46 days previously.

Reaction.—A normal temperature was maintained for 7 days up to 3rd June. The temperature was on the 4th 103.6 and 105.6°, the 5th (a.m.) 106°, but from the day thereafter it was normal again.

Immunity test.—On 20th June, 24 days after the injection of the mosquitoes, the sheep was subcutaneously injected with 1 c.c. blood from sheep 31570. A short, but quite typical reaction commenced to appear 7 days later, lasting about 3 days, with 107.4° as maximum.

Result.—The experiment has to be regarded as *negative*. The short reaction following the injection of mosquitoes cannot be considered as a bluetongue reaction and the sheep proved to be susceptible later when tested for immunity.

C. EXPERIMENTS WIHT *Aedes lineatopennis*.

At the time that these experiments were carried out *A. lineatopennis* was very difficult to obtain and not more than 10 specimens could be induced to engorged themselves on the virus sheep. Of these a few specimens were refed on a susceptible sheep after a period of 15 days and longer. After 45 days 2 specimens were still alive and they were injected into a sheep.

Virus sheep 14 and 15 were used and mosquito groups:

Group 17.—Fed on virus sheep 15 during the night of 11th to 12th April. First or second day of fever. Temperature 106.8-105.8°. 3 specimens (reared from larvae) engorged. Used for experiment 32.

Group 18.—Fed on virus sheep 14 during the same night. First day of fever. Temperature 107.8°. 3 specimens (reared from larvae) engorged. Used for experiment 37.

Group 19.—Fed on the same virus sheep one night later. First day after the end of the febrile reaction. Temperature 104-104.2°. 4 specimens (reared from larvae) engorged. Used for experiments 37 and 38.

EXPERIMENT 37 (B.T. 27). *Aedes lineatopennis*.
Feeding. *Interval* 15-29 *days*. *Sheep* 34053.

During the night of 26th to 27th April the combined groups 17 and 18 were put on to this sheep, but only 1 (out of 3) specimens fed. The following night batch 19 was put on and 2 (out of 4) mosquitoes fed. The same lot of mosquitoes was again put on to the sheep during the night of 10th to 11th May and 2 specimens probably fed. This point was not ascertained with certainty, however. During the following night 1 specimen (out of 2) from groups 17-18 engorged itself.

The sheep was, therefore, bitten by 3 specimens after a period of 15 days, by 2 (?) after 27, and by 1 specimen after 29 days.

Reaction.—No definite fever reaction followed the feeding of these mosquitoes. From time to time (29th April, 21st, 28th May, 3rd, 4th June) short elevations varying between 103.8 and 105° were noticed, but they were irregular and not connected with one another as a reaction.

Immunity test.—On 8th June, 43 days after the first, and 29 days after the last, feeding of the mosquitoes the sheep was injected subcutaneously with 1 c.c. blood of sheep 31570 (virus sheep 15). On 14th June, 6 days later, the temperature rose, reaching 106° the same day, 106.4° the following, and 107° the day thereafter, however, with daily remissions to 104°. On 12th June, the reaction came to an end.

The *result* of this experiment was *negative*. No reaction followed the feeding of the mosquitoes. The reaction of the immunity test was not very marked, probably on account of the low virulence of this strain.

EXPERIMENT 38 (B.T. 41). 2 *Aedes lineatopennis.*
Injection. Interval 45 *days. Sheep* 34410.

On 27th May, 2 *A. lineatopennis* from group 2 were still alive and they were injected on that day together with 1 specimen infected with the Kameelfontein strain into sheep 34410.

Reaction.—No fever reaction followed the injection of the mosquitoes. The highest temperature being registered during the period of 23 days was 104.2°.

Immunity test.—On 20th June, the sheep was injected subcutaneously with 1 c.c. blood from sheep 31570 (virus sheep 15). No reaction, however, followed during the next 20 days, the temperature not exceeding 104°. On 11th July the sheep was injected with 1 c.c. of the same material and this time, after 5 days, a typical reaction commenced, lasting 3-4 days, with 106.5° as maximum.

Result.—The experiment has to be regarded as *negative.* The result of the immunity test was, however, very remarkable. The first injection was absolutely negative, whereas a subsequent injection with the same material was followed by a typical reaction.

D. EXPERIMENT WITH *Aedes durbanensis.*

With this relatively rare species only a single experiment with 1 specimen could be carried out.

Virus sheep 14 was used and mosquito group:

Group 2.—Fed on virus sheep 14 during the night of 12 to 13th April. First day after the end of the febrile reaction. Temperature 104-104.2° 2 specimens (reared from larvae) engorged. Used for experiment 39.

EXPERIMENT 39 (B.T. 29). *Aedes durbanensis.*
Feeding. Interval 16 *days. Sheep* 34567.

During the night of 27th to 28th April the 2 *A. durbanensis,* which had fed 16 days previously, were put on to sheep 34567 and one specimen fed.

Reaction.—The sheep was kept under observation for 31 days, but no temperature reaction occurred, 104° being exceeded only once for ½ day.

Immunity test.—On 28th May, the sheep was injected subcutaneously with 1 c.c. blood of virus sheep 14. After 7 days the temperature began to rise, reaching 107.2°, its highest point, on 5th June. Then it began to drop, but was followed by clinical symptoms, red coronets, mucous discharges from the mouth, and excoriations of the gums.

The *result* of this experiment is clearly *negative.* No reaction followed the mosquito feeding, whereas the immunity test showed a marked reaction with clinical symptoms. The appearance of these symptoms was quite unexpected, as the original sheep, from which the virus was derived, showed only a slight reaction and other immunity tests carried out with the same material gave only reactions of medium intensity.

