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The Influence of Solar Radiation on the Course of Bluetongue.

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INTRODUCTION.

THE control of bluetongue in areas where this disease occurs enzootically has been practised in South Africa during the last 35 years with a vaccine prepared at Onderstepoort from a strain of bluetongue virus which is known to give mild reactions in the stable as well as under field conditions. More than 50 million doses of vaccine have been issued up to the present. The results of the immunization was usually satisfactory, but unfavourable reports were received periodically not only from stockowners who used this vaccine for the first time, but also from others who had applied this prophylactic measure for many years with good results. The complaints were that as many as 50 per cent. of their Merino and other European breeds of sheep showed alarming symptoms, and that the convalescent period lasted several weeks. Although the percentage mortality in these inoculated sheep was frequently much lower than in those which contracted the disease naturally, the financial losses due to the poor wool production as the result of the unthriftiness were sometimes considerable.

Intercurrent infections and nutritional disturbances are known to have a provocative influence on the course of bluetongue; but the severe reactions in the field after injection with bluetongue vaccine were also observed on farms where the control of verminosis, the supply of food and the application of hygienic measures, generally practised in this country, were beyond reproach. Biological tests were frequently carried out in Merino sheep at the laboratory with either the remainder of the vaccine or with blood collected from the sheep which had reacted severely in the field, but at no time were such severe reactions produced in the stable. When all these tests had been completed, one was still faced with the question why the vaccine had produced these extraordinary reactions on individual farms.

Since no satisfactory explanation could be obtained from the reports submitted on such occasions, one was compelled to look for other factors responsible for this puzzling aspect of the problem. On considering the environmental factors prevailing in a stable as compared with those in the field, it was obvious that the most striking difference between the two localities was the absence or presence of direct solar radiation. As the result of the investigations which are described in this article sunlight has now to be included as one of the most detrimental influences on the course of bluetongue. The authors, moreover, believe that the exposure to solar radiation may also have a detrimental influence on the course of other infectious diseases which stock contract in the field.

EXPERIMENTAL WORK.

. In order to prove whether solar radiation has a detrimental influence on the course of bluetongue, one is dependent on the weather conditions for a period of 3 weeks, i.e., the duration of incubation, reaction and convalescent periods. Since it is impossible to predict the weather for such a long period it was considered necessary to increase the sensitivity of sheep against sunlight in order to intensify the effect and consequently any specific reaction which the experimental animal might show. Methyleneblue was chosen for the purpose of rendering the sheep more sensitive against light, because it is known that adequate doses of this dye-stuff have otherwise no harmful effect. It was important, however, to use a dose which would not increase the sensitivity to such an extent that ill effects would be caused by irradiation only. A preliminary experiment was, therefore undertaken in order to ascertain a suitable dose of methyleneblue for this purpose.

1. Preliminary Experiment, February, 1942.

Three healthy sheep were shorn along the back over an area of about 1,200 sq. cm. Two of these sheep were injected with methyleneblue intravenously, one receiving 0.5 gm. and the other 0.25 gm., while the third sheep acted as a control. The sheep were exposed to solar radiation immediately after the administration of the drug and subsequently for three successive days in order to ascertain how long the photosensitivity would persist. The sheep which received 0.5 gm. methyleneblue was exposed for 36 minutes, during which period an amount of 54 gram calories impinged per square centimetre of a horizontal surface. The exposure resulted in a very marked sensitivity, which was already noticed after 4 minutes. After 11 minutes the animal was flinching severely, assumed a crouching position and became exceedingly restless. The animal was so distressed, that for humane reasons it was soon returned to the stable. The body temperature had risen from 103.0° F. to 105.4° F. The symptoms of photosensitization rapidly subsided in the stable and after 5 minutes a decided improvement was observed.

The second sheep, which received 0.25 gm. methyleneblue was exposed to an amount of 50 gram calories per square centimetre during a period of 34 minutes. The exposure resulted in marked sensitivity which was noticed after 7 minutes. The symptoms later became more pronounced, but never as marked as in the previously described animal. After being returned to the stable, the sheep showed a rapid improvement and after 5 minutes practically all the symptoms of photosensitization had subsided.

