

Observations on Blue Tongue in Cattle and Sheep.

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DURING the course of an investigation into the nature of certain phenomena noted in connection with Theileriasis in cattle (de Kock, van Heerden, du Toit, Neitz, 1936), certain interesting reactions both in cattle and subsequently in sheep, following upon the injection of material collected from reacting bovines, were observed. The investigations into Theileriasis referred to were undertaken in 1934 at Tzaneen in the Transvaal and the subsequent work, with material collected from cattle exposed at Tzaneen, was conducted at Onderstepoort. Following upon the data obtained in 1934 a further exposure experiment with cattle was carried out during the earlier portion of 1935 and the results obtained with the material from the cattle exposed at Tzaneen during these two seasons constitute the subject matter of the present paper.

As shown by Bekker, de Kock, and Quinlan (1934) the disease blue tongue had been looked upon until 1932, as being confined specifically to sheep. These authors demonstrated the susceptibility of cattle to various strains of the virus both by natural infection in the field and by artificial means. Blood collected from cattle in the field suffering from what had been called "pseudo foot and mouth disease" was injected into blue tongue susceptible sheep and typical cases of blue tongue resulted which were subsequently confirmed by cross immunity tests with blue tongue viruses of sheep extraction.

Shortly after the exposure of the cattle mentioned above to tick infestation on the Tzaneen town lands in 1934 (a study of Theileriasis) very definite temperature reactions were noted. From the beginning it was noticed that the temperature of these animals, recorded morning and evening, showed a tendency to fluctuate abnormally, evening temperatures of 104° F. to 105° F. being a very common occurrence. These temperatures were probably due to a number of factors, e.g., the early heavy infestation with ticks, the long railway journey to which the animals had been subjected,

the complete change in environment to which the animals were immediately subjected on arrival, etc. The town of Tzaneen lies in what is known as the Transvaal Lowveld at an elevation of slightly over 2,000 feet with a climate which is entirely tropical, whereas the cattle came from Vryburg and the Pretoria district respectively with elevations of 4,000 feet and over and temperate climates.

Apart from the initial abnormally fluctuating temperatures noted in the exposed cattle, which had to be regarded as due to the conditions stated above, very definite febrile reactions commenced towards the end of the first week of exposure and reached their height in most cases during the third week, when temperatures of over 108° F. were recorded in some cases. In some cases the temperatures gradually returned to the comparatively high fluctuating conditions noted at the commencement whereas in many others extremely high temperatures were sustained.

As stated previously the primary object of this exposure experiment had been the study of certain phases of Theileriasis, and inter-current infections, except those tick borne infections, e.g., Piroplasmosis, anaplasmosis, etc., which could normally be expected to occur in this area, were to some extent overlooked. Blood from a number of the cattle which showed abnormally high temperatures was collected at the end of the fifth week of exposure and injected, after having been pooled, into susceptible bovines in an attempt to transmit the Theileriasis which had already made its appearance in the exposed animals. The bovines so injected succumbed to tickborne infections transmitted in the inoculum but blood collected from one of these animals prior to death and injected into blue tongue immune sheep produced reactions which later proved to be blue tongue.

In the light of this experience pooled blood from a number of the exposed cattle was subsequently injected into sheep and from the ensuing reactions a strain of blue tongue was obtained which could be passaged successfully through a large number of generations of sheep.

In 1935 a similar experiment to that conducted in 1934 was carried out at Tzaneen and the observations recorded in 1934 were confirmed and the work amplified by further experimentation at Onderstepoort.

SCHEME OF EXPERIMENTS.

Experiment 1.

The work in connection with the exposure experiments carried out during the two years in question, so far as blue tongue was concerned, consisted primarily of the recovery of the virus from cattle exposed to natural infection in the veld and the demonstration of the identity of the virus so recovered in sheep as that of blue tongue. During the course of the work it was known that the immunity in sheep produced by the laboratory vaccine strain of virus could be broken down easily by the virus recovered from cattle although some basal immunity persisted in sheep as evidenced by the fact that only very rarely did sheep (vaccine immune) except for thermal reactions, display symptoms indicative of blue tongue.

Under this heading the main experiments in the actual passage of the virus through a number of generations were performed as follows:—

- (1) Passage of the virus recovered from cattle through blue tongue vaccine immune sheep.
- (2) Passage of the virus through susceptible sheep.
- (3) Passage of the virus through bovines.

Experiment 2.

Various cross immunity experiments were performed wherein the following strains of blue tongue virus were utilised:—

- (1) *Laboratory vaccine strain.*—This consisted of a strain of the virus which, by repeated passages through a large number of generations in susceptible sheep, had become considerably attenuated and is the strain utilised at Onderstepoort for the preparation of blue tongue vaccine purposes.
- (2) *Novo strain.*—This strain had been recovered by Bekker (1933) from cattle at the farm Novo in Transvaal. It was found to be a strain possessing considerable virulence and capable of causing a large percentage of deaths in sheep.
- (3) *Kromdraai strain.*—This strain had also been recovered by Bekker (1933) from cattle on the farm Kromdraai, Transvaal, and was found to be approximately equivalent to the Novo strain in virulence.
- (4) *Tzaneen strain.*—This strain might be sub-divided under different headings, according to attempts on our part to pass it through (a) vaccine immune sheep, (b) susceptible sheep, (c) bovines, the object being to note whether any changes in the virulence or immunological structure occurred, as (a) Tzaneen strain, late, (b) Tzaneane strain, early and (c) Tzaneen bovine strain respectively.

Experiment 3.

Experiments were conducted to ascertain the length of time that the virus persists in the animal body (1) in cattle and (2) in sheep.

Experiment 4.

The filterability of the virus was determined so far as we were able by the use of Berkefeld filters.

Experiment 5.

Attempts were made to deposit or concentrate the virus by means of high speed centrifugation.

For the sake of convenience the work performed during 1934 and that of 1935 will be discussed separately.

EXPERIMENTS CONDUCTED DURING 1934.

The 1934 experiments established the fact that blue tongue virus could be recovered from cattle exposed to natural infection at Tzaneen and subsequently, that the infection could be established in sheep which showed the typical clinical evidence of blue tongue. As mentioned previously our object had been, primarily, the study of Theileriasis in bovines and blue tongue was merely identified as an intercurrent infection.

On three separate occasions pooled blood from exposed cattle injected into blue tongue vaccine immune sheep and susceptible sheep and the infection was demonstrated in each case (see Appendix I). Various cross immunity experiments were performed, as indicated in tabular form in the Appendix IA, wherein the virus recovered at Tzaneen was tested against the laboratory vaccine strain of virus and in certain cases against the Novo and Kromdraai strains isolated from cattle by Bekker and others as mentioned previously. An analysis of the result obtained in these cross-immunity experiments reveals the fact that—

- (a) 15 animals previously immunized against the laboratory vaccine strain of blue tongue reacted to the Tzaneen strain after periods varying between 49 and 433 days, i.e., after vaccination;
- (b) 6 animals failed to react to the injection with Tzaneen virus;
- (c) 1 animal previously immunized with the Kromdraai strain of blue tongue virus failed to react when subsequently injected with the Tzaneen strain;
- (d) 1 animal previously immunized with the Novo strain of blue tongue virus failed to react to a subsequent injection with the Tzaneen strain.

In certain cases additional immunity tests were performed on some of the animals which had reacted to each of the two previous injections, using the Tzaneen and Kromdraai strains of virus.

1. Of the 15 sheep under (a) mentioned above 7 were injected with the Kromdraai strain of blue tongue at intervals of from 26 to 67 days after their reaction to the Tzaneen virus. These sheep all failed to react.

2. One animal mentioned above under (b) failed to react to an injection of the Kromdraai strain administered 61 days after the injection with Tzaneen virus.

3. Two sheep, which had both reacted to the injection with the Tzaneen strain were again injected with the Tzaneen strain at intervals of from 30 to 38 days after this reaction and one of these sheep reacted again, the reaction, however, being mild in nature and showing very irregular temperatures.

CONCLUSIONS ARRIVED AT FROM THE 1934 EXPERIMENTS.

1. Blue tongue virus can be recovered from cattle exposed to natural infection at Tzaneen and subsequently passed through a number of generations in sheep.

2. The virus of blue tongue recovered from cattle at Tzaneen is capable of breaking down the immunity produced by the laboratory vaccine strain of blue tongue.

3. A fair degree of immunity is produced by the laboratory vaccine strain of virus as evidenced by the fact that only very rarely are clinical symptoms produced as a result of a reaction produced by the Tzaneen strain in a B.T.V. immune sheep.

4. From the immunological aspect the Kromdraai strain of blue tongue recovered by Bekker in 1932 is similar to that recovered from cattle at Tzaneen.

EXPERIMENTS CONDUCTED DURING 1935.

The 1935 experiments were to some extent a repetition of those conducted in 1934 but the work was considerably amplified and the nature of the reactions observed in sheep and the behaviour of the virus in sheep and cattle more closely studied.

In *Experiment 6* (see Appendix) the virus recovered from cattle was passed through 15 generations in *blue tongue vaccine immune sheep*. The immune sheep so infected throughout the course of this passage all showed clearly defined temperature reactions after an average incubation period of 2·37 days. In only a few cases were clinical symptoms of blue tongue, such as swelling and hyperaemia of the lips and buccal mucous membrane, noted. The sheep which showed the clinical evidences of the disease to which they were reacting revealed these clinical symptoms in an extremely mild form only. The greater majority of the animals showed no clinical symptoms whatsoever, the well defined temperature reactions and subsequent cross immunity tests affording thorough proof of the infection.

From time to time during the course of this passage the virulence of the virus strain was tested in *blue tongue susceptible sheep*. In all cases the nature of the reactions was much more marked than in the case of the vaccine immune sheep. Clinical symptoms, such as swollen lips and hyperaemic mucous membranes with frequently and sero-haemorrhagic discharge from the nostrils together with dullness were for the most part very much in evidence. In all 19 susceptible sheep were utilised during the course of this passage of which 6 showed clinical evidence of blue tongue and 13 were either killed in extremis or died as the result of the infection.

From this experiment it appears evident, therefore, that during a passage of 15 generations through blue tongue vaccine immune sheep no demonstrable change in the virus, recovered originally from cattle, occurred; certainly no attenuation so far as virulence is concerned. It appears, further, that blue tongue vaccine immune sheep retain a fair degree of immunity to subsequent reinfection with the Tzaneen strain of blue tongue and, although capable of setting up a definite and even a severe temperature reaction, this strain is capable of producing mild clinical symptoms of blue tongue in only a few isolated cases in such immune sheep. On the other hand in the case of blue tongue susceptible sheep clinical symptoms are the rule and the vast majority succumb to infection with this strain.

Experiment 7.

This experiment served to demonstrate the fact that the virus of blue tongue was capable of being passed through a series of normally susceptible bovines, three generations of the virus through such bovines having been accomplished.

No very clearly defined temperature reactions were noted but for a period of a week or longer after the injection of the virulent blood from a reacting sheep very irregular temperatures were recorded. The incubation period between the injection of virulent blood and the onset of the above-mentioned period of irregular temperatures correspond very closely with that observed in sheep, viz., 2 to 3 days. Clinical symptoms were not observed, the animals feeding normally and showing no sign of ill health.

Experiment 8.

The object of this experiment was to note whether blood of bovines experimentally infected with blue tongue could produce the disease in sheep by injection. It was also attempted to ascertain how long bovines would harbour the virus in their blood by withdrawing blood at various intervals from infected bovines and injecting this into sheep.

I. In the case of bovine 5563 (see Appendix I, Experiment 8, Generation 1) blood was removed from this animal at intervals of 6 days, 8 days, 11 days, 12 days, 15 days, 22 days, and after the original injection of virulent sheep blood into this animal and injected at each of these intervals into blue tongue virus immune sheep. Blood removed at intervals of from 6 to 22 days was capable of setting up the disease in these sheep but that removed 8 weeks after the original injection failed to set up the infection.

II. Blood from bovine 5653 (see Appendix I, Experiment 8, Generation 2) was removed at intervals of 2 and 11 days after the injection of virulent blood into it and injected on these two occasions into blue tongue virus immune sheep. The sheep so injected reacted to blue tongue, some showing slight clinical symptoms of the disease.

Experiment 9.

In planning this experiment the fact was taken into consideration that virulent blue tongue virus collected from reacting sheep which had contracted the disease naturally in the field, is capable of being attenuated by passage through a sufficient number of blue tongue susceptible sheep. The virus was admittedly not taken through a sufficient number of generations in order to arrive at very definite conclusions but the fact that at the 9th generation a typical uncomplicated case of blue tongue terminating in death resulted is significant.

Experiment 10.

While testing the nature of the reaction observed in sheep 42436 (see Appendix, Experiment 6, Generation 6) by intraperitoneal injection of an emulsion of organs (brain, gland and liver) obtained at autopsy, it was observed that the resulting symptom complex in the two sub-inoculated sheep resembled more closely an intoxication than a true blue tongue reaction. The post-mortem examination disclosed a fibrinous peritonitis in each.

An attempt was then made to ascertain the effect of brain material injected intraperitoneally under as aseptic conditions as possible. Brain material was collected from one of these sheep as aseptically as possible, emulsified in saline and injected intraperitoneally into two sheep. Dullness, cessation of feeding and elevated temperatures followed in both cases from one to two days after the injection, both animals succumbing within six days. In one case hyperaemia and oedema of the lungs together with a fatty degeneration of the liver and general venous stasis were the only post-mortem lesions observed, which strongly suggest an intoxication following upon the injection of brain material. The other animal showed a fibrinous peritonitis and pleuritis.