E. RESULTS OF EXPERIMENTS WITH THE IXOPO STRAIN OF BLUETONGUE.

At the beginning of April, material from a natural case of bluetongue was obtained from Ixopo. When injected into two sheep only very slight reactions, less than those with the ordinary vaccine virus, were obtained and therefore only a limited number of experiments were carried out.

In all, 413 *Aedes caballus,* 10 *A. linneatopennis* and 2 *A. durbanensis* engorged themselves either during or directly after the short febrile reaction shown in the virus sheep. After this feed the mosquitoes were kept in our warm room on sugar water in cages or jars following the usual method. They were refed after an interval of at least 15 days on susceptible sheep, and after 1½ months

the remaining specimens were injected into sheep. Seven experiments were carried out and in each case after a sufficient period had elapsed the immunity was tested by subcutaneous injections with the same strain of virus.

In these experiments the following results were obtained:—

Aedes caballus, experiment 33. After an interval of 15 days 13 specimens fed. Result *negative*.

A. caballus, experiment 34. After the same interval 14 specimens fed. The result was also *negative*.

A. caballus, experiment 35, was carried out with the remaining mosquitoes of the two preceding experiments. 4 specimens fed after an interval of 23-24 days. Result *negative*.

A. caballus, experiment 36, 2 specimens injected after an interval of 45-46 days. Result *negative*.

A. lineatopennis, experiment 32, 3 specimens fed after a period of 15 days, 2 (uncertain) after 27 and 1 specimen after 29 days. Result *negative*.

A. lineatopennis, experiment 28, 2 specimens injected after 45 days. Result *negative*.

A. durbanensis, experiment 39. Only 1 specimen fed after an interval of 16 days. Result *negative*.

In these 7 experiments 27 *A. caballus* fed after a period of 15 days, and 4 after 23-24 days, 3 *A. lineatopennis* after 15, 2 (?) after 27, and 1 after 29 days, and 1 *A. durbanensis* after 16 days, 2 *A. caballus* and 2 *A. lineatopennis* were injected after 45-46 days. All experiments were negative. The virus sheep on which these mosquitoes had taken their original feed, showed, however, only an extremely mild and short reaction, and part of the mosquitoes had actually fed when the reaction had already (earlier than could have been expected) come to an end. The conditions were, therefore, very unfavourable for conducting transmission experiments and may have influenced their results.

In one of the experiments the injection of virus failed to give an infection, whereas a subsequent injection of the same material gave a typical reaction.

VIII. EXPERIMENTS WITH A STRAIN FROM KAMEELFONTEIN.

At the beginning of April, 1932, nearly at the same time as the Ixopo strain, material from another natural case of bluetongue, which had occurred in lambs on the farm Kameelfontein was obtained through the courtesy of the Extension Officer of the Pretoria District. The blood was injected into several sheep and infections of medium and marked intensity were produced, resembling very much the ordinary vaccine strain reactions. By means of further subinoculations in one case at least clinical symptoms were obtained. The strain could be regarded as suitable,

although our hope of getting a strain giving clear cut reactions and regularly slight clinical symptoms at least, was not fulfilled.

As in the case with the Ixopo strain *Aedes caballus* was the only species available in larger numbers at the time we obtained the Kameelfontein strain. In all, 402 specimens engorged themselves on the virus sheep. Thereafter they were kept in the usual way in cages in our warm room, till they were refed on susceptible sheep. The mortality amongst the mosquitoes during the period between these two feeds was very considerable.

Only a few specimens of *A. linneatopennis* were at our disposal, and of these 12 could be induced to feed.

The experiments were conducted in the ordinary manner. The mosquitoes were refed on susceptible sheep after an interval of at least 14 days and afterwards the immunity of the sheep was tested by injection of the same strain of virus.

A. Virus Sheep.

In all, 5 sheep were injected with the Kameelfontein strain, and of these the following 3 were used for feeding mosquitoes on.

Virus sheep 16=sheep 31717. Injected on 5th April, 1932, subcutaneously with 2 c.c. preserved blood from the original case at Kameelfontein.

Result.—The temperature remained normal up to 11th April. On the 12th it was 106 and 105.2°, the 13th 106.2 and 105°, the 14th 105.8 and 105°, the 15th 106 and 105°, the 16th 105.2 and 105.2°, and the 17th (a.m.) 104.8°. From the next day onwards normal temperatures were maintained. The reaction lasting 6 days was marked.

Virus sheep 17=sheep 32345 was injected together with the preceding animal with the same material.

Result.—The temperature remained normal or nearly normal (not exceeding 104.5°) up to 15th April. On the 16th it was 103.7 and 105.1°, the 17th (a.m.) 104.5°, the 18th 107.6 and 108.2°, the 19th 106.8 and 106.6°, and the 20th 105 and 104.4°. Thereafter a normal temperature was regained. Notwithstanding a maximum of 108.2°, the reaction was only of medium intensity. The incubation period, lasting 11 days, was longer than usual.

Virus sheep 18=sheep 34498 was injected on 13th April subcutaneously with 1 c.c. blood, taken from virus sheep 34498 during the fever reaction.

Result.—A normal temperature was maintained up to 17th April. On the 18th it was 103.8 and 105.1°, the 19th 105.4 and 106.7°, the 20th 105.8 and 107°, the 21st 107 and 108.2°, the 22nd 107 and 104.5°, the 23rd 104.1 and 104.2°, the 24th (a.m.) 104.7°, the 25th 105 and 105°, the 26th 103.4 and 103.3°, and the 27th 105°, when the animal was killed. This sheep showed quite a severe reaction accompanied by clinical symptoms, red coronets and discharges from the nostrils. During the last days it showed a markedly ill appearance.

B. Experiments with *Aedes caballus*.

On all three virus sheep batches of *A. caballus* were fed, in all 402 specimens. One batch from each sheep was used for the experiments, of which 5 were carried out. The mosquitoes were refed on the susceptible sheep for the first time after 15 days and part of them for a second time 5-10 days later.

Experiments 40 and 41 were carried out with mosquitoes infected on virus sheep 16, experiments 42-43, with specimens from sheep 17 and the last experiment with mosquitoes fed on virus sheep 18.

The following mosquito groups were used:—

Group 26.—Fed on virus sheep 16 during the night of 14th to 15th April. Third to fourth day of fever. Temperature 105-106°. 45 specimens engorged (reared from larvae). Used for experiments 40 and 41.

Group 27.—Fed on virus sheep 17 during the night of 18th to 19th April. Third day of fever. Temperature 108.2-106.8°. 89 specimens (reared from larvae) engorged. Used for experiments 42-43.

Group 33.—Fed on virus sheep 18 during the night of 21st to 22nd April. Fourth day of fever. Temperature 108.2-107.3°. 30 specimens (reared from larvae) engorged. Used for experiment 47.

EXPERIMENT 40 (B.T. 31). 15 *Aedes caballus*.

Feeding. Interval 15 *days. Sheep* 34587.

On 29th April, *A. caballus* group 26 was put on sheep 34587 and 15 (out of 32) specimens engorged themselves during the following night. They had fed 15 days previously on virus sheep 16 during the third to fourth day of fever.

Reaction.—During an observation period of 28 days no reaction followed, the highest temperature registered being 103.8°.

Immunity test.—On 28th May, the sheep was injected subcutaneously with 1 c.c. blood from sheep 34498 (virus sheep 18). A marked reaction commenced 4 days later, lasting 6-7 days, with 107.8° as maximum.

The *result* of this experiment was clearly *negative*. No reaction followed the feeding of the mosquitoes, and the sheep afterwards proved to be normally susceptible.

EXPERIMENT 41 (B.T. 38). 4 *Aedes caballus*.

Feeding. Interval 25 *days. Sheep* 34100.

On 9th May, the remaining specimens of the same batch of *A. caballus* were fed on sheep 34100 and 4 (out of 12) specimens engorged themselves. The mosquitoes had had their original feed 25 days previously.

Reaction.—A normal temperature was maintained for 14 days. On 24th May, 105° was registered, but the following morning the temperature had dropped again. Thereafter it remained somewhat unstable, 104.8° being noticed on 2nd June, and 105° on 5th. These high temperatures were, however, not maintained for more than one day.

Immunity test.—On 10th June the sheep was injected with 1 c.c. blood from sheep 34498 (virus sheep 18). 11 days of normal temperature followed. On 22nd June a sudden rise up to 105° occurred, lasting, however, only ½ day and followed by 4 days of normal temperature. On 27th May, 17 days after the injection, the bluetongue reaction started at last, lasting 7 days with 107.3° as maximum.

Result.—The experiment has to be regarded as *negative*, as only a few short and unmaintained rises of temperature occurred after the feeding of the mosquitoes. The reaction of the immunity test was somewhat atypical through the exceptionally long incubation period.

EXPERIMENT 42 (B.T. 32). 31 *Aedes caballus*.

Feeding. Interval 15 *days. Sheep* 34464.

On 3rd May *A. caballus* group 27 was fed on sheep 34464 and the following night 31 (out of 36) specimens engorged themselves. These mosquitoes had fed on virus sheep 17 during the third day of the bluetongue reaction.

No reaction at all followed the feeding of the mosquitoes during an observation period of 24 days, 104° being the highest temperature registered.

Immunity test.—On 28th May 1 c.c. blood from sheep 34498 was injected subcutaneously and 5 days later a typical bluetongue reaction followed, lasting 5 days with 107.8° as maximum.

The *result* of this experiment was clearly *negative*.

EXPERIMENT 43 (B.T. 39). 9 *Aedes caballus*.

Feeding. Interval 21 *days. Sheep* 34184.

On 5th May, the remaining specimens of the same batch of *A. caballus* used in the preceding experiment were put on sheep 34184 and 9 (out of 12) specimens fed. These mosquitoes had taken their initial feed 21 days previously

No *reaction* followed the feeding of the mosquitoes, 104° being the highest temperature registered during an observation period of 29 days.

Immunity test.—On 8th June, the sheep was injected with 1 c.c. blood from sheep 34498. A marked reaction, lasting 4-5 days with 108° as maximum occurred after a prolonged incubation period of 9 or 12 days.

The *result* of this experiment was also *negative.*

EXPERIMENT 44 (B.T. 36). 4 *Aedes caballus.*
Feeding. Interval 15-20 *days. Sheep* 34045.

On 6th May *A. caballus* group 33 was put on sheep 34045 and 3 (out of 12) specimens fed during the following night. The remainder of the same batch were fed again on the same sheep during the night from 11th to 12th May and 1 (out of 3) specimens fed. The interval was in the first case 15 and in the second 20 days. The mosquitoes had had their original feed on virus sheep 18 during the fourth day of fever.

No *reaction* followed during an observation period of 37 days after the first feeding of the mosquitoes. A temperature of 104° was reached or slightly exceeded on several occasions, however, only for short periods, the sheep generally running an elevated temperature.

Immunity test.—On 13th June 1 c.c. blood of sheep 34498 was injected subcutaneously. A reaction of medium intensity commenced 9 days later, lasting about 3 days with 107° as maximum.

The *result* was *negative*, as in the preceding experiments.