A further intraperitoneal injection of brain material from one of the above-mentioned sheep was made into two sheep. Symptoms similar to those described above were noted, in one case being complicated by a chronic fibrinous peritonitis whereas the other animal showed no trace of peritonitis, symptoms of toxæmia only being in evidence.

It would appear, therefore, that an emulsion of brain material of sheep injected intraperitoneally into the same species of animal is capable of bringing about the death of the animal by virtue of toxic properties contained in such brain material.

Experiment 11.

In order to produce additional evidence regarding the identity of the virus obtained from cattle at Tzaneen as that of blue tongue, filtration experiments were conducted by means of Berkefeld filters.

Blood from reacting sheep (see Appendix, Experiment 11) was laked in distilled water and passed through paper pulp in order to remove as much fibrin as possible. This blood so filtered was then passed through a Berkefeld candle together with a broth culture of *B. proteus* in order to test the effectiveness of the candle. Cultures were made from the filtrate which proved to be sterile after two weeks incubation in both cases.

In all one blue tongue virus immune sheep, one blue tongue susceptible sheep and one Tzaneen strain immune sheep were injected with the filtrate, suitable controls being introduced to prove the virulence of the original blood and that passed through paper pulp.

Both the blue tongue virus immune and blue tongue susceptible sheep reacted but the Tzaneen strain immune sheep failed to react. The control sheep all reacted typically with the exception of one which had previously been immunized against a strain of blue tongue recovered from cattle on the farm Novo in the Transvaal in 1933.

It may be concluded that the virus of blue tongue obtained from cattle at Tzaneen is capable of retaining a culture of *B. proteus*.

Experiment 12.

Four attempts were made to concentrate or deposit the Tzaneen blue tongue virus by means of high speed centrifugation at 15,000 revolutions per minute (see Appendix, Experiment 12).

This experiment was undertaken in collaboration with Alexander and Mason, the object being to endeavour to introduce blue tongue successfully into small laboratory animals. It was held that, should it be possible to concentrate or deposit the virus, the possibility of its establishing itself in the animal body upon introduction by the intercerebral or intraperitoneal route would be very much greater.

No success was achieved in the four experiments which were conducted and it was impossible to arrive at any conclusion regarding any tendency towards concentration or deposition of the virus.

It is thought that this angle of investigation into the properties of blue tongue virus in conjunction with filtration through suitable collodion membranes, which were not available to us at the time, might be pursued with profit at a later date, and with this object in view mention only has been made of the small amount of work done by us.

Conclusions arrived at from the 1935 Experiments.

1. In these experiments it has been possible to confirm the results obtained in 1934.

2. Blue tongue virus was recovered from cattle exposed to natural infection at Tzaneen.

3. This virus is capable of producing febrile reactions and in a small number of cases slight clinical symptoms in sheep immunized with the laboratory strain of blue tongue.

4. The injection of Tzaneen blue tongue virus into susceptible blue tongue sheep produces febrile reactions, clinical symptoms and death in the majority of cases.

5. No attenuation was noticed of Tzaneen blue tongue virus after the passage through 15 generations in sheep immunized with the laboratory vaccine strain of blue tongue.

6. No attenuation was noticed after passaging this virus through 9 generations in sheep susceptible to blue tongue.

7. Cattle injected with Tzaneen blue tongue virus show mild febrile reactions but no clinical symptoms. The incubation period corresponds to that observed in sheep.

8. Cattle may harbour the virus up to 22 days after infection but not for 8 weeks.

9. Brain emulsions injected intraperitoneally are capable of bringing about death probably due to the toxic properties of the brain material.

10. Tzaneen blue tongue virus can pass through a Berkefeld filter and is capable of retaining a culture of *B. proteus*.

11. It was not possible to cause a deposition or a concentration of the virus after centrifugation at 15,000 revolutions per minute.

12. All attempts failed to prove that the multiple necrosis observed in the lungs of some of the sheep that died from blue tongue could be attributed to a specific organism.

DISCUSSION.

Blue tongue virus recovered from cattle exposed at Tzaneen confirmed the work of Bekker, de Kock, and Quinlan in which the presence of the well-known virus causing blue tongue in sheep was described in the blood of cattle under certain conditions. In none of the Tzaneen exposed cattle carriers of the virus were the characteristic symptoms and lesions in cattle described by Bekker, de Kock, Quinlan, seen, namely in the buccal and nasal cavities, teat and udder symptoms, skin changes, feet lesions, etc. The marked thermal reactions observed in the exposed cattle at Tzaneen and from which the virus was recovered, must be attributed to other agencies (de Kock, van Heerden, du Toit, Neitz).

This virus recovered from cattle and subsequently passaged through a number of blue tongue vaccinated and susceptible sheep revealed a number of interesting data.

The Tzaneen recovered blue tongue virus was capable of breaking down the immunity produced by the laboratory vaccine strain of virus in sheep, however, without the occurrence of typical symptoms and mortality in spite of the fact that some of the sheep tested had been immunized considerable periods previously (see Appendix II, A and B). The degree of virulence of this virus as demonstrated in blue tongue susceptible sheep indicated that some basal immunity persisted in sheep vaccinated with the Onderstepoort strain.

The marked early thermal reactions in a number of blue tongue vaccinated sheep, and the absence of typical clinical symptoms gave the impression at first that another virus had probably been recovered from the Tzaneen exposed cattle and not blue tongue. In this connection it is interesting to note (see Appendix II, A and B) the thermal reactions in such previously vaccinated sheep (e.g. sheep 41566, 41002, 40943, 40955, 40933, 42768, 41004, etc.), characterised by a short incubation period, sudden rise of temperature often with a maximum of 106° F. and more, and which usually persisted for 4-6 days. These reactions resembled those described by Bekker, de Kock, Quinlan (in Chart 1, Appendix D), in susceptible blue tongue sheep, with the exception, however, that no mortality or typical blue tongue lesions were observed. This peculiar behaviour of the virus in blue tongue vaccinated sheep confused the earlier studies. Definite conclusions that this Tzaneen strain of virus was that of blue tongue, were only subsequently arrived at when the virus was passaged through a number of susceptible sheep.

A test was carried out to ascertain whether this virulency displayed by the Tzaneen virus could be attributed to the large dose (5 c.c.) inoculated exclusively *intravenously*. It showed, however, that smaller doses (2 c.c.) injected *subcutaneously* brought about

identical results, not only in susceptible, but also in vaccinated blue tongue sheep. (This experiment was carried out in sheep 41722, 43751, 41762, and 44228, see Appendix II B).

A number of Tzaneen strain viruses was prepared according to the method outlined by du Toit (1929) and stored. From time to time inoculations were carried out with these stored viruses. In two instances when tested on blue tongue susceptible sheep, it was found to be virulent after 8 and 8½ months respectively [(a) sheep 44989 and 45004 were tested on the 13.11.35 with the virus of sheep 41002 (18.3.35), 41768 (16.3.35), and 41566 (12.3.35); (b) sheep 41709 and 41791 were tested on 22.11.35 with the virus of sheep 41566 (4.3.35)]. In one instance reaction was obtained after 10½ months' storage, but in this case the test was carried out in blue tongue vaccinated sheep. In this respect it is interesting to recall the test referred to by du Toit in which a reaction on two out of three sheep was obtained with a 10½ years old blue tongue vaccine.

A passage of 15 generations through *blue tongue vaccinated sheep* revealed no demonstrable changes in the virus recovered from cattle at Tzaneen, certainly no attention as far as virulency is concerned. In fact it appeared as if the virulency of this virus had actually become enhanced. At the ninth (9th) generation a typical uncomplicated case of death occurred in a passage experiment of the Tzaneen strain through blue tongue susceptible sheep. It is admitted that in the latter instance the passage was not taken through a large number of generations, nevertheless these observations are of interest, especially those in connection with the blue tongue vaccinated sheep when reviewed in light of the results obtained by du Toit (1929). From the 12th to the 41st generation he obtained no mortality from blue tongue and believed that the virus had become attenuated to such an extent that no mortality occurred after the 11th generation. It is essential that a parallel experiment be repeated with strains of virus recovered from cattle and from sheep in susceptible as well as in vaccinated sheep to ascertain to what these differences in virulency could be attributed.

From Appendix II A and B it becomes evident that the blue tongue vaccine at present in use at Onderstepoort was able to maintain a certain degree of basal immunity even for considerable periods after immunization, in spite of the fact that an exceedingly virulent strain was utilised in the test. In this respect the results resemble those of du Toit (1929) who found that twelve months after immunization with blue tongue vaccine the immunity of sheep is still fairly strong; a certain proportion will react and these will generally show a fever reaction only.

It was found that cattle may harbour the Tzaneen strain of virus for 22 days, whereas in sheep it was possible to recover the virus up to 60 days. Du Toit (1929) is of opinion that the virus may be present in the blood of recovered sheep for say about four months; it would also appear that during this period there may be intervals during which the virus cannot be demonstrated in the blood. In this respect the following observations are certainly of interest. It was

found that sheep injected with Tzaneen virus reacted and reacted a second time when subsequently reinjected with the same strain of virus (see the following table):—

D.O.B. No. of Sheep.	Injection.		Immunity Test.		
	Material.	Result.	Injection Virus.	Result.	Refer Table.
44983	Tzaneen cattle virus, 5563	Reacted	S. 44995, Tzaneen virus	Reacted	Blue tongue susceptible table 1935.
44989	Sheep Tzaneen virus, 41566, 41768 and 41003	"	S. 45341, Tzaneen virus	"	" "
43481	Sheep Tzaneen virus	"	S. 44995, Tzaneen virus	"	Blue tongue immune table 1935.
44297	" "	"	S. 42738, Tzaneen virus	"	" "

At present the nature of these second reactions with the same strain of virus is not understood. In this respect the observations of Thomas and Migall (1935) with the virus of influenza in ferrets are of interest. They showed that those animals recovering from infection with one strain have been found to be actively immune to reinfection with either homologous or heterologous strain; infrequently, however, reinfection has occurred in animals receiving a second intranasal inoculation within a period of two months; in these cases the only evidence of reinfection was the persistence of fever for 2-3 days after the second inoculation and all attempts to recover the virus from these animals have in general been unsuccessful. In view of the shortage of accommodation and the lack of suitable animals, it was found impossible and impracticable at present to pursue these studies in sheep in respect of infectivity of the blood, neutralising properties of the serum at different stages. An attempt is, however, being made to transmit this virus to Laboratory animals on which these tests could then be advantageously carried out.

The macroscopical and microscopical findings are enumerated in Appendix III. A very complete symptomatology and pathology of blue tongue as well as the course of the disease, etc., was given by Bekker, de Kock, Quinlan. Only certain variations from these need be stressed here. In the blue tongue susceptible sheep the disease was of a very acute nature with high mortality. Death occurred as early as the fifth day in sheep 45007 which died suddenly and which at post-mortem revealed marked oedema of the lungs together with multiple necrotic foci in the lungs. On the seventh day death was recorded in two cases, 45337 and 45745. In the former extensive oedema and swelling of the tongue, lips and lymph glands were observed, besides multiple haemorrhages in connection with the lips and tongue. Oedema of the subcutis was pronounced. Like in Dikkop horsesickness this oedema was mainly confined to the intra-mandibular spaces, ventral aspect of the neck and extending on to

the shoulder region. In sheep 45745 swelling of the tongue with early ulceration was identified as well as extensive blood extravasations in respect of the abomasum, duodenum and both endocardiums. Sheep 44993 was killed in extremis on the seventh day, with more or less similar lesions. The number of red cells in this particular case on the day of death manifested 12 million per cubic mm. whereas 4,000 leucocytes were numerated. On the 8th, 9th and 10th days the majority of sheep died, namely 45341, 45420, 45741, 45005, 44995 45343. In 45341 extensive transudation was observed in the subcutis in the region of the neck, intra-mandibular space, region of the shoulder as well as extensive oedema of the lips and tongue with multiple haemorrhages and hydrothorax, hydropericardium and extensive oedema of the lungs. These latter changes in many respects resembled the Dunkop form of horsesickness, in which case the lymphoid vessels of the lungs were markedly distended with a greenish-yellow lymph and in places the pleura revealed oedematous-like sugillations. Enlargement and oedema of the majority of lymph glands were also observed. In sheep 45420 besides the extensive oedema, marked extravasations were also observed in connection with the epicard and both endocards, as well as marked oedema of the lungs and extensive hydrothorax and hydropericardium. In sheep 45741 no swelling of the lips and tongue occurred and this case resembled the Dikkop form of horsesickness in that it showed extensive hydropericardium with marked extravasations in connection with both endocardiums. Sheep 45343 besides the marked oedema of the lung revealed multiple pneumonic foci with necroses. The following sheep, 44972, 44985, 45731, 45734, and 32729 were killed in extremis between the 11th and 14th days. Besides well marked oedema in the subcutis they showed a cachexia. These sheep were in good conditions at the time of inoculation and in this particular instance the cause of the extreme symptoms of weakness and debility must be contributed to blue tongue because no other causes were identified to explain the condition. Similarly two sheep 42346 and 44974 were killed in extremis as a result of debility three weeks after inoculation. No lesions were found to account for the prostration and these cases should also be regarded as killed in extremis as a sequel to blue tongue.

The lesions referred to above closely resemble those encountered in horsesickness except that in some sheep with one strain of virus it resembled the Dunkop form whereas in others it was more of the nature of Dikkop, while in some both conditions occurred in the same sheep. The extensive local lesions of the tongue and lips observed by Bekker, de Kock and Quinlan were not manifested to the same extent and in some sheep they were entirely absent. Besides the extensive extravasations referred to in the subcutis, lips, tongue, lungs, epicardium, thorax, and the blood extravasations noted in respect of epicardium and both endocardiums, parenchymatous organs revealed very slight regressive changes. In a number of the sheep slight fatty changes were identified and in a few slight proliferative changes in the periphery of the liver lobules. In a few localised pneumonic areas with necrosis were identified. Very careful histological examination was undertaken in connection with various levels of the central nervous system, for instance, medulla, pons,

thalamus, hippocampus, corpus striatum, cerebellum, cerebrum; etc., to ascertain whether changes could be observed in the vascular system or the ganglion cells. Except for slight distension of the blood vessels no pathological changes were manifested. Various different stains were employed, including the Giemsa stain, without being able to identify any form of "elementary" or "reaction bodies".

The virus of blue tongue in many respects resembled that of horsesickness, especially in relation to the pathological changes. These viruses are characterised by extensive transudation and at the present moment the pathogenesis of the prostration and the cause of the sudden death of the blue tongue cases could not be explained.

Rivers (1935) is of opinion that inflammation in virus maladies is a secondary phenomenon, while hyperplasia and necrosis of the affected cells are the primary, most important and most characteristic reactions of cells to this type of infection. In the acute blue tongue the action of the virus, as Rivers expresses it, would be "rapid and explosive" characterised by those extensive transudations.

Morphologically it is extremely difficult to establish a type of lesion in the vascular system responsible for this flow of fluid from the vessels to the tissues. It is felt that some other form of approach should be undertaken to study these phenomena and how death of the animal is brought about. Nothing of the nature of a hyperplasia or a necrobiosis could be identified in the blue tongue. The only lesions besides the transudations and extravasations of blood were of the nature of a very slight fatty change in the liver. The significance of the proliferative changes in the periphery of the lobule of the sheep's liver is not fully understood. This has been observed to lesser or greater extent in sheep which were regarded as clinically healthy and for that reason it cannot be regarded as of any significance in this condition. It is significant how frequently such proliferations in the periphery of the liver lobule are also met with in bovines.

CONCLUSIONS.

1. Experiments are described in which it was possible to recover blue tongue virus from laboratory cattle exposed on the veld at Tzaneen. In these cattle no clinical evidence of blue tongue was manifested.

2. This Tzaneen strain of virus is capable of "breaking the immunity" of sheep immunized with the Onderstepoort vaccine, however, without any mortality and without the occurrence of typical blue tongue symptoms.

3. There is no doubt that some basal immunity persisted in these vaccinated sheep, even for considerable periods after immunization and utilizing exceptionally virulent viruses for the various tests.

4. The significant thermal reactions provoked in vaccinated sheep by this Tzaneen strain were referred to, and contrasted with the marked reactions and mortality in the blue tongue susceptible sheep.

5. These marked reactions could not be attributed to the size of the dose and the method of inoculation.

6. In one instance the virus prepared according to the existing method of preserving the virus, remained virulent for susceptible blue tongue sheep for a period of 8½ months.

7. A passage of 15 generations through blue tongue vaccinated sheep and 9 generations through blue tongue susceptible sheep failed to attenuate the virus in respect of its virulency.

8. It was shown that cattle could remain carriers of the virus without manifesting symptoms for a period of 22 days after injection and in sheep up to 60 days.

9. The significance of first and second reactions in the same blue tongue susceptible sheep with the same strain of Tzaneen virus is referred to.

10. The nature of the most important lesions was considered as well as their pathogenesis and close relationship to the various forms of horsesickness in horses

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APPENDIX I.

EXPERIMENTS CONDUCTED DURING 1934.

Experiment 1.

This experiment consisted of the recovery of blue tongue virus from the exposed cattle and its subsequent passage in sheep. The cattle had been exposed at Tzaneen from 21.3.34.

20.4.34.—Pooled blood cattle 4954, 4980, 5524, 5214 and 5224 was injected into two susceptible cattle, 5409 and 5454 (i.e. about one month after the commencing date of the exposure).

Result.—5409 and 5454 both died of a mixed infection of red-water and heartwater on 13.5.34 and 22.5.34 respectively.

9.5.34.—Blood collected from 5454 on this date was injected intravenously into bovine 5188 and two blue tongue vaccine immune sheep 38876 and 38905.

Result.—5188 died of redwater on 14.5.34.

38876 showed very variable temperatures from the day following the injection. Temperatures remained variable fluctuating between 102° and 105.8° until the 24.5.34, when a more or less definite reaction with a maximum temperature of 107° set in culminating in death on the 27th. The post-mortem revealed *heart-water* as the cause of death which was confirmed by examination of jugular intima smears and the demonstration of Rickettsiae.

38905.—A reaction commenced on the fifth day following injection which persisted for five days with a maximum temperature on the 16.5.34 of 107°. No clinical symptoms of blue tongue were apparent.

15.5.34.—An emulsion of spleen of bovine 5188 collected at post-mortem was injected intravenously into two blue tongue vaccine immune sheep 38210 and 37111.

Result.—38210.—On the fifth day following injection the temperature rose to 105° and remained elevated for four days. A second reaction set in on 12.6.35 which persisted for four days.

Immunity Test.—On 21.7.34, 5 c.c. blood of 40980 (Kromdraai strain) was injected into this sheep. No reaction resulted.

37111.—A reaction set in on the fourth day after injection which persisted for four days. On 22.5.34 the sheep was observed to be off its feed and rather dull but no other evidence of blue tongue was noted.

Immunity Test.—(1). On 22.6.34 this animal received 10 c.c. blood of 40698 (see Experiment 5373). Six days after this injection a rise of temperature to 104.8 occurred and the temperature remained elevated for two days. On 5.7.34 a temperature of 106 was recorded for one day and again on the 12th elevated temperatures were noted over a period of 3 days.

(2) On 21.7.34 this sheep was injected with 5 c.c. blood of 40980 (Kromdraai strain) to which it proved to be immune.

22.6.34.—Blood of 37111 was injected intravenously into two blue tongue vaccine immune sheep 37625 and 38206.

Result.—37625.—Failed to react. An immunity test consisting of an injection of 5 c.c. blood of 40980 (Kromdraai strain) on 21.7.34 gave no reaction up until 9.8.34 when the animal was discharged.

38206.—A definite febrile reaction set in three days after injection which lasted for five days, the maximum temperature being 106.8°.

An *immunity* test consisting of an injection of 10 c.c. blood intravenously of 40698 (see Experiment S.5373) gave no reaction. On 21.7.34 this animal received 5 c.c. blood of 40980 (Kromdraai strain). No reaction resulted up until the date of discharge on 9.8.34.

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Experiment 2.—To recover the virus of blue tongue from exposed cattle and pass it into sheep.

Generation 1.

2.6.34.—Pooled blood of cattle 4954, 4984, 4987, 4988, 5526, 5198, 5200, 5210 and 5225 collected on 9.5.34 was injected intravenously into two vaccine immune sheep 38182 and 37848, 10 c.c. into each.

Result.—38182.—The day following injection the temperature rose to 106° in the afternoon. It dropped the following day to 102.4° a.m. and 103.2° p.m. A definite reaction, with a maximum of 106°, set in on the 5.6.34, which persisted until the 9th when it gradually subsided. On the 13th the animal was noted to be off colour and not feeding well.

Immunity Test.—On 21.7.34 the animal received 5 c.c. blood of 40980 (Kromdraai strain) to which it failed to react.

37848.—A definite reaction set in on the third day following injection. A maximum temperature of 107° was recorded on the fifth day and the animal was killed on the ninth day. The sheep was off its feed from the seventh day and the buccal mucous membrane showed swelling and reddening, i.e. clinical evidence of blue tongue.

Generation 2.

8.6.34.—5 c.c. blood of 37848 was injected i.v.* into 35171 and 38850 (blue tongue susceptible), and 35781 and 38937 (blue tongue vaccine immune).

Result.—35171.—This sheep had previously been in a jaagsiekte experiment. After a period of fluctuating temperatures a reaction set in on the fifth day following injection. Marked cyanosis of the buccal mucous membrane and conjunctivae was shown accompanied with respiratory distress and the animal died on 15.6.34, the post-mortem examination revealing pulmonary lesions of jaagsiekte.

38850.—A reaction set in on the afternoon following injection which persisted for eight days, the maximum temperature recorded being 106.8°. Slight reddening of the buccal mucosa was shown and the animal showed dullness and inappetence for one day.

Immunity Test.—On 21.7.34 the animal received 5 c.c. blood of 40980 (Kromdraai strain) to which it failed to react.

35781.—A very slight reaction commencing on the third day after injection and lasting four days, with maximum temperature of 104.4° was noted.

Immunity Test.—Similar to above. No reaction resulted.

38937.—A mild reaction set in on the third day following injection, maximum 106, which persisted for six days. No *immunity test* was applied.

Generation 3.

15.6.34.—Blood of 38850, collected on 11.6.34, was injected into two blue tongue susceptible sheep 40805 and 40899.

Result.—40805.—A reaction set in two days after injection, the temperature remaining elevated up until the 30.6.34. The maximum temperature recorded was 107.2. Clinical symptoms of blue tongue were observed from the eighth day following injection.

Immunity Test.—On 21.7.34 the animal received 5 c.c. blood of 40980 (Kromdraai strain). On the fourth day following this injection the temperature commenced rising, 106° being recorded on the fifth and sixth days. Elevated temperatures persisted until 9.8.34, when the animal was discharged.

* Intravenously.

40899.—Four days after injection a febrile reaction commenced which lasted for six days and showed a maximum temperature of 106.4°. Hyperaemia of the buccal mucosa, with swollen lips and dullness were noted, gradual improvement thereafter taking place. On 13.8.34 a second temperature reaction occurred which showed a maximum temperature on this day of 106.2°. This reaction persisted for three days and could not be accounted for. No immunity test was applied.

Generation 4.

2.8.34.—Blood of 40899 collected on 22.6.34 was injected subcutaneously into two blue tongue susceptible sheep 41015 and 41034, 5 c.c. into each.

Result.—41015.—Four days after injection a definite reaction set in which lasted about six days and showed a maximum temperature on 9.8.34 of 106.4°.

Immunity Test.—On 1.9.34 this sheep received 5 c.c. blood of sheep 40633 (Kromdraai strain). A slight reaction commenced on the fifth day after this injection, which lasted three days.

41034.—A reaction, lasting three days, with maximum of 107° set in on the sixth day following injection.

Immunity Test.—On 1.9.34 the animal's immunity was tested with the Kromdraai strain (as above). No reaction resulted.

18.8.34.—Pooled blood of 41015 and 41034 was injected into two blue tongue susceptible sheep, 40994 and 41044.

Result.—Both these sheep failed to react and the passage was discontinued at this stage, the virus having died out.

Experiment 3.—To recover the virus of blue tongue from cattle exposed at Tzaneen and pass it into sheep.

25.6.34.—Blood collected on 11.6.34 from cattle 4954, 4984, 4987, 4988, 5798, 5200, 5225, 5526 and 5210 which had been exposed to natural infection at Tzaneen was pooled and injected subcutaneously and intravenously (10 c.c. by each route) into two blue tongue susceptible sheep, 40623 and 40850.

Result.—40623 gave no appreciable reaction.

40850 showed a distinct reaction commencing four days after the injection and persisting for seven-and-a-half days with a maximum temperature of 106.4. No clinical symptoms of blue-tongue were apparent.

Immunity Test.—On 2.8.34, 5 c.c. blood of 40899 (see Experiment 5408) was injected subcutaneously into these two sheep. No reaction resulted, tending to indicate that sheep 40623 was immune to start with. On 29.8.34 a further immunity test with Kromdraai strain virus (sheep 40633) was applied. A mild but definite reaction set in five days later, the maximum temperature recorded being 106 and the reaction persisting for four-and-a-half days.

3.7.34.—Blood of 40850 was injected i.v. into two blue tongue susceptible sheep, 40653 and 40654 (10 c.c. into each).

Result.—40653.—A marked reaction set in on the third day after injection which showed a maximum temperature of 108° and lasted for five-and-a-half days. Definite clinical symptoms were apparent.

40654.—A slight reaction, persisting over a period of about eight days, commenced on the fourth day after inoculation, the maximum temperature recorded being 104.6°.

Immunity Test.—(1) On 2.8.34 both 40653 and 40654 received 5 c.c. blood of sheep 40899 collected on 22.6.34 (see Experiment 2, generation 3), to which they failed to react.

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(2) On 1.9.34 the immunity of these two sheep was again tested by means of a subcutaneous injection of 5 c.c. blood of 40633 (Kromdraai virus). Both sheep reacted mildly, 40653 on the third day after injection showing a maximum temperature of 106.6° and 40654 on the second day with a maximum temperature of 105°. No clinical symptoms were noted.

Experiment 4.—To recover the virus of blue tongue from cattle exposed to natural infection at Tzaneen and pass it into sheep.

27.6.34.—Pooled blood of cattle 4954, 4984, 4987, 4988, 5526, 5198, 5200, 5210 and 5225 collected on 9.5.34 was injected subcutaneously into two blue tongue susceptible sheep, 40677 and 40739.

Result.—Both these sheep failed to react.

Immunity Test.—On 2.8.34 these sheep received 5 c.c. blood of sheep 40899 (see Experiment 2, generation 2) collected on 22.6.34. Both reacted with typical reactions, after incubation periods of five and six days, respectively, and showed hyperaemia of the buccal mucosa.