C. EXPERIMENTS WITH *Aedes lineatopennis.*

With this species, which had become rare during the time of these experiments, only two experiments could be carried out, one with feeding after an interval of 14-21 days, and the other by injection of 1 specimen being left after 38 days.

Virus sheep 18 was used and the mosquito group:

Group 20.—Fed on virus sheep 18 during the night of 19th to 20th April. Second day of fever. Temperature 106.7-105.8°. 12 specimens engorged (reared from larvae). Used for experiments 45 and 46.

EXPERIMENT 45 (B.T. 34). 6 *Aedes lineatopennis.*
Feeding. Interval 14-21 *days. Sheep* 34329.

During the night of 3rd to 4th May, *A. lineatopennis* group 20 was fed on sheep 34329 and 5 (out of 7) specimens engorged themselves. The remaining mosquitoes of the same batch were refed 7 days later, 10th to 11th May, and 1 specimen took up blood. The first five specimens had fed 14 days and the last specimen 21 days after the initial feed on virus sheep 18.

Reaction.—A normal temperature was maintained for 22 days after the first feeding of mosquitoes. Then a short rise to 105° occurred, lasting only ½ day, whereafter the temperature remained somewhat elevated, exceeding 104°, however, only once for ½ day.

Immunity test.—On 10th June, 38 days after the first feeding of the mosquitoes, the sheep was injected subcutaneously with 1 c.c. blood from sheep 34498 (virus sheep 18). Ten days later a marked reaction appeared, lasting 5 days with 107° as maximum.

The *result* of this experiment was *negative.* The slight temperature reaction noticed was not typical at all and the sheep proved afterwards to be normally susceptible.

EXPERIMENT 46 (B.T. 41). 1 *Aedes lineatopennis.*
Injection. Interval 38 *days. Sheep* 34410.

On 27th May, 1 *A. lineatopennis* of group 20 was still alive and it was injected (with two other specimens fed on a sheep infected with Ixopo virus) into sheep 34410.

The *result* of the experiment was *negative.* The reaction and the immunity test have been described already under experiment 38.

D. Results of the Experiments with the Kameelfontein Strains of Bluetongue.

At the beginning of April, material from a case of bluetongue, which had occurred in a lamb at Kameelfontein (Transvaal) was obtained. It promised to be a suitable strain for our purposes, as at least in one of the injected animals slight clinical symptoms appeared.

The season, however, was already nearing its end, and *Aedes caballus* was the only species obtainable in large numbers, breeding in our experimentally flooded breeding places. Besides this species only a very limited number of *A. lineatopennis* could be obtained.

In all, 402 *A. caballus* and 12 *A. lineatopennis* were fed on the virus sheep. Unfortunately the mortality amongst the former species was considerable. The mosquitoes were kept in our warm room on sugar water as usual. After an interval of at least 14 days they were fed on susceptible sheep and part of them refed after a longer period. One specimen of *A. lineatopennis* was injected into a sheep more than 1 month later. All sheep were afterwards tested for immunity by injections with the same strain of virus.

The following results were obtained in these experiments.

Aedes caballus, experiment 40. 15 specimens fed after 15 days. Result *negative*.

Aedes caballus, experiment 41. After 25 days 4 specimens belonging to the same batch as those in the preceding experiment, fed. Result *negative*.

A. caballus, experiment 42. After an interval of 15 days 31 specimens fed. Result *negative*.

A. caballus, experiment 43. After 21 days 9 specimens belonging to the same group as those of experiment 42, fed. Result *negative*.

A. caballus, experiment 44. After an interval of 15 days 3 specimens and after 20 days 1 specimen belonging to the same group fed. Result *negative*.

A. lineatopennis, experiment 45. After 14 days 5 specimens and after 21 days 1 specimen fed. Result *negative*.

A. lineatopennis, experiment 46. Injected after 38 days 1 specimen. Result *negative*.

In these 7 experiments 49 *A. caballus* fed after 15 days, 10 after 20-21, and 4 after 25 days, 5 *A. lineatopennis* after 14 and 1 after 21 days, whereas one specimen of the latter species was injected after 38 days. *All experiments were negative.*

The experiments with *Aedes caballus* were carried out with sufficiently large numbers. The strain, which had shortly before been isolated from a natural case of bluetongue, was virulent enough. The result, therefore, must have a definite value.

DISCUSSION OF THE RESULTS OBTAINED IN BLUETONGUE EXPERIMENTS.

During the summer 1931/32 a number of experiments with bluetongue in sheep was carried out at Onderstepoort together with work on horsesickness.

The epizootological evidence at hand pointed out that the transmission of both diseases must be very similar and that most likely mosquitoes were involved in the transmission. It must be stated, however, that these epizootological evidences—the non-contagious character of the disease, its restriction mainly to the summer months, the correlation between the amount of rain and the number of cases, the protection afforded by stables at night—are somewhat meagre, fitting well, however, with the assumption of mosquitoes being the carriers.

During the same time a mosquito survey was carried out at Onderstepoort, the results of which have been described in the first paper of this series. It was found that amongst the mosquitoes some *Aedes* species best fulfilled the epizootological requirements. These species are limited to the summer months, and their numerical appearance depends more than that of any other genus on the amount of rain. *Aedes caballus*, *A. lineatopennis* and *A. hirsutus*, were regarded as the most suitable transmitters, *A. vittatus*, *A. dentatus* and some other species as possible transmitters of secondary importance.

No transmission experiments have been undertaken before with this disease.

Strains of Bluetongue Virus.

Three different strains were used for these experiments, most of which were carried out with the ordinary laboratory vaccine strain, as the first material from natural cases was only obtained at the beginning of April.