Experiment 5.—To establish the identity of the reactions noted in sheep injected with blood of cattle exposed at Tzaneen either direct or in series through sheep by means of cross immunity tests.

This experiment may best be recorded by placing the animals in tabular form. It will be noted that in the table of cross immunity tests which follows a number of sheep will be found which are not recorded in the foregoing portion of this appendix. These are sheep which received injections from reacting sheep during the course of one or other of the passage experiments where for some or other reason it became necessary to verify the actual reaction noted. The animals, therefore, upon which cross immunity experiments were performed, furnish additional evidence which is of value in establishing the identity of the infection originally obtained from cattle.

EXPERIMENTS CONDUCTED DURING 1935.

Experiment 6.—To recover blue tongue virus from cattle exposed at Tzaneen and pass it through a number of generations in sheep.

In the course of this passage through sheep animals were used which had previously been immunised against the laboratory vaccine strain of the virus, with only a few exceptions when blue tongue susceptible sheep were made use of.

Generation 1.

25.2.35. Bovine 6252 (exposed at Tzaneen) was bled and 5 c.c. blood injected subcutaneously into two blue tongue vaccine immune sheep, 41027 and 41566.

Result.—41027.—Five days after injection a good reaction, with maximum temperature of 105.6°, lasting four-and-a-half days. No clinical symptoms apparent.

41566.—Good reaction commencing the third day after injection. Maximum temperature recorded 107.2°. Reaction lasted six days. No clinical symptoms.

Generation 2.

4.3.35.—5 c.c. blood of 41566 was injected subcutaneously into two blue tongue vaccine immune sheep, 41002 and 41025.

Result.—41002.—A good reaction set in on the third day which lasted for about five-and-a-half days showing a maximum temperature of 106.8°. No clinical symptoms recorded. 41025.—No reaction.

Generation 3.

12.3.35.—5 c.c. blood of 41002 was injected subcutaneously into two B.T.V.* immune sheep 41768 and 41841.

Result.—41768.—A good reaction set in on the third day following injection, lasting four days: The temperature, however, remained between 103 and 104° up until the 29.3.35 before returning to normal. From the 16th March hyperaemia of the buccal mucosa, dullness and disinclination to feed were noticed. The maximum temperature recorded was 106·6°.

41841.—After an initial rise to 104·6° on the afternoon of injection, the temperature dropped to 103·2° the following morning but rose again that afternoon to 103·8°. Elevated temperatures, with a maximum of 106·4° on the 15th March, were maintained until the 20th when the reaction subsided. Slight but definite clinical symptoms were noted.

Generation 4.

18.3.35.—10 c.c. blood of 41768 was injected intravenously into a blue tongue susceptible sheep, 41748 and 20 c.c. intravenously and 2 c.c. into the prescapular lymphatic gland of bovine 5441.

Result.—41748.—With exception of temperatures of 105° a.m. and 103·8 p.m. on the second day following injection, the reaction proper did not commence until the 23rd March. A marked reaction set in which persisted for a period of about 15 days with occasional drops to about 103°. The maximum recorded was 107·4° and clinical symptoms of swollen lips, hyperaemic mucous membranes, dullness etc., were well marked.

5441.—Temperatures fluctuated markedly from the afternoon of the day of injection until the 31.3.35. On the morning of the 21.3.35, 105·2° was recorded and 106·4° on the afternoon of the 29th but it was difficult to actually distinguish a definite reaction.

Generation 5.

30.3.35.—20 c.c. blood of 41748 was injected into a blue tongue susceptible sheep 42359 intravenously.

Result.—Four days after injection the temperature rose to 104 a.m. and 104·4° but dropped next day to 102 and 102·6°. On 5th April temperature remained at 103 and 102·8°, the 6th 102·6 and 103·8, the 7th 103 (a.m.), 8th 104 and 105 and the 9th 107 and 107·2. Thereafter the temperature commenced subsiding gradually, the animal being killed on the 13th for collection of material. Animal was slightly dull on the 10th but no symptoms of blue tongue were noted.

Generation 6.

10.4.35.—10 c.c. blood of 42359 was injected intravenously into each of the following sheep: 40943 (B.T.V. immune), 40955 (B.T.V. immune) and 42436 (blue tongue susceptible).

Result.—40943.—On the day following injection a good reaction set in which lasted for 8 days and showed a maximum of 107°. No clinical symptoms noted.

40955.—A good reaction commenced on the day following injection, with maximum of 107, and lasting 5 days. No clinical symptoms noted.

42436.—On the second day after injection the temperature rose to 105 (a.m.) and 104 (p.m.). Temperatures between 103 and 104 were recorded for the following three days. On the 16th, 105 and 106·8, 17th, 105·8° and 105·4°. The temperature then dropped to 103° but remained irregular with elevations to 104°. The animal ceased to feed on the 16th and gradually lost condition eventually becoming paralysed on the 1st May. It was killed on 2.5.35 in a very poor condition and unable to rise.

* B.T.V. = Blue tongue vaccine.

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Generation 7.

26.7.35.—5 c.c. blood of 40943 (collected on 13.4.35) was injected intravenously into two B.T.V. Immune sheep 41807 and 41858.

(These sheep had each received 25 c.c. blood of 40955 intravenously on 27.6.35, the object being to note whether a sheep (i.e. 40955) recovered from a reaction produced by the virus recovered at Tzaneen, harboured *Spirochaeta theileri* in its blood. These two sheep failed to react to this injection although 41858 showed numerous *Eperythrozoon ovis* in blood smears together with anaemia).

Result.—41807 and 41858 both showed marked reactions commencing on the third and first day after injection with maximum temperatures of 107.4° and 107.6° respectively. 41858 showed no clinical symptoms, apart from the anaemia occasioned by *Ep. ovis*, but 41807 showed dullness, disinclination to feed and slight reddening of the buccal mucosa on the eighth day following injection.

Generation 8.

7.8.35.—5 c.c. blood of 41807 was injected intravenously into each of two B.T.V. immune sheep, 43870 and 44545 and Bovine 5563.

Result.—Both sheep reacted typically, the reactions commencing on the third day after injection with maximum temperature of 107.4° and 108° respectively. 43870 showed swollen lips, hyperaemia of the buccal mucosa and was off its feed for a few days.

Generation 9.

13.8.35.—10 c.c. of the pooled blood of 43870 and 44545 was injected intravenously into two B.T.V. immune sheep 40933 and 42768.

Result.—Both sheep reacted typically on the second day after injection showing maximum temperatures of 108° and 107.2° respectively. No clinical symptoms were displayed.

Generation 10.

19.8.35.—10 c.c. of the pooled blood of 40933 and 42768 was injected intravenously into a B.T.V. immune sheep, 41104.

Result.—A reaction commenced on the 3rd day after injection showing a maximum temperature of 106.6°. No clinical symptoms were noted.

Generation 11.

26.8.35.—10 c.c. blood of sheep 41104 was injected intravenously into a B.T.V. immune sheep 44233.

Result.—A reaction commenced the day following injection, the maximum temperature being 107°. No symptoms were noted.

Generation 12.

31.8.35.—10 c.c. blood of 44233 was injected intravenously into a normal sheep 45005.

Result.—A reaction commenced on the second day following injection, the temperature rising rapidly to 107.4° on the seventh day with *death* on the afternoon of this day. From the day preceding death the animal ceased to feed and showed marked depression with swollen lips and hyperaemic mucous membranes.

Generation 13.

7.9.35.—10 c.c. of an emulsion of liver, kidney, spleen and lymphatic glands of 45005 was injected intravenously into a B.T.V. immune sheep, 43481.

Result.—A reaction commenced on the day following injection which showed a maximum temperature of 108° on the second day. No clinical symptoms were noted.

Generation 14.

13.9.35.—5 c.c. blood of 43481 was injected intravenously into 42738 (B.T.V. immune).

Result.—A good reaction set in on the day following injection showing a maximum temperature of 105.8° on the second day. The animal was visibly ill and off its feed for a few days.

Generation 15.

20.9.35.—5 c.c. blood of 42738 was injected intravenously into the following B.T.V. immune sheep: 42676, 43660, 44209, 44297, 42698.

Result.—The first four sheep gave extremely mild reactions and in the case of 43660 it is doubtful whether the animal reacted at all.

42698 showed a rise of temperature on the fourth day after injection, the maximum being 105.2° . The reaction persisted for three days.

Generation 16.

29.9.35.—5 c.c. blood of 42698 was injected intravenously into a B.T.V. immune sheep 43835.

Result.—A good reaction set in on the third day after injection with maximum of 106.6° and lasting four days. No clinical symptoms were noted.

Generation 17.

8.10.35.—5 c.c. blood of 43835 was injected intravenously into 43229 (B.T.V. immune).

Result.—A marked reaction set in on the third day after injection which showed a maximum of 106.4° . No clinical symptoms were noted.

Generation 18.

19.10.35.—5 c.c. blood of 43229 was injected intravenously into a normal sheep 44995.

Result.—A marked reaction set in on the second day after injection which showed a maximum of 106° . Clinical symptoms of blue tongue including subcutaneous oedema were pronounced and death supervened on the 29.10.35, that is ten days after injection.

Generation 19.

28.10.35.—5 c.c. blood of 44995 was injected intravenously into a blue tongue susceptible sheep 44993.

Result.—The temperature rose on the second day following injection to reach the maximum during the afternoon of 106.6° . Irregular temperatures were recorded during the following four days varying between 103° and 106.4° . Death occurred on the 4.11.35 with all the typical symptoms of blue tongue.

Generation 20.

4.11.35.—5 c.c. blood of 44993 was injected intravenously into a blue tongue susceptible sheep 44994.

Result.—A reaction commenced on the second day after injection with a maximum on this day of 106° . Temperatures were not very high. Marked clinical symptoms of swollen mucous membranes, etc., were shown with death on the 13.11.35.

Generation 21.

13.11.35.—5 c.c. blood of 44994 was injected intravenously into a normal sheep, 44977.

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Result.—The temperature rose to 106° on the second day after injection. Clinical symptoms became manifest from the 19.11.35 until about the 30th, the animal showing marked swelling of the buccal mucosa, sero-haemorrhagic discharge from the nostrils, accelerated respiration and inappetence. The ninth day after injection the temperature dropped to normal but returned to 105° the following afternoon and thereafter showed frequent rises to 105° until the 7.12.35 when it remained, thereafter, within normal limits.

Generation 22.

22.11.35.—2 c.c. blood of 44977 was injected subcutaneously into each of 41722 (blue tongue susceptible) and 43751 (B.T.V. immune) and 5 c.c. intravenously into each of 41762 (blue tongue susceptible) and 44228 (B.T.V. immune).

Result.—All four sheep reacted well, the maximum temperatures varying between 106.4° and 107°. Clinical symptoms were present in the case of the two B.T.V. immune sheep in the form of slight reddening and swelling of the buccal mucosa. In the case of the two normal sheep clinical symptoms were very marked, both showing accelerated respiration with muco-purulent discharge from the nostrils, disinclination to feed and marked swelling of the lips. 41722 was killed *in extremis* on 30.11.35 showing characteristic symptoms of blue-tongue. 41762 was killed on 9.12.35 showing extreme depression, inability to rise and skin myiasis.

Generation 23.

30.11.35.—5 c.c. of the pooled blood of 41722 and 41762 was injected intravenously into a blue tongue susceptible sheep, 45343.

Result.—A reaction commenced on the second day after injection. Irregular temperatures, varying between 103° and 105°, were recorded during the five days following. On 7.12.35 the temperature rose to 107 (p.m.) and reached 108° the following day at which point death supervened. At post-mortem typical lesions of blue tongue were noted complicated by necrotic foci in the lungs.

Generation 24.

9.12.35.—5 c.c. blood, collected from 45343 at post-mortem, was injected intravenously into a normal sheep, 45337.

Result.—Two days after injection the temperature rose to 106°, this being the maximum of a reaction which persisted for six days culminating in death on 16.12.35. The autopsy revealed marked pulmonary oedema and haemorrhagic enteritis together with the typical external symptoms of blue tongue.

Generation 25.

16.12.35.—5 c.c. blood 45337 was injected intravenously into a blue tongue susceptible sheep, 45371.

Result.—On the second day after injection the temperature rose to 107°. Thereafter it commenced falling and reached 103° on the fifth day when death supervened. The animal showed a marked diarrhoea on the 19th and 20th December with slight reddening of the buccal mucosa and disinclination to move and feed and went down in the sternal position on the 21st showing dyspnoea. The post-mortem examination revealed a local gangrenous myositis at the site of injection, presumably associated with the injection, together with a pseudo membranous pleuritis on the left side.

Generation 26.

18.1.36.—5 c.c. blood of 45347 and 45423 pooled, collected on 21.12.35 was injected intravenously into each of two blue tongue susceptible sheep, 45347 and 45423.

Result.—45347.—Except for a rise of temperature to 107° on the afternoon of injection which was not maintained, the reaction proper did not commence until the fifth day. A reaction of medium intensity, with maximum of 106.6° was noted. Slight swelling of the lips and reddening of the buccal mucosa was shown.

45423.—A rise of temperature to 104.6° was noted on the day after the day of injection. This temperature was maintained the following morning but dropped during the afternoon. On the fifth day after injection a reaction commenced which showed a maximum of 106.2° . The lips were swollen and reddened but feeding continued normally.

Generation 27.

16.3.36.—5 c.c. blood of 45423 collected on 25.1.36 was injected into each of the following sheep intravenously: 44594 (B.T.V. immune), 45745 (blue tongue susceptible) and 45752 (blue tongue susceptible).

Result.—44594.—On the second day following injection the temperature rose to 106° . The maximum of 107° was recorded on the fifth day after which recovery rapidly followed. No clinical symptoms were observed.

45745 and 45752.—Marked reactions set in on the second day following injection. Clinical symptoms were marked and death from typical uncomplicated blue tongue followed on the seventh and ninth days respectively following the injection.

Generation 28.

20.3.36.—5 c.c. blood of 44594 was injected subcutaneously into each of two blue tongue susceptible sheep, 45754 and 45748.

Result.—45754.—A good reaction commenced on the sixth day and reached its maximum of 107° on the ninth day.

45748.—A reaction commenced on the third day with a maximum on this day of 107.4° . Death from typical uncomplicated blue tongue, characterised by subcutaneous oedema, followed on the ninth day. At this stage the passage was discontinued.

Experiment 7.—To note the effect of passage through bovines of the virus recovered at Tzaneen from bovines.

Generation 1.

7.8.35.—5 c.c. blood of sheep 41807 (see Experiment 1, Generation 8) was injected intravenously into bovine 5563.

Result.—The second day following injection the temperature rose to 102.6° and 103° was recorded on the morning of the 9.8.35. Thereafter, a period of about two weeks followed in which the temperature remained irregular with elevations, principally during the afternoons, to 103° and 103.4° .

Generation 2.

23.8.35.—10 c.c. blood of bovine 5563 was injected intravenously into bovine 5651.

Result.—The third day after injection the temperature rose to 105.2° , which constituted the maximum. Temperatures of over 103° were recorded for an additional two days after which irregular temperatures were noted with evening exacerbations to 103° and even 104.2° . No clinical symptoms were evident.

Generation 3.

31.8.35.—10 c.c. blood of bovine 5651 was injected intravenously into bovine 5653.

Result.—On the evening of the second day following injection the evening temperatures registered 102.6° . Irregular temperatures persisted for the following three days varying between 100.4° (a.m.) to 102.4° and 103° (p.m.). On the sixth day after injection the evening temperature registered 105.6° , the 7th day (a.m.) 103.4° after which it dropped to remain irregular with occasional elevations to 103° for a period of about two weeks. No clinical symptoms were noticed.

Experiment 8.—To test the infectivity of bovine blood for sheep.

13.8.35.—10 c.c. blood of bovine 5563 (see Experiment 1, generation 1) was injected intravenously into two B.T.V. immune sheep, 41029 and 44186.

Result.—41029.—Two days after the injection the temperatures recorded were 105.8° a.m. and 107 p.m. Thereafter the temperature dropped by degrees, normality being reached on the 21.8.35. No clinical symptoms were noted.

44186.—A reaction set in on the 16th August (i.e. three days after the injection) which reached its maximum of 107° on the 17th. Temperatures remained elevated until the 20th when the reaction subsided. No clinical symptoms were noted.

15.8.35.—10 c.c. blood of bovine 5563 was injected intravenously into two B.T.V. immune sheep, 37175 and 40972.

Result.—Good reactions commenced on the second day following injection with maximum temperatures of 107.2° and 106.6° on the fifth day respectively. The reactions persisted for seven to nine days. No clinical symptoms.

18.9.35.—5 c.c. blood of bovine 5563 was injected intravenously into two sheep 43665 (B.T.V. immune) and 44983 (normal).

Result.—43665 failed to react.

44983.—A reaction set in on the fifth day after injection with a maximum of 106.4° on the tenth day. The reaction lasted for seven-and-a-half days. Clinical symptoms were not recorded.

19.8.35.—10 c.c. blood of bovine 5563 was injected intravenously into two B.T.V. immune sheep 41090 and 44242. The blood thus injected was taken from 5563, twelve days after the original injection (see Experiment 2, generation 1).

Result.—41090.—A reaction commenced on the second day after injection which lasted for six-and-a-half days and showed a maximum of 107.2°. No clinical symptoms.

44242.—A reaction commenced on the second day after injection, lasting about four days with maximum of 106.2°. No clinical symptoms.

22.8.35.—10 c.c. blood of 5563 was injected intravenously into two B.T.V. immune sheep, 43349 and 43634. (15 days after original injection).

Result.—Good reactions set in on the fourth day after injection showing maximum temperatures of 107° and 107.4° and lasting five and three-and-a-half days respectively. No clinical symptoms were noted.

29.8.35.—10 c.c. blood of bovine 5563 was injected intravenously into a B.T.V. immune sheep, 43236. (22 days after original injection).

Result.—The second day after injection the temperature rose to 106°, the maximum, the reaction persisting for seven days. No clinical symptoms recorded.

2.10.35.—5 c.c. blood of bovine 5563 was injected intravenously into two B.T.V. immune sheep, 43678 and 43882 (i.e. eight weeks after the original injection).

Result.—Neither of these two sheep reacted. Both sheep proved to be susceptible when tested on the 23.10.35 with Tzaneen blue tongue virus collected from sheep 44995 (Experiment 1, generation 18).

A further test of the infectivity of bovine blood for sheep was conducted with blood collected from bovine 5653 (see Experiment 7, generation 3).

2.9.35.—5 c.c. blood of 5653 was injected intravenously into two B.T.V. immune sheep, 43298 and 43313.

Result.—43298.—A reaction set in on the third day after injection which lasted five days and showed a maximum temperature of 105.8°. No symptoms were recorded.

43313.—The sheep reacted on the third day after injection, the reaction lasting five-and-a-half days with maximum of 106.2° . No clinical symptoms were noted. On 17.9.35 (i.e. 15 days after injection) a second febrile reaction set in which lasted seven days and showed a maximum of 106° . No symptoms noted.

11.9.35.—5 c.c. blood of 5653 was injected intravenously into two B.T.V. immune sheep, 42174 and 42696.

Result.—42174.—A reaction commenced on the third day after injection which lasted six days and showed a maximum of 107.4° . The animal showed reddening and swelling of the buccal mucous membrane and a slight disinclination to feed for a few days.

42696.—A reaction set in on the third day after injection which persisted for five days and showed a maximum of 107° . Slight reddening of the buccal mucosa only was noted.

Experiment 9.—To note the effect of passage through normal sheep upon the virus of blue tongue recovered from cattle at Tzaneen.

Generation 1.

22.11.35.—Blood collected from sheep 41566 on 4.3.35, i.e. almost nine months previously (see Experiment 6, generation 1), was injected intravenously into two blue tongue susceptible sheep, 41709 and 41791.

Result.—41709.—The temperature rose on the afternoon of the day of injection to 104.6° and remained fluctuating between 103° and 104° until the 27.11.35 when an evening temperature of 105° was recorded. This constituted the commencement of a reaction which attained a maximum of 107° on the 30th and lasted 4 days.

41791.—A reaction commenced on the third day after injection which lasted nine days and showed a maximum of 106.2° . Very irregular temperatures were recorded during this period.

Generation 2.

9.12.35.—5 c.c. Pooled blood of 41709 and 41791 (collected 4.12.35) was injected intravenously into a blue tongue susceptible sheep, 45375.

Result.—A reaction set in on the second day following injection which lasted eleven days and showed a maximum of 106° . On the 19.12.35 the animal showed slight reddening of the buccal mucosa and diarrhoea.

Generation 3.

18.12.35.—Blood of 45375 was injected intravenously into a blue tongue susceptible sheep, 45341.

Result.—A marked reaction set in on the third day after injection showing a maximum of 105° with death on the 26.12.35. Clinical symptoms were noted from the 25th, the animal showing markedly swollen lips, cyanotic mucous membranes and dyspnoea. The post-mortem examination revealed typical blue tongue characterised by subcutaneous oedema and blood extravasations of the heart.

30.12.35.—Blood collected from 45341 at post-mortem on the 26.12.35 was injected intravenously (5 c.c. each) into the following B.T.V. immune sheep, 43830, 44098, 44105, 44114, 44126.

Result.—These sheep all reacted on about the third day after injection, the reactions lasting about 9 days with maximum temperatures varying between 106° and 108.2° . Clinical symptoms were very mild, slight swelling and reddening of the lips only being noticeable.

BLUE TONGUE IN CATTLE AND SHEEP.

Generation 4.

18.1.36.—5 c.c. blood of 45341 collected on 24.1.36 was injected intravenously into two blue tongue susceptible sheep, 45349 and 45369.

Result.—45349.—A reaction set in on the second day after injection which persisted for about a fortnight and showed a maximum of 107°. The lips became swollen and reddened and the external nares reddened and tender.

45369.—The temperature rose rapidly on the second day after injection to reach the maximum of 107° on the seventh day. Death supervened on the 26.1.36. The lips were markedly swollen and reddened and the external nares red and excoriated. The post-mortem reveals sub-epi- and endocardial haemorrhages, lips and tongue swollen and oedematous and the presence of ulcers in the abomasum.

Generation 5.

24.1.36.—Blood of 45349 was injected intravenously into 5 blue tongue susceptible sheep, 45409, 45403, 46422, 45445, 45520, one B.T.V. immune sheep, 43751 and one sheep immunised against a strain of blue tongue recovered from cattle on the farm Novo during 1932.

Result.—For reactions in the case of 43751, 45409 and 45403 see experiment 11b (filtration) and for 45422, 45445, 45351 and 45420 see appendix experiment 12b (spinning).

Generation 6.

11.2.36.—5 c.c. blood of 45409 collected on 1.2.36 was injected intravenously into one blue tongue susceptible sheep, 45404 and one Tzaneen strain immune sheep, 45443.

Result.—Both sheep reacted well, the reactions commencing on the second day after injection and showing maximum temperature of 106° in both cases. The reactions persisted for eight days.

Generation 7.

17.2.36.—Pooled blood of 45443 and 45404 was injected intravenously into 8 blue tongue susceptible sheep, 45734, 45753, 45731, 45717, 45727, 45741, 45739 and 45758.

Result.—For reactions see appendix experiment 12 c.

Generation 8.

27.2.36.—Blood of 45731 was injected intravenously into 9 blue tongue susceptible sheep, 45718, 45728, 45732, 45751, 45729, 45742, 45759, 45719 and 45743.

Result.—For temperature reactions see appendix experiment 12 d.

Generation 9.

16.3.36.—Blood collected from 45729 on 8.3.36 was injected intravenously into two blue tongue susceptible sheep 45726 and 45757 and one B.T.V. immune sheep, 43942.

Result.—43942 reacted on the third day after injection, the reaction lasting six days and showing a maximum of 107.2°.

45726.—A marked reaction set in on the fourth day which lasted eight days and showed a maximum of 107.4°. Slight clinical symptoms were noted.

45757.—A severe reaction set in on the second day after injection with a maximum of 106.2°. Death supervened on the 23.3.36. Clinical symptoms of blue tongue were apparent, the diagnosis being confirmed on post-mortem.

Experiment 10.—To study the effect of brain material from sheep reacting to blue-tongue injected into normal and B.T.V. immune sheep.

In order to ascertain that sheep 42436 (see experiment 6, generation 6) was reacting to blue-tongue blood of this animal was collected on the 17.4.35, at the height of the reaction, and 15 c.c. injected intravenously into 42532, a blue tongue susceptible sheep, and 41872, a B.T.V. immune animal.

Result.—42532.—A very slight ill-defined reaction commenced on the fifth day after injection. Temperatures remained irregular during the following three weeks and no symptoms were recorded.

41872.—A well defined reaction set in on the third day following injection which showed a maximum of 106.6° and lasted nine days. No clinical symptoms were recorded.

2.5.35.—At the death of 42436 on this date an emulsion of organs (brain, gland and liver) was injected into 42526, a normal sheep, and 41574 a B.T.V. immune animal. These two sheep received 20 c.c. of the emulsion intraperitoneally and 5 c.c. into the prescapular gland.

Result.—42526.—The day following the injection the animal was dull and not feeding. The temperature rose steadily to 105.2° on the fourth day after injection when death occurred. The post-mortem revealed no evidence of peritonitis and death was attributed to the toxic effect of the brain material injected.

41574.—The day following the injection the animal was dull and not feeding and the temperature rose rapidly to reach 106.2° on the second day. Death occurred on the 5.5.35. The post-mortem showed an acute fibrinous peritonitis.

7.5.35.—20 c.c. of an emulsion of the brain of 42526 was injected intraperitoneally into 41833, B.T.V. immune and 42493, blue tongue susceptible.

Result.—41833.—The animal was dull and not feeding on the day following the injection. 106.2° was recorded on the third day with death at this point. The post-mortem examination revealed hyperaemia and oedema of the lungs, fatty degeneration of the liver and generalised venous stasis.

42493.—The temperature rose to 105.2° (the maximum) on the second day after injection, the animal being dull and not feeding well. High temperatures were maintained until the 13.5.35. when death occurred. The post-mortem revealed fibrinous peritonitis and pleuritis.

10.5.35.—20 c.c. of a 10 per cent. brain emulsion in saline of 41833 was injected intraperitoneally into 39642, B.T.V. immune and 42884, blue tongue susceptible.

Result.—39642.—The animal was dull on the second day after injection. The following day the temperature commenced rising and a well defined reaction, lasting seven days and showing a maximum of 106° set in. The sheep remained dull, showed a disinclination to feed normally and the abdomen showed tenseness. A progressive loss in condition followed which terminated in death on the 17.6.35. The post-mortem revealed chronic peritonitis.

42884.—The day after the injection the temperature rose to 104°. The animal was dull and not feeding normally. On the third day the temperature dropped to subnormal and then rose sharply on the fourth day to 105° when death occurred. The post-mortem revealed evidences of toxæmia although peritonitis was absent.