The vaccine strain had been isolated from a natural case in February, 1927, and had passed in the meantime through 48 generations. In 86 per cent. of the sheep temperature reactions were shown after injections with this strain, of which 20 per cent. had to be regarded as slight reactions. Only 8 deaths had occurred in the early generations, and from the 12th generation onwards no clinical symptoms were recorded. Temperature reactions were, therefore, the only guide for the interpretation of the results in our work, and in a number of experiments no definite opinion as to the result could be reached.

At the beginning of April, 1932, material from two natural cases of bluetongue was received, one from Ixopo, Natal, and the other from the farm Kameelfontein in the Pretoria District. At that time the bluetongue season was already nearing its end. All efforts to get material from spontaneous cases earlier in the season had failed.

Through injection of the Ixopo strain in the first generation only mild reactions were obtained, whereas the Kameelfontein material gave infections of medium to marked intensity, and in one of the further subinoculations even slight clinical symptoms.

Experimental Animals.

The sheep used for these experiments came from a known bluetongue-free area of the Cape Province. It is very exceptional to find an immune animal amongst these sheep. To be as safe as possible, however, all sheep were tested for immunity after the experiment had been finished with the same strain as was used for infecting the respective mosquitoes.

The *mosquitoes* used in the experiment were obtained by catching the larvae and pupae or the adults in the field.

They were fed on the infected and later on the susceptible sheep in small cages covered with mosquito-netting. These cages were fixed on clipped areas of the skin by pieces of tape, attached to the surrounding wool. They were covered with a piece of wet cotton wool to ensure a sufficient degree of humidity inside the cages.

The mosquitoes were kept between the feedings in a warm room on sugar water in jars or cages. In order to ensure that a sufficient degree of humidity was obtained, the jars were placed in larger jars containing damp cotton wool and the cages were surrounded with damp hessian.

A full description of the technique has been given in the second paper of this series.

In all, 2,892 specimens of 6 different species of mosquitoes were fed on the infected sheep, viz., 2,124 *A. caballus*, 639 *A. lineatopennis*, 52 *A. hirsutus*, 50 *A. rittatus*, 24 *A. dentatus*, 3 *A. punctothoracis* and 2 *A. durbanensis*. More than double the number of specimens had to be caught and handled to obtain these results.

Scheme of Experiments.

The clean mosquitoes were fed on sheep experimentally infected with bluetongue virus during the actual temperature reaction. Later on, sheep infected or supposed to be infected by mosquitoes were also used. The mosquitoes were generally put on to the animals late in the afternoon and taken off again the following morning.

These infected mosquitoes were injected into susceptible sheep in the form of an emulsion in serum, or refed on susceptible sheep after an interval of at least 14 days, to allow for a certain development or multiplication of the virus to take place in the mosquito. Some of the mosquitoes were refed again after longer intervals.

The injections of crushed mosquitoes were mainly made as preliminary experiments to ascertain the presence or absence of the virus in some part of the body of the mosquito. To prove that a certain species is a transmitter, feeding experiments are naturally necessary. The exclusion of a species from the list of probable transmitters is more easily done by injection experiments.

When the experiment was concluded, every sheep was tested for immunity by means of subcutaneous injections of 1 or 2 c.c. bluetongue material from the same strain as that used for the infection of the mosquitoes. The immunity test was never applied earlier than 3 weeks after the feeding or injection of the mosquitoes in order to allow sufficient time for a reaction to appear.

First Series of Experiments.

We started our experiments with the ordinary *vaccine strain of bluetongue virus*. At first, in order to ascertain the presence of virus, the mosquitoes were injected into susceptible sheep after periods ranging between ½ and 19 days. In all, the following 12 experiments were made, in which about 250 mosquitoes, 124 *Aedes caballus*, 3 *A. dentatus*, 2 *A. hirsutus*, 112 *A. lineatopennis*, 1 *A. punctothoracis* and 9 *A. vittatus* were injected into 10 sheep.

Exp. 1. 5 *A. caballus* injected after ½ day. Result positive.
Exp. 2. 20 *A. caballus* injected after 5 days. Result doubtful.
Exp. 3. 30 *A. caballus* injected after 5 days. Result negative.
Exp. 4. 40 *A. caballus* injected after 7 days. Result negative.
Exp. 5. 14 *A. caballus* injected after 7 days. Result negative.
Exp. 6. 15 *A. caballus* injected after 16-17 days. Result negative.
Exp. 7. 20 *A. lineatopennis* injected after 5 days. Result negative.
Exp. 8. 30 *A. lineatopennis* injected after 7 days. Result negative.
Exp. 9. 2 *A. lineatopennis* injected after 16 days. Result negative.
Exp. 10. 60 *A. lineatopennis* injected after 17-19 days. Result positive.
Exp. 11. 6 *A. vittatus* injected after 5 days. Result negative.
Exp. 12 { 3 *A. vittatus* injected after 7 days.
3 *A. dentatus* injected after 7 days.
2 *A. hirsutus* injected after 7 days.
1 *A. punctothoracis* injected after 7 days. } Result negative.

In the first preliminary experiment 5 *Aedes caballus* were injected about 18 hours after they had fed on an infected sheep, with a positive result, showing that the 5 specimens had taken up sufficient virus to reproduce the disease. This result proved that the manner in which the mosquitoes were fed was efficient.

Negative results were obtained by injecting 99 *A. caballus* 5-17 days after they had fed on virus sheep; 52 *A. lineatopennis* after 5-16 days, 9 *A. vittatus* after 5-7 days, 3 *A. dentatus* after 7 days, 2 *A. hirsutus* after 7 days, and 1 *A. punctothoracis* after 7 days.

The injection of 30 *A. caballus* after 5 days was doubtful, the sheep showing a slight reaction not followed by immunity.

One experiment, in which 60 specimens were injected after 17-19 *days was clearly positive*, showing a typical fever reaction, complete immunity and clearly positive results with immunity in two subinoculations (experiment 10).

From these experiments we can conclude that the *bluetongue virus is capable of persisting in* Aedes lineatopennis *in a fully virulent form for periods of at least* 17 *days. This, however, was not regularly the case.* In 52 specimens of the same species no virus was present after 5-16 days.

Second Series of Experiments.

In one of the experiments of the first series a doubtful reaction was obtained after an injection of *A. caballus*, the temperature suggesting a mild infection with bluetongue, which, however, showed no immunity when tested later. On this case a number of *A. dentatus*, *A. hirsutus* and *A. vittatus* were fed and the following 5 experiments carried out:—

Exp. 13. 9 *A. dentatus* injected after 5-6 days. Result doubtful.
Exp. 14. 20 *A. hirsutus* injected after 5-6 days. Result doubtful.
Exp. 15. 6 *A. hirsutus* injected after 16 days. Result negative.
Exp. 16. 14 *A. vittatus* injected after 5 days. Result negative.
Exp. 17. 8 *A. vittatus* injected after 20 days. Result doubtful.

Two of these experiments (15 and 16) with 6 *A. hirsutus* injected after 16 and 14 *A. vittatus* after 5 days were certainly negative.

In the 3 other experiments (13, 14, 17) with specimens of *A. denatus*, *A. hirsutus* and *A. vittatus* injected after 5-20 days reactions resembling more or less the true bluetongue reactions were noticed after the injections of the mosquitoes, but no immunity followed or at any rate the immunity conferred was very weak. Subinoculations into 8 susceptible sheep failed.

In the last experiment (17), in which 8 *A. vittatus* were injected after an interval of 20 days the reaction that followed was, except for the short incubation period (4 days), indistinguishable from a true bluetongue reaction and was also accompanied by slight clinical symptoms. The immunity reaction was quite weak, suggesting that a certain degree of immunity had developed. However, as 4 subinoculations of blood taken during the fever period, failed, this experiment also has to be regarded as doubtful.

Third Series of Experiments.

Besides the doubtful case used for the second series a true case of bluetongue had been obtained in the first series of experiments by means of injections of 60 *A. lineatopennis* 17-19 days after their feeding. The virus had therefore undergone *one passage through mosquitoes*. On this sheep and two subinoculated animals 446 *Aedes caballus*, 136 *A. lineatopennis* and 10 *A. hirsutus* were fed, and with this material the following 9 experiments were carried out, in which this time the mosquitoes were refed on susceptible sheep after a period of at least 14-15 days had elapsed. Some of the mosquitoes were fed again after a longer interval or injected later into sheep as an emulsion.

Exp. 18. 16 *A. caballus* fed after 14-15 days. Result negative.
Exp. 19. 25 *A. caballus* fed after 14-15 days. Result doubtful.
Exp. 20. 10 *A. caballus* fed after 18-19 days. Result doubtful.
Exp. 21. 1 *A. caballus* injected after 35-36 days. Result negative.
Exp. 22. 8 *A. lineatopennis* fed after 14-20 days. Result negative.
Exp. 23. 10 *A. lineatopennis* injected after 22 days. Result doubtful.
Exp. 24. 15 *A. lineatopennis* fed after 14-15 days. Result positive.
Exp. 25. 16 *A. lineatopennis* fed after 18-26 days. Result doubtful.
Exp. 26. 3 *A. hirsutus* fed after 17 days. Result doubtful.

One of the experiments (No. 24) was regarded as *positive*. In this case 15 specimens fed after an interval of 14-15 days. 12 days later a temperature reaction, however, not very marked, occurred. When tested 18 days later for immunity, the animal proved to be normally susceptible. Blood taken during the short febrile reaction, was injected into two sheep. One of these animals developed a marked fever reaction, accompanied by slight but typical clinical symptoms, and when tested for immunity it proved to be totally immune. Virus must, therefore, have been present in the original mosquito infected sheep, although no immunity was conferred to it.

Clearly negative results were obtained in only 3 experiments, in which 16 *A. caballus* were fed after 14-15 days, 8 *A. lineatopennis* after 14-20 days, and in which 1 *A. caballus* was injected after 35-36 days.

In the remaining 5 experiments the results were doubtful. In these experiments 35 *A. caballus* had been fed after 14-19, 16 *A. lineatopennis* after 18-26, and 3 *A. hirsutus* after 17 days, whereas 10 *A. lineatopennis* were injected after 22 days. In one of the experiments with *A. caballus* a short reaction occurred after the feeding of the mosquitoes, followed later by an immunity reaction of a similar intensity. In the second experiment a fairly typical reaction commenced 11 days after the feeding of the mosquitoes but it did not confer any immunity to the sheep. In both cases subinoculations into susceptible sheep failed to give a positive result. In the experiment in which 10 *A. lineatopennis* were injected, no definite reaction followed the injection, but the result of the immunity test was not reliable. In the other experiment in which specimens of the same species were fed after 16-18 days, quite a typical temperature reaction occurred, but it was not followed by immunity and subinoculations of blood into susceptible sheep were negative. In the experiment with *A. hirsutus* the result of the immunity test was very doubtful. The animals used in these experiments normally showed a regular temperature.

Fourth Series of Experiments.

Three of the sheep of the preceding series of experiments were used for feeding further mosquitoes. Two of them had shown temperatures more or less closely resembling bluetongue reactions, but not followed by immunity after the feeding of *A. caballus* or *A. lineatopennis*, and the third animal had had a true bluetongue reaction after being injected with blood from another sheep. The vaccine strain had thus passed twice, truly or supposedly, through mosquitoes.