BLUE TONGUE IN CATTLE AND SHEEP.

Experiment 11A.—To ascertain whether the virus of blue-tongue is filterable.

1.10.35.—Blood of 44983 (see experiment 8, 18.9.35), was laked by dilution with distilled water, 1:10, and passed through paper pulp. After this preliminary filtration the blood was passed through a Berkefeld filter candle together with a culture of *B. proteus*.

The original blood, the blood passed through paper pulp and the Berkefeld filtrate were injected intravenously into B.T.V. immune sheep. A sterility test of the filtrate by culture in broth for seven days proved the filtrate to be sterile.

43761 received 50 c.c. of the original blood in distilled water 1:10, 44555 received 50 c.c. of the diluted blood after passing through paper pulp and 42695 received 50 c.c. of the Berkefeld filtrate.

Result.—42695.—A rise of temperature to 105° occurred on the day after injection but was not maintained. The reaction proper commenced on the fourth day after injection. This reaction persisted for seven days and showed a maximum of 107°.

43761.—The injection was marked by a rise of temperature during the afternoon to 105°. Thereafter the temperature gradually returned to normal. On the seventh day a mild but well defined reaction commenced which lasted for four-and-a-half days and showed a maximum of 104·8°.

44555.—An initial rise of temperature was noted on the day of injection. A good reaction set in on the fourth day which lasted for four-and-a-half days and showed a maximum of 107·4°.

In order to test the reaction of 42695, 5 c.c. blood of this animal was injected into 42504 (a B.T.V. immune animal) on 8.10.35. A good reaction set in on the second day after injection which lasted seven days and showed a maximum of 107·2°. No clinical symptoms were noted.

Experiment 11B.—To ascertain whether the virus of blue-tongue is filterable.

A second filtration experiment was conducted on 24.1.36 when blood of sheep 45349 (see experiment 9, generation 4) was laked in distilled water (1:8) and passed through a Berkefeld candle together with a broth culture of *B. proteus*. The actual filtration proved to be sterile on subsequent bacteriological examination of the filtrate.

5 c.c. of the original blood was injected intravenously into one blue tongue susceptible sheep 45403 and one sheep immune to a strain of blue tongue obtained from the farm Novo, 41890.

40 c.c. of the Berkefeld filtrate (equivalent to 5 c.c. of the original blood) was injected into one blue tongue susceptible sheep, 45409, and one Tzaneen blue tongue strain immune sheep, 43751.

Result.—45403.—A good reaction commenced on the second day following injection which lasted for eleven-and-a-half days and showed a maximum temperature of 107°. No clinical symptoms noted.

41890.—On the second day following injection the temperature rose to 104·4° during the afternoon. On the third day 103° (a.m.) and 104° (a.m.) were recorded. Thereafter temperatures remained normal. The reaction (if any) was very indefinite.

45409.—A good reaction set in on the second day after injection which lasted nine days and showed a maximum of 107·2°.

43751 failed to react.

Experiment 12.—To ascertain whether blue tongue virus can be deposited by spinning at 15,000 revolutions per minute.

In order to determine this question four experiments were conducted:—

(a) 10.1.36.—Blood of sheep 41531 (a blue tongue immune sheep which reacted to an injection of Tzaneen blue tongue virus) collected at the height of the reaction was defibrinated and centrifuged at 4,000 r.p.m. to separate out the cellular constituents.

The serum so obtained was spun at 14,000 r.p.m. for 35 minutes and the following sheep injected with the supernatant fluid, the deposit and (as controls) with the original blood:—

45336 Tzaneen B.T. strain immune	}	each received 4 c.c. of the supernatant fluid intravenously.
44120 B.T.V. immune		
44124 B.T.V. immune		
45435 B.T. susceptible		
45443 B.T. susceptible	}	each received 3 c.c. of the deposit diluted with normal saline intravenously.
41890 Tzaneen B.T. strain immune		
44140 B.T.V. immune		
45440 B.T. susceptible		
44117 B.T.V. immune	}	each received 5 c.c. of the original defibrinated blood intravenously.
45370 Tzaneen B.T. strain immune		
45415 B.T. susceptible		

Result.—The five sheep which received supernatant fluid all failed to react with the exception of 44120, which showed a reaction on the twenty-first day after injection. This reaction was of medium intensity, with a maximum of 106.2° , and lasted for a fortnight. On account of the delayed nature of this reaction and the total absence of any clinical symptoms it is very doubtful whether it could be connected in any way with the injection.

The three sheep which received the deposit all failed to react.

In the case of the three sheep which received the original blood all reacted, 44117 showing a good reaction from the third to the eighth day after injection with a maximum of 107.2° . 45370 showing a very mild reaction from the seventh to the 9th day with a maximum of 104.6° and 45415 showing a pronounced reaction commencing on the third day after injection, with a maximum of 107° , and ending in death with all the typical symptoms of blue tongue on the twelfth day.

(b) 24.1.3.—Blood of sheep 45349 (see experiment 9, generation 4) was treated in a similar way to that of the preceding experiment. Spinning at 15,000 r.p.m. was carried out for 10 minutes only. The following sheep were injected:—

45445 B.T. susceptible	}	each received 1 c.c. of the deposit diluted with saline, subcutaneously.
45336 Tzaneen B.T. strain immune		
45351 Novo strain of B.T. immune	}	each received 3 c.c. of the supernatant fluid subcutaneously.
45420 B.T. susceptible		
41890 Tzaneen B.T. strain immune	}	each received 5 c.c. defibrinated blood of 45349 subcutaneously.
45403 B.T. susceptible		
45422 B.T. susceptible		

Result.—45445.—A marked reaction set in on the third day after injection which showed a maximum of 106.8° . Death supervened on the eighth day from acute uncomplicated blue tongue.

45336.—A reaction set in on the third day which reached its peak of 107° on the fourth day. This reaction persisted for ten days. No clinical symptoms were noted.

45351.—A reaction commenced on the fourth day which lasted for four-and-a-half days and showed a maximum of 108°. No clinical symptoms were observed.

45420.—An acute reaction set in on the second day which culminated in death from blue tongue on the seventh day after injection. The maximum recorded was 107°.

41890.—A slight rise of temperature to 104.2° occurred on second day after injection. Temperatures fluctuated between 102.2° and 104° for a few days and then subsided. It is doubtful whether this may be looked upon as a reaction.

45403.—A reaction set in on the second day after injection which persisted for twelve days and showed a maximum of 107°. No clinical symptoms were noted.

45422.—A very mild reaction commenced on the fourth day after injection which lasted for about four days and showed a maximum of 105.2°. No clinical symptoms were recorded.

(c) 17,236.—Pooled blood of sheep 45404 and 45443 (see experiment 9; generation 6) was spun at 4,000 r.p.m. for 30 minutes. Equal parts of serum and laked red corpuscles so obtained was spun at 15,000 r.p.m. for 30 minutes. The deposit obtained after the second spinning was washed by spinning at 15,000 r.p.m. for 30 minutes on two occasions in a large excess of saline.

The following injections were made into blue tongue susceptible sheep:—

45739	}	each received 3.5 c.c. of the supernatant fluid of the spinning at 15,000 r.p.m. intravenously.
45758		
45733	}	each received 4.3 c.c. of the washed deposit obtained after spinning at 14,000 r.p.m. intravenously.
45734		
45731	}	each received 3.5 c.c. of the mixture of plasma and laked corpuscles after the spinning at 4,000 r.p.m. intravenously.
45717		
45741	}	each received 3.5 c.c. of the original pooled blood defibrinated intravenously.
45727		

Result.—45739.—A good reaction set in on the fifth day which lasted eight days and showed a maximum of 107°.

45758.—A slight reaction commenced on the ninth day after injection, which showed a temperature of 106° on one afternoon only and persisted for three days.

45753.—A reaction set in on the fourth day which ended in death from blue tongue on the 10th day and showed a maximum of 106.6°.

45734.—A reaction set in on the fifth day which showed a maximum of 107° and ended in death on the fourteenth day. The animal was killed *in extremis* and showed the typical symptoms of blue tongue.

45731.—A reaction set in on the fourth day which showed a maximum of 107°. The animal was killed *in extremis* on the fourteenth day showing clinical evidence of blue tongue.

45717.—A reaction commenced on the fourth day which persisted for ten days and showed a maximum of 106.2°.

45741.—A marked reaction set in on the third day which resulted in death on the ninth day with the typical symptoms of blue tongue. The maximum recorded was 106°.

45727.—A reaction commenced on the third day which persisted until the animal was killed *in extremis* on the fourteenth day. The maximum recorded was 107° and all the symptoms of blue tongue were present.

(d) 27.2.36.—Defibrinated blood of sheep 45731 (see experiment 9, generation 7) was spun at 4,000 r.p.m. for one-and-a-half hours. The supernatant fluid so obtained was spun at 15,000 r.p.m. for thirty minutes. The deposit obtained from the second spinning was washed twice by spinning at 15,000 r.p.m. for thirty minutes on two occasions with a large excess of normal saline.

The following injections were made into blue tongue susceptible sheep:—

- 45759 received .1 c.c. subcutaneously of supernatant fluid spun at 4,000 r.p.m. for one-and-a-half hours.
- 45729 received .01 c.c. subcutaneously of supernatant fluid spun at 4,000 r.p.m. for one-and-a-half hours.
- 45742 received .001 c.c. subcutaneously of supernatant fluid spun at 4,000 r.p.m. for one-and-a-half hours.
- 45732 received 1 c.c. of supernatant obtained from spinning at 15,000 r.p.m. for half-an-hour.
- 45751 received .1 c.c. of supernatant obtained from spinning at 15,000 r.p.m. for half-an-hour.
- 45728 received .01 c.c. of supernatant obtained from spinning at 15,000 r.p.m. for half-an-hour.
- 45718 received .001 c.c. of supernatant obtained from spinning at 15,000 r.p.m. for half-an-hour.
- 45719 received 12.5 c.c. (= half original volume) of washed deposit after washings at 15,000 r.p.m. reconstituted with saline to original volume.
- 45743 received 1 c.c. (= 1/23 original volume) of washed deposit after washings at 15,000 r.p.m. reconstituted with saline to original volume.

Result.—45759.—A reaction set in on the sixth day which showed a maximum of 106.4° and lasted for six days.

45729.—A reaction set in on the sixth day which showed a maximum of 107.4° and lasted for seven days.

45742.—A reaction set in on the sixth day which showed a maximum of 107.2° and lasted for nine days.

45732.—A reaction set in on the fifth day which showed a maximum of 107.6° and lasted for six days.

45751.—A reaction set in on the seventh day which showed a maximum of 105.4° and lasted for seven days.

45728.—Failed to react.

45718.—Failed to react.

45719.—Failed to react.

45743.—Failed to react.

Experiment 13.—To ascertain whether bacterial cultures made from the organs of sheep 45007 are capable of setting up a reaction in injected sheep. Six sheep were injected with these cultures but in none were any reactions observed. One of the sheep was subsequently injected with Tzaneen blue tongue virus and reacted.

Conclusions.—This experiment indicated that the bacteria isolated from the organs did not play any rôle in setting up the febrile reactions observed in sheep injected with Tzaneen blue tongue virus.

Previous History.									
D.O.B.	Source.	Date of Injection.	Experiment No.	B.T.		Incubation Period.	Reaction and Duration.	Highest Temperature.	Symptoms Recorded.
				Virus.	Qual.				
38876	Karoo	23. 2.34	5291	S. 38941	Vacc.	5 days	** 9 days	106.8	Nil
38905	"	5. 3.34	5291	S. 38898, 38876	"	6 days	** 5 days	105.2	Nil
38210	"	9. 1.34	5247	Batch 884	"	9 days	** 10 days	107.0	Nil
37111	"	19. 4.33	4997	S. 34068	"	± 24 hrs.	** 9 days	106.8	Nil
37625	"	6. 5.33	4997	S. 34072	"	2 days	** 9 days	104.8	Nil
38206	"	9. 1.34	5247	Batch 882	"	5 days	** 7 days	106.0	Slight symp.
37848	"	15. 5.33	5035	Batch 848	"	—	* (?)	104.6	—
		21. 6.33	5035	Batch 848	"	—	—	—	—
		11. 7.33	5035	S. 35004	—	—	—	—	—
		21. 9.33	5137 (H.W.)	S. 37385	(H.W.)	—	—	—	—
		24.11.33	5137 (H.W.)	S.32704	(H.W.)	—	—	—	—
38182	"	9. 1.34	5247	Batch 882	Vacc.	3 days	** 10 days	107.2	Very slight
37879	"	15. 5.33	5035	Batch 854	"	—	*	—	—
		1. 9.33	5135 (H.W.)	Org. Em. 37353	(H.W.)	—	—	—	—
37289	"	26. 4.33	4997	S. 34068	Vacc.	4 days	**	106.2	Nil
		7. 7.33	5052 (H.W.)	S. 38055	(H.W.)	15 days	*	106.2	Nil
		10. 8.33	5052 (H.W.)	S. 37301, 172	"	8 days	** 9 days	107.8	V. Sl.
		2. 9.33	5052 (H.W.)	S. 36899, 37487	"	—	—	—	—
		13.10.33	5137 (H.W.)	S. 37309, 37397	"	—	—	—	—
37096	"	19. 4.33	4997	S. 34068	Vacc.	7 days	**	105.4	—
		22.11.33	4377	S. 35004, 37754	(H.W.)	—	—	—	—
		20. 1.34	4377	S. 37368, 38077	"	12 days	** 12 days	107.8	Nil
38937	"	9. 2.34	5247	Batch 902	Vacc.	4 days	**	107.0	Nil
		11. 4.34	5350 (L.III.)	Tick emuls	L.III.	—	—	—	—
		2. 5.34	5350 (L.III.)	L.I. 11, Vs.	—	—	R.L.III.	—	—
36881	"	15. 4.33	4997	S. 34068	Vacc.	5 days	**	105.6	—
		6. 3.34	5302 (L.III.)	Tick emuls	(L.III.)	—	** L.III.	—	—
		2. 5.34	5302 (L.III.)	L.I. 11, Vs.	—	—	—	—	—
35781	"	1. 4.33	4997	S. 34068	Vacc.	3 days	**	106.8	—
		25.10.33	5160 (H.W.)	S. 35798	(H.W.)	—	—	—	—
39466	"	26. 4.34	5291	S. 39498	Vacc.	6 days	** 6 days	106.0	—
		28. 5.34	5291	S. 40381	"	—	—	—	—
39648	"	26. 4.34	5291	S. 39498	"	6 days	** 7 days	105.4	—
		28. 5.34	5291	S. 40381	"	—	—	—	—
39270	"	10. 4.34	5345	S. Doringpoort	—	—	—	—	—
		28. 4.34	5345	S. 37691	Kromdraai	3 days	** 8 days	106.4	Sl.
		5. 6.34	5345	S. 39399	Bekker	—	—	—	—
39399	"	10. 4.34	5345	S. Doringpoort	—	—	—	—	—
		28. 4.34	5345	Novo.	—	5 days	** 6 days	106.8	Nil
		5. 6.34	5345	40788	Vacc.	—	—	—	—
39202	"	5. 5.34	5291	S. 39466, 648	"	—	*	105.0	—
		5. 6.34	5291	S. 39399	"	—	—	—	—
39665	"	5. 5.34	5291	S. 39466, 648	"	—	* Irreg.	105.0	—
		5. 6.34	5291	S. 39399	"	—	—	—	—
40889	"	19. 5.34	5353	S. 39447	"	4 days	**	106.0	—
		28. 6.34	5414 (E.Ovis)	S. 37429	(E. Ovis)	—	—	—	—
40368	"	8. 5.34	5353	S. 39447	Vacc.	4 days	**	107.0	—
		28. 6.34	5414 (E.Ovis)	S. 37429	(E. Ovis)	—	—	—	—
40127	"	7. 5.34	5353	S. 39152	Vacc.	4 days	**	105.6	—
		28. 6.34	5414 (E.Ovis)	S. 37429	(E. Ovis)	—	*	—	—
39853	"	28. 4.34	5353	S. 39152	Vacc.	5 days	**	106.4	—
		28. 6.34	5414 (E.Ovis)	S. 37429	(E. Ovis)	—	—	—	—

K.O..... = Killed for collection of specimens.
 B.T..... = Blue Tongue.
 Qual..... = Character of the B.T. virus.

Vacc.....
 (H.W.).....
 V. Sl.....



APPENDIX II A. (cont.)

TZANEEN EXPERIMENT 1934. (BLUE-TONGUE IMMUNE SHEEP).

Experiment with Tzaneen Virus.								
Interval between B.T. and Tzaneen. (days.)	Date of Injection.	Experiment No.	Tzaneen Virus.	Incubation Period.	Reaction and Duration.	Highest Temperature.	Symptoms Recorded.	Result.
75	9. 5.34	5373	C. 5454	±24 hrs.	* * * 10 days	106.0	Nil	Died H.W., 28.5.34
65	9. 5.34	5373	C. 5454	4 days	* * * 10 days	107.0	Nil	—
126	15. 5.34	5373	C. 5188	±4 days	* * * irregular	107.0	Nil	—
411	15. 5.34	5373	C. 5188	3 days	* * * 5 days	106.4	V. Sl.	—
381	22. 5.34	5373	S. 37111	—	—	—	—	—
133	22. 5.34	5373	S. 37111	2 days	* * 5 days	106.8	Nil	—
346	2. 6.34	5361	C. 4954, 4984, 4985, 4986, 4987, 4988, 5198, 5200, 5210, 5225, 5520, 5526	2 days	* * 6 days	107.0	Nil	K.O. 4. 6.34
—	—	—	—	—	—	—	—	—
144	2. 6.34	5361	—	±24 hrs.	* * Sl. irreg.	106.0	—	—
386	5. 6.34	5361	S. 37848 "	1 day	* * 7 days	107.0	Nil	—
405	5. 6.34	5361	S. 38182	5 days	* * 5 days	106.6	—	—
—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—
412	5. 6.34	5361	S. 38182	4 days	* *	107.0	Sl.	—
—	—	—	—	—	—	—	—	—
119	8. 6.34	5350 (L. Ill.)	S. 37848	2 days	* * 6 days	106.0	Nil	—
—	—	—	—	—	—	—	—	—
420	8. 6.34	5302 (L. Ill.)	S. 37848	±24 hrs.	* * 6 days	106.8	Nil	—
—	—	—	—	—	—	—	—	—
433	8. 6.34	5361	S. 37848	2 days	* 4 days	104.4	—	—
50	15. 6.34	5361	S. 38850 (see Susc. Sheep Chart)	2 days	* * 4 days	107.0	Nil	—
—	—	—	—	—	—	—	—	—
50	15. 6.34	5361	S. 40698 (see Susc. Chart)	2 days	* * 9 days	106.6	Nil	—
74	23. 6.34	5407	S. 40698 (see Susc. Chart)	—	—	—	—	—
—	—	—	—	—	—	—	—	—
74	23. 6.34	5407	S. 40698 (see Susc. Chart)	—	—	—	—	—
—	—	—	—	—	—	—	—	—
49	23. 6.34	5407	S. 40698 (see Susc. Chart)	4 days	* * 4 days	106.4	Nil	—
49	23. 6.34	5407	—	—	—	—	—	—
68	26. 7.34	5414 (E. Ovis)	S. 39466	—	—	—	—	K.O.
—	—	—	—	—	—	—	—	—
79	26. 7.34	5414 (E. Ovis)	S. 39466	—	—	—	—	E. Ovis compl.
—	—	—	—	—	—	—	—	—
80	26. 7.34	5414 (E. Ovis)	S. 39466	—	—	—	—	—
—	—	—	—	—	—	—	—	—
89	26. 7.34	5414 (E. Ovis)	S. 39466	—	—	—	—	—
—	—	—	—	—	—	—	—	—

ABBREVIATIONS.

acc..... = Onderstepoort B.T. vaccine strain.
H.W.)..... = Heartwater experiment.
V. Sl..... = Very slight.

Source..... = Where sheep came from.
L.Ill..... = Louping ill.
S..... = Sheep.
C..... = Cattle.

*..... = Slight temperature.
**..... = More marked temperature.
***..... = Marked temperature.

Subsequent History.									
Interval between Tz. Inj. and Test. (days.)	Date of Injection.	Experi- ment No.	Virus.		Incuba- tion Period.	Reaction.	Highest Tempera- ture.	Symptoms Recorded.	Result.
			Orig.	Qual.					
---	---	---	---	---	---	---	---	---	---
67	21. 7. 34	S. 5373	S. 40980	---	---	---	---	---	---
38	22. 6. 34	S. 5373	S. 40698	Tz. (see Susc. Chart)	6 days	*	106	Intermittant	nt reaction.
30	21. 7. 34	S. 5373	S. 40980	Bekker	---	---	---	---	---
61	21. 7. 34	S. 5373	S. 40980	Bekker	---	---	---	---	---
30	22. 6. 34	S. 5373	S. 40698	Tz. (see Susc. Chart)	---	---	---	---	---
30	21. 7. 34	S. 5373	S. 40980	Bekker	---	---	---	---	---
For collection of specimens, post-mortem negative, although clinical evidence of Blue Tongue.									
---	---	---	---	---	---	---	---	---	---
49	21. 7. 34	S. 5361	S. 40980	Bekker	---	---	---	---	---
---	---	---	---	---	---	---	---	---	---
47	21. 7. 34	S. 5361	S. 40980	---	---	---	---	---	---
---	---	---	---	---	---	---	---	---	---
---	---	---	---	---	---	---	---	---	---
47	21. 7. 34	S. 5361	S. 40980	---	---	---	---	---	---
---	---	---	---	---	---	---	---	---	---
---	---	---	---	---	---	---	---	---	---
---	---	---	---	---	---	---	---	---	---
---	---	---	---	---	---	---	---	---	---
43	21. 7. 34	S. 5361	S. 40980	---	---	---	---	---	---
26	21. 7. 34	S. 5361	S. 40980	---	---	---	---	---	---
---	---	---	---	---	---	---	---	---	---
26	21. 7. 34	S. 5361	S. 40980	---	---	---	---	---	---
---	---	---	---	---	---	---	---	---	---
---	---	---	---	---	---	---	---	---	---
---	---	---	---	---	---	---	---	---	---
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---	---	---	---	---	---	---	---	---	---
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---	---	---	---	---	---	---	---	---	---
---	---	---	---	---	---	---	---	---	---
63	28. 9. 34	4377 (H.W.)	S. 37119, 41120	---	---	---	---	---	K.O.
---	---	---	---	---	---	---	---	---	---
35	30. 8. 34	4377 (H.W.)	S. 37847, 855	---	---	---	---	---	D. (H.W.)
---	---	---	---	---	---	---	---	---	---
63	28. 9. 34	4377 (H.W.)	S. 37119, 41120	---	---	---	---	---	---
---	---	---	---	---	---	---	---	---	---

Slight temperature reaction.
 More marked temperature reaction.
 Marked temperature reaction.

K.E. = Killed in extremis.
 D. = Died.
 R. = Reacted.

Experiment with Tzaneen Virus.									
D.O.B. No.	Source.	Date of Injection.	Experiment No.	Tzaneen Virus.	Incubation Period.	Reaction and Duration.	Highest Temperature.	B.T. Symptoms Recorded.	Result.
40834	Karoo.....	5. 6. 34	5361	S. 38182 (see Immune Sheep Chart)	3 days	** 8 days	107.0	Sl.	—
35171	Onderstepoort	8. 6. 34	5361	" "	± 24 hrs.	** 8 days	106.8	V. sl.	D. (Jaags. × Tz.) 16. 6. 34
38850	"	8. 6. 34	5361	" "					
40850	Karoo.....	15. 6. 34	5361	S. 38850.....	2 days	** 7 days	107.2	Sl.	—
40899	"	15. 6. 34	5361	" "	3 days	**	106.4	Sl.; irreg.	—
40647	"	15. 6. 34	5361	" "	2 days	** 7 days	106.2	Sl.	K.O.....
40698	"	15. 6. 34	5361	" "	1 day	**	106.0	Sympt.	D.B.T.....
40726	"	20. 6. 34	5403	S. 39486 (see Immune Chart).....	5 days	**	106.0	Sl.	—
40835	"	20. 6. 34	5403	S. 39204 (B.T.V. cattle)	24 hrs.	** 7 days	107.0	Sl.	—
40623	"	25. 6. 34	5408	C. 4954, 4984, 4987, 4988, 5198, 5200, 5225, 5526, 5210	—	—	—	—	—
40850	"	25. 6. 34	5408	" "	4 days	** 7 days	106.4	Nil	—
40739	"	27. 6. 34	5413	C. 4954, 4984, 4987, 4988, 5198, 5200, 5225, 5526, 5210, 5520, 4986, 4985	—	*	—	—	—
40677	"	27. 6. 34	5413	" "	—	—	—	—	—
40654	"	3. 7. 34	5408	S. 40850.....	3 days	** 8 days	104.6	—	—
40653	"	3. 7. 34	5408	" "	2 days	** 6 days	108.0	Sl.	—
41180	Onderstepoort	14. 7. 34	5433	S. 40647.....	—	** compl.	—	—	D.....
35450	"	21. 7. 34	5414	S. 3466 (see Immune Chart).....	—	* <i>E. ovis</i>	—	—	K.O.....
35449	"	21. 7. 34	5414	" "	—	observed	—	—	K.O.....
53448	"	21. 7. 34	5414	" "	—	* <i>E. ovis</i>	—	—	K.O.....
41015	Karoo.....	2. 8. 34	5405	S. 40899.....	3 days	6 days	106.4	—	—
41034	"	2. 8. 34	5405	" "	5 days	3 days	107.0	—	—
40908	"	18. 8. 34	5361	" "	—	—	—	—	—
41087	"	18. 8. 34	5361	" "	—	—	—	—	—
40994	"	18. 8. 34	5470	S. 41015, 41034.....	—	—	—	—	—
41044	"	18. 8. 34	5470	" "	—	—	—	—	—
40987	"	5. 7. 34	5420	S. 40850 (serum).....	—	—	—	—	—
41010	"	5. 7. 34	5420	" "	—	—	—	—	—
41041	"	5. 7. 34	5420	" "	—	—	—	—	—
41116	"	5. 7. 34	5420	" "	—	—	—	—	—
40985	"	31.10.34	5470	S. 40994.....	13 days	* 2 days	—	—	—
40995	"	31.10.34	5470	" "	2 days	** 5 days	107.4	Sl.	K. Compli.
41546	"	20.11.34	5470	S. 40985.....	—	*	—	—	K. Compli.
41573	"	20.11.34	5470	" "	—	—	—	—	—
40942	"	31.10.34	5470	" "	—	—	—	—	—
41060	"	31.10.34	5470	" "	4 days	** 5 days	107.4	Sl.	—

34. (BLUE-TONGUE SUSCEPTIBLE SHEEP).