On these three sheep 382 *A. caballus* and 75 *A. lineatopennis* were fed and they were refed in the following 6 experiments after intervals ranging between 14 and 20 days.

Exp. 27. 2 *A. caballus* fed after 15 days. Result negative.
Exp. 28. 41 *A. caballus* fed after 15 days. Result doubtful.
Exp. 29. 36 *A. caballus* fed after 21 days. Result negative.
Exp. 30. 40 *A. caballus* fed after 14 days. Result doubtful.
Exp. 31. 17 *A. caballus* fed after 20 days. Result negative.
Exp. 32. 10 *A. lineatopennis* fed after 15 days. Result negative.

None of these experiments were positive.

Clearly negative results were obtained in 3 experiments, in which 55 *A. caballus* fed after 15-21 days, and 10 *A. lineatopennis* after 15 days.

Doubtful results were obtained in 2 experiments, in which respectively 41 and 40 *A. caballus* fed after 15 and 14 days. In one of these experiments a temperature reaction set in just prior to the immunity test and continuing through its incubation period, whereas in the other experiment just before the application of the immunity test, which was positive, another reaction of equal strength had occurred.

Fifth Series of Experiments.

At the beginning of April, 1932, material from a natural case of bluetongue was received from *Ixopo*. Blood from this case injected at Onderstepoort into 2 sheep gave only weak reactions. On these sheep 413 *A. caballus*, 10 *A. lineatopennis* and a few *A. durbanensis* were fed. At that time of the year *A. caballus* was the only species obtainable in fair numbers. The mosquitoes were refed in the following experiments after intervals ranging from 15 to 29 days and those remaining alive after 1½ months were injected into sheep.

Exp. 33. 13 *A. caballus* fed after 15 days. Result negative.
Exp. 34. 14 *A. caballus* fed after 15 days. Result negative.
Exp. 35. 4 *A. caballus* fed after 23-24 days. Result negative.
Exp. 36. 2 *A. caballus* injected after 45-46 days. Result negative.
Exp. 37. 6 *A. lineatopennis* fed after 15-29 days. Result negative.
Exp. 38. 2 *A. lineatopennis* injected after 45 days. Result negative.
Exp. 39. 1 *A. durbanensis* fed after 16 days. Result negative.

In all, 31 *A. caballus* were fed after 15-24 days and 2 were injected after 45-46 days, 6 *A. lineatopennis* (of which 2 were doubtful) fed after 15-29 days, 2 were injected after 45 days and 1 *A. durbanensis* fed after 16 days. All the experiments were negative, whereas the sheep used proved to be normally susceptible.

Sixth Series of Experiments.

At the beginning of April, 1932, we received material from another natural case, which had occurred at the farm Kameelfontein in the Pretoria District. Injected at Onderstepoort into sheep, it gave temperature reactions of medium to marked intensity and slight clinical symptoms in one of the further subinoculations.

On three virus sheep 402 *A. caballus* and 12 *A. lineatopennis* could be fed and they were refed in the following experiments after intervals lasting at least 14 days.

Exp. 40. 15 *A. caballus* fed after 15 days. Result negative.
Exp. 41. 4 *A. caballus* fed after 25 days. Result negative.
Exp. 42. 31 *A. caballus* fed after 15 days. Result negative.
Exp. 43. 9 *A. caballus* fed after 21 days. Result negative.
Exp. 44. 4 *A. caballus* fed after 15-20 days. Result negative.
Exp. 45. 6 *A. lineatopennis* fed after 14-21 days. Result negative.
Exp. 46. 1 *A. lineatopennis* injected after 38 days. Result negative.

In these 7 experiments 63 *A. caballus* were refed on sheep after intervals ranging between 15 and 21 days, whereas finally 1 *A. lineatopennis* was injected after 38 days. All the experiments were negative and the 7 sheep used proved later to be normally susceptible to the Kameelfontein strain.

General Discussion of the Results.

In all, 46 experiments were made with three different strains of bluetongue, a vaccine strain and two others derived from fresh spontaneous cases.

In these experiments 324 specimens were injected after periods ranging between ½ and 45 days and infected mosquitoes were refed 346 times after 14-29 days, viz.:—

A. caballus, 127 injected, 281 refed.
A. lineatopennis, 125 injected, 61 refed.
A. rittatus, 31 injected.
A. hirsutus, 28 injected, 3 refed.
A. dentatus, 12 injected.
A. punctothoracis, 1 injected.
A. durbanensis, 1 refed.

Three positive results were obtained. In the first case some *Aedes caballus* were injected the day after their feeding and thus the result has no relation to the actual capacity for transmission. In the second experiment a positive infection was obtained by the injection of 60 *Aedes lineatopennis* 17-19 days after their feeding on a virus sheep and this infection could be transmitted further through subinoculations. The third case was not absolutely clear cut. 15 *A. lineatopennis* had fed 14-15 days after their original infection, the feeding being followed by a slight temperature reaction, but not by immunity. Through subinoculations, however, the presence of virus could be definitely traced during the febrile reaction.

The results were doubtful in 10 experiments in which 30 *A. caballus*, 10 *A. lineatopennis*, 20 *A. hirsutus*, 8 *A. rittatus* and 9 *A. dentatus* were injected after 5-22 days and 116 *A. caballus* fed after 14-26 days. In these experiments temperature reactions, not followed by immunity and not confirmed by subinoculations, were observed, or the result of the immunity test was open to doubt.

32 Experiments were negative, the vast majority, in which 182 mosquitoes were injected and 199 specimens refed. 92 *A. caballus*, 55 *A. lineatopennis*, 23 *A. rittatus*, 8 *A. hirsutus*, 3 *A. dentatus* and 1 *A. punctothoracis* were injected after intervals of 5-46 days, whereas 165 *A. caballus*, 30 *A. lineatopennis*, 3 *A. hirsutus* and 1 *A. durbanensis* were refed after 14-29 days. In 5 of these experiments, in which 14 *A. rittatus* and 6 *A. hirsutus* were injected and 38 *A. caballus* and 10 *A. lineatopennis* refed, the mosquitoes had been originally fed on cases in which there could be some doubt as to the true nature of the temperature reaction.

As negative experiments, of which there is no doubt, there remain 27, in which 162 specimens, 92 *A. caballus*, 55 *A. lineatopennis*, 9 *A. rittatus*, 2 *A. dentatus*, 2 *A. hirsutus* and 1 *A. punctothoracis* were injected and 151 specimens, 127 *A. caballus*, 20 *A. lineatopennis* were refed after the same intervals as stated above.

Only with *A. caballus* and *A. lineatopennis* could experiments with large numbers be made.

Both positive experiments were obtained with *Aedes lineatopennis*. Against one positive case obtained by injection of 60 specimens after 17-19 days, there are negative results with 50 specimens injected after 5-7 and 5 after 16-45 days, and against the positive result obtained through feeding stand 3 negative experiments, in which 20 specimens were refed after 14-29 days.

All the experiments with *A. caballus* were negative. 92 specimens were injected, 74 after 5-7 days, 15 after 16-17, and 3 after 35-46 days. 127 specimens were refed, 92 after 14-15 days and 35 after 20-25 days. The material used was large enough to allow of a definite conclusion being arrived at.

Some facts may have influenced the results of these experiments. The vaccine strain, with which most of the work was done, was not a very suitable one. It had been isolated about 5 years previously and passed through nearly 50 generations by means of direct inoculations. Through these direct inoculations it had been profoundly altered, attenuated, and it is possible that at the same time its developmental capacity in insects had also been changed, viz., reduced. The Ixopo strain, though recently isolated from a natural case, gave very slight reactions, suggesting that during the temperature reactions the amount of virus present in the blood was not very large. It may further be possible that only a certain period in the duration of the febrile reaction is suitable for the infection of the insects.

In the interpretation of the results we were as careful as possible, and some of those regarded as doubtful may actually have been positive.

Arriving at the final conclusions we may say:—

Aedes lineatopennis seems *to be a natural transmitter of bluetongue*. Our results do not allow us to state whether this species is an important transmitter or only a more or less accidental carrier, as the number of positive results was too small.

Unfortunately, when we did obtain material from natural cases towards the end of the season this species was only available in very small numbers.

From an epizootological point of view, *Aedes lineatopennis* would be a very suitable transmitter. Breeding only in temporary water, it depends absolutely on the amount of rain. It breeds during the summer after good rains in a large number of marshy

spots in the veld, provided thay are covered with grass. After the mosquitoes have hatched out, the adults remain for some time at or near the breeding places, which usually lie in vleis. The breeding habits and behaviour of the adults is just what one would expect of an efficient transmitter.

Aedes caballus does not seem to be a transmitter at all. The number of negative experiments was sufficient to warrant this conclusion.

The material used of other species is too small to allow of any definite conclusion. However, according to the negative results, although small in numbers, it is improbable that they are very important carriers.

In future work the real importance of *A. lineatopennis* will first of all have to be decided, and if this should happen not to be in favour of this species, more work with the *Stegomyia* group and with Anophelines will have to be carried out. The possibility still remains that Arthropoda other than mosquitoes may be involved in the transmission of this disease.

SUMMARY.

During the summer 1931/32 transmission experiments with bluetongue in sheep were carried out at Onderstepoort. Owing to lack of rain, the season was very unfavourable for our work.

The result of a mosquito survey had pointed to *Aedes* species as being very suitable transmitters from an epizootological point of view, and some species of this genus were used for our experiments.

Three strains of virus were used, the vaccine strain of the laboratory and 2 strains from natural cases, obtained towards the end of the season.

Nearly 3,000 clean mosquitoes were fed on 18 infected sheep, the majority belonging to *Aedes caballus* and *A. lineatopennis*, and only relatively small numbers of 5 other species including *A. hirsutus*, *A. rittatus* and *A. dentatus*.

In 22 experiments, 324 specimens were injected into sheep after periods ranging between ½ and 45 days, 127 *A. caballus*, 125 *A. lineatopennis*, the remainder belonging to *A. rittatus*, *A. hirsutus*, *A. dentatus* and *A. punctothoracis*.

Infected mosquitoes were refer 346 times after 14-29 days on susceptible sheep at periods varying between 14 and 29 days, viz., 281 *A. caballus*, 61 *A. lineatopennis* and 4 *A. hirsutus* and *A. durbanensis*.

Three positive results were obtained. In the first case 5 mosquitoes were injected shortly after their infection, showing only that sufficient virus was taken up. In the second case 60 *A. lineatopennis*

were injected after 17-19 days, and in the last experiment 15 specimens of the same species refed after 14-15 days. In the last case no immunity was acquired but the presence of virus could be ascertained by subinoculation.

All the other experiments were either negative or the results doubtful.

Aedes lineatopennis seems to be a transmitter of bluetongue, very adapted for this purpose from an epizootological point of view owing to its breeding habits and behaviour in the adult stage. It could not be ascertained if this species is an important or only a more or less accidental transmitter.

Aedes caballus does not seem capable of transmitting the disease.

The work carried out up to now does not present a solution of the problem of the natural transmission of bluetongue.