Subsequent History.									
Interval between Tz. Inj. and Test. (days.)	Date of Inj.	Experiment No.	Virus.	Incubation Period.	Reaction and Duration.	Highest Temperature.	B.T. Symptoms Recorded.	Result.	
46	21. 7. 34	5361	S. 40980 Bekker...	4 days	** 5 days	107.0	V. Sl.	—	
—	—	—	—	—	—	—	—	—	
34	12. 7. 34	5430	Jaags. Ob.....	—	—	—	—	—	
9	21. 7. 34	5361, 5430	S. 40980 Bekker...	—	—	—	—	—	
—	16. 4. 35	5652	Jaags. Dibenz.....	—	—	—	—	—	
36	21. 7. 34	5361	S. 40980 Bekker...	3 days	** 3 days	106.0	Nil	—	
—	—	—	—	—	—	—	—	—	
—	—	—	—	—	—	—	—	—	
—	—	—	—	—	—	—	—	—	
—	—	—	—	—	—	—	—	—	
—	—	—	—	—	—	—	—	—	
38	25. 8. 34	5398	Jaags.....	—	—	—	—	—	
—	2. 8. 34	5408	S. 40899 Tz.....	—	—	—	—	—	
38	2. 8. 34	5408	S. 40899 Tz.....	—	—	—	—	—	
30	1. 9. 34	5408	S. 40633 Bekker...	4 days	** 4 days	106.0	Nil	—	
36	2. 8. 34	5413	S. 40899 Tz.....	6 days	** 4 days	107.0	V. Sl.	—	
30	1. 9. 34	5413	S. 40633 Bekker...	—	—	—	—	—	
36	2. 8. 34	5413	S. 40899 Tz.....	5 days	** 5 days	106.2	V. Sl.	—	
30	1. 9. 34	5413	S. 40633 Bekker...	5 days	** 3 days	106.0	Nil	—	
30	2. 8. 34	5408	S. 40899 Tz.....	—	—	—	—	—	
30	1. 9. 34	5408	S. 40633 Bekker...	1 day	*	105.0	—	—	
30	2. 8. 34	5408	S. 40899 Tz.....	3 days	** 4 days	106.6	Nil	—	
30	1. 9. 34	5408	S. 40633 Bekker...	—	—	—	—	—	
—	—	—	—	—	—	—	—	—	
—	—	—	—	—	—	—	—	—	
—	—	—	—	—	—	—	—	—	
—	—	—	—	—	—	—	—	—	
30	1. 9. 34	5405	S. 40633 Bekker...	4 days	3 days	—	Nil	—	
30	1. 9. 34	5405	S. 40633 Bekker...	—	—	—	—	—	
37	24. 9. 34	5291	S. 41050.132 Vacc.	3 days	** 8 days	105.4	Nil	—	
37	24. 9. 34	5291	S. 41050.132 Vacc.	3 days	** 7 days	106.0	Nil	—	
11	29. 8. 34	5470	S. 40899 Tz.....	2 days	** 8 days	107.0	Sl.	—	
11	29. 8. 34	5470	"	4 days	** 3 days	106.4	Sl.	—	
55	29. 8. 34	5420	"	5 days	** 3 days	106.0	Sl.	—	
55	29. 8. 34	5420	"	2 days	*** 10 days	108.4	Sympt.	—	
55	29. 8. 34	5420	"	3 days	** 5 days	107.0	Sympt.	—	
55	29. 8. 34	5420	"	4 days	** 6 days	106.8	Sympt.	—	
—	—	—	—	—	—	—	—	—	
—	—	—	—	—	—	—	—	—	
—	—	—	—	—	—	—	—	—	

APPENDIX II B

Previous History.										
D.O.B.	Source.	Date of Injection.	Experiment No.	B.T.		Incubation Period.	Reaction and Duration.	Highest Temperature.	B.T. Symptoms Recorded.	Inter B.T. a
				Virus.	Qual.					
40964	Karoo	5. 6.34	5390	B. 912	Vacc.	5 days	** 8 days	106.0	—	
40948	"	23.10.34 5. 6.34	5390 5390	S. 40633 B. 914	Bekker Vacc.	— 6 days	— ** 4 days	— 106.2	— Sl.	
41027	"	23.10.34 5. 6.34 23.10.34	5390 5390 5390	S. 40633 B. 911 S. 40633	Bekker Vacc. Bekker	12 days 5 days —	* ** 9 days —	105.0 106.0 —	— V. Sl. —	
41566	"	7.12.34	5291	S. 41543, 41556	Vacc.	5 days	*** 8 days	107.0	—	
37156	"	21. 4.35	4997	S. 34068	Vacc.	—	*	107.0	—	
41531	"	19.11.34	5390	S. 40980	Bekker	4 days	*** 13 days	107.6	Sl.	
41002	"	5. 6.34	5390	B. 911	Vacc.	7 days	** 5 days	106.2	V. Sl.	
41025	"	23.10.34 5. 6.34	5390 5390	S. 40633 B. 914	Bekker Vacc.	— 6 days	— *** 7 days	— 107.0	— —	
41814	"	23.10.34	5390	S. 40633	Bekker	—	—	—	—	
41814	"	21.11.34	5390	B. 968	Vacc.	6 days	** 4 days	106.2	V. Sl.	
41838	"	21.11.34	5390	B. 966	Vacc.	7 days	*** 4 days	107.4	Sl.	
41845	"	23.11.34	5390	B. 962	Vacc.	4 days	*** 7 days	107.0	Sl.	
41768	"	23.11.34	5390	B. 971	Vacc.	6 days	** 6 days	106.0	—	
41841	"	21.11.34	5390	B. 963	Vacc.	4 days	*** 6 days	106.6	Sl.	
38908	"	31. 1.34	5247	B. 894	Vacc.	6 days	** 3 days	106.0	Hyperaemia	
41811	"	21.11.34	5390	B. 955	Vacc.	6 days	** 3 days	106.4	Nil	
41801	"	24.12.34	5390	B. 983	Vacc.	6 days	** 4 days	106.0	Sl.	
40943	"	5. 6.34	5390	B. 927	Vacc.	7 days	** 4 days	106.0	—	
40955	"	5. 3.35 5. 6.34	5390 5390	S. 40980 B. 928	Bekker Vacc.	— 7 days	— ** 5 days	— 106.0	— —	
41872	"	5. 3.35	5390	S. 40980	Bekker	—	—	—	—	
41874	"	21.11.34	5390	B. 907	Vacc.	5 days	*** 10 days	106.6	Sl.	
41574	"	11.10.34	5390	B. 946	Vacc.	5 days	** 7 days	106.0	Sl.	
41833	"	29.10.34	5518 (<i>E. ovis</i>)	S. 40968	(<i>E. ovis</i>)	—	*	—	Compl.	
41833	"	21.11.34	5390	B. 966	Vacc.	5 days	** 5 days	107.0	<i>E. ovis</i>	
39642	"	23. 4.34	553	S. 39152	Vacc.	—	*	—	Sl.	
41724	"	24.12.34 29. 1.35	5390 5572 (<i>E. ovis</i>)	B. 982 S. 41839	Vacc. (<i>E. ovis</i>)	6 days —	** 4 days —	106.4 —	Sl. Compl.	
41836	"	24.12.34 29. 1.35	5390 5572 (<i>E. ovis</i>)	B. 984 S. 41839	Vacc. (<i>E. ovis</i>)	6 days —	** 6 days —	106.0 —	Nil. Compl.	
41875	"	21.11.34 29. 1.35	5390 5572 (<i>E. ovis</i>)	B. 959 S. 41839	Vacc. (<i>E. ovis</i>)	4 days —	*** 6 days —	106.4 —	<i>E. ovis</i> Sl.	
41879	"	21.11.34 29. 1.35	5390 5572 (<i>E. ovis</i>)	B. 964 S. 41839	Vacc. (<i>E. ovis</i>)	5 days 2 days	** 7 days —	106.0 —	Sl. <i>E. ovis</i>	
32720	Born O'Poort	14. 8.31	4479	S. 31930	Vacc.	—	—	—	—	
		28. 4.32	Splenectomise d.							
		29. 6.32	4664 (A.cent.)	C. 3725 (A.cent.)	—	—	—	—	—	
		17. 1.34	5255	Vs. Mal. ovinus	—	—	—	—	—	
			(Rickettsia)							
		23. 3.34	5332 (<i>E. ovis</i>)	S.38884,86.96.97 S.38910,11.33.43	—	—	—	—	<i>E. ovis</i>	
		1. 4.35	5638 (Theil)	C. 4676	—	—	—	—	<i>E. ovis</i>	

APPENDIX II B. (cont.)

TZANEEN EXPERIMENT 1935. (BLUE-TONGUE IMMUNE SHEEP).

Experiment with Tzaneen Virus.								
Interval between B.T. and Tzaneen (days.)	Date of Injection.	Experiment No.	Tzaneen Virus.	Incubation Period.	Reaction and Duration.	Highest Temperature.	B.T. Symptoms Recorded.	Result.
267	27. 2.35	5611	C. 5665	2 days	** 4 days	105.4	—	—
—	—	—	—	—	—	—	—	—
267	27. 2.35	5611	C. 5665	3 days	** 4 days	106.0	—	—
—	—	—	—	—	—	—	—	—
265	25. 2.35	5611	C. 6252	4 days	*** 4 days	105.4	—	—
—	—	—	—	—	—	—	—	—
80	25. 2.35	5611	C. 6252	2 days	*** 6 days	107.2	—	—
314	4. 3.35	5611	C. 5648	—	*	—	—	—
92	4. 3.35	5611	C. 5648	4 days	** 2 days	105.0	—	—
272	4. 3.35	5611	S. 41566	2 days	*** 5 days	106.8	—	—
—	—	—	—	—	—	—	—	—
272	4. 3.35	5611	S. 41566	—	—	—	—	—
—	—	—	—	—	—	—	—	—
111	12. 3.35	5611	C. 5742	—	—	—	—	—
111	12. 3.35	5611	C. 5742	—	—	—	—	—
109	12. 3.35	5611	C. 5742	—	—	—	—	—
109	12. 3.35	5611	S. 41002	2 days	** 5 days	106.8	Definite	—
111	12. 3.35	5611	S. 41002	1 day	*** 6 days (irreg.)	106.4	Sl.	—
—	—	—	—	—	—	—	—	—
407	14. 3.35	5611	C. 5726, 5665, 5648, 5629	1 day	** 4 days	106.0	Sl.	—
113	14. 3.35	5611	C. 5726, 5665, 5648, 5629	—	*	105.0	—	—
80	14. 3.35	5611	C. 5665, 5748 (Spl. & Gld.)	± 24 hrs.	*** 7 days	106.4	Sl. sympt.	K.O. 21/3/35
319	10. 4.35	5611	S. 42359	± 24 hrs.	*** 8 days	107.0	Nil	—
—	—	—	—	—	—	—	—	—
319	10. 4.35	5611	S. 42359	1 day	** 5 days	107.0	Nil	—
—	—	—	—	—	—	—	—	—
147	17. 4.35	5611	S. 42436	2 days	**	106.6	—	—
203	2. 5.35	5611	S. 42436 Brain	± 24 hrs.	** 3 days	106.2	—	Died 6/5/35
—	—	—	—	—	—	—	—	—
178	7. 5.35	5611	S. 42526 Brain	± 24 hrs.	** 3 days	106.2	—	Died 10/5/35
382	10. 5.35	5674	S. 41833 Brain	2 days	** 7 days	106.0	Compl. peritonitis	Died 17/6/35
—	—	—	—	—	—	—	—	—
184	27. 6.35	5700	S. 40943	—	—	—	—	—
—	—	—	—	—	—	—	—	—
184	27. 6.35	5700	S. 40943	—	—	—	—	—
—	—	—	—	—	—	—	—	—
218	27. 6.35	5700	S. 40943	2 days	*** 7 days	107.8	Nil	—
—	—	—	—	—	—	—	—	—
218	27. 6.35	5701	S. 40943	1 day	** 8 days	107.0	Symptoms	—
—	—	—	—	—	—	—	—	—
1441	26. 7.35	5638	S. 40943	5 days	*** 5 days	107.0	Symptoms	K.E. 6/8/35 Splenectomised.

APPENDIX II B (cont.)

Subsequent History.									
Interval between Tz. Inj. and Test. (days.)	Date of Injection.	Experi- ment No.	Virus.		Incuba- tion Period.	Reaction and Duration.	Highest Tempera- ture.	B.T. Symptoms Recorded.	Result.
			Orig.	Qual.					
3	30.12.35	5838	Bld. S. 45351	Novo	—	—	—	—	—
	3. 2.36	5866	S. 45007	Orig. cult.	—	—	—	—	—
—	30.12.35	5838	Bld. S. 45351	Novo	—	—	—	—	—
—	3. 2.36	5866	S. 45007	Orig. cult.	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—
Injected with Anthrax vac cinc.									
5th and 8th weeks Remissions.									
—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—
Died, sequel injection brain material.									
—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—
Died, sequel injection brain material.									
Died, sequel injection brain emulsion.									
29	26. 7.35	5700	S. 40943	Tz.	3 days	** 5 days	106.4	—	—
—	—	—	—	—	—	—	—	—	—
29	26. 7.35	5700	S. 40943	Tz.	2 days	*** 5 days	107.4	—	—
—	—	—	—	—	—	—	—	—	—
29	26. 7.35	5701	S. 40943	Tz.	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—
29	26. 7.35	5701	S. 40943	Tz.	—	—	—	—	—
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