The control sheep was simultaneously exposed, without methyleneblue injection for a period of 36 minutes. No symptoms of photosensitization were observed.

Four hours later the temperatures of all three sheep were back to normal. During the following three days they were again exposed to sunlight, but apart from the rise in temperature of 1 to 2° F. and accelerated respiration no other symptoms were observed.

Conclusion.—Since the reaction to sunlight in the sheep which was injected with 0.25 gm. methyleneblue was moderate, it was decided to use this dose which would enhance the influence of the solar radiation without necessarily bringing about other complications in the subsequent experiments.

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When the correct dose of methyleneblue had been determined, four experiments were carried out on small groups of sheep during various seasons of the year. In order to gain a clear concept of the environmental factors which influenced the sheep during the periods of exposure, meteorological observations were taken, and the data collected by the South African Solar Radiation Survey were used for the determination of the amount of solar radiation influencing the experimental animals. The figures referring to the amount of solar radiation represent the total amount of sun and sky radiation impinging on one square centimetre of a horizontal surface during the period of exposure. The intensity is given in gram calories. The wind could not be registered, but had to be taken into consideration because of its cooling effect. The combined effect of wind, air temperature and radiation on animals exposed in the open can not yet be determined. There exists no adequate formula or instrument which summarizes the effects of these three factors satisfactorily and which allows recording over a period. With regard to human beings this problem has partly been solved by the construction of the various types of cooling balls, e.g., the frigorimeter by Thilenius and Dorno (1925) and the frigorigraph by Pfleiderer (1933) and Büttner (1938). Since the latter instrument is available at Onderstepoort, readings of the cooling ball temperature were used for comparing the prevailing conditions during the various periods of exposure.* It must be realized, however, that they are, strictly speaking, not applicable to sheep, but only represent relative values of the combined effect of wind, air temperature and radiation.

2. Four Experiments Demonstrating the Influence of Solar Radiation on the Course of Bluetongue during Various Seasons of the Year.

In the following four experiments, the "Bekker" strain of virus, which is known to produce fairly typical symptoms of bluetongue in sheep in the stable, was used.

An area over the back of the sheep was shorn as described in the previous experiment. The sheep in each of the experiments were divided into groups. Some were kept as controls in the stable, while the others were exposed to sunlight daily during the different periods of the course of the disease, i.e., during the incubation, the reaction or the incubation and reaction periods. Of the exposed sheep the majority were treated with methyleneblue on alternate days. The exposure followed immediately after the administration of the dye-stuff.

The details of the treatment of each animal with remarks on the amount of solar radiation to which they were exposed, as well as particulars concerning the clinical symptoms and the lesions observed at autopsy are given in Tables 1-4 in the appendix. In Table 1 in the text a summary of the results of these tests is given. They can shortly be summarized as follows:

Experiment 1. April, 1942.

The clinical symptoms of bluetongue were decidedly more pronounced in the photosensitized sheep exposed to solar radiation than is usually observed in sheep kept under stable conditions. The severest clinical symptoms were observed in the sheep exposed during the incubation and the reaction periods (Group 2). The general weakness during and after the

* A description of the principle on which the cooling ball temperature is based and details on measurements in South Africa, can be found in the publication by Riemerschmid, 1941.

reaction to bluetongue, which stockowners frequently reported in their immunized sheep was also noticed. This symptom was at the time ascribed to the coronitis, a condition which was observed in all the sick animals. The severity of the reactions, furthermore, was clearly indicated by the fact that two sheep (1 out of Group 2 and 1 out of Group 3) died. At autopsy typical lesions of bluetongue were noticed. Intramuscular haemorrhages in the pillars of the diaphragm were noticed in one sheep, a lesion which had not been observed previously in bluetongue.

Experiment 2, September, 1942.

The weather conditions did not permit a complete study because after 7 days of sunshine the sky became overcast and prohibited further exposure. Nevertheless it was again observed that the sheep which were treated with methyleneblue and subsequently exposed to solar radiation showed more severe clinical symptoms than those exposed but not treated with methyleneblue. The mildest reactions were observed in the control sheep in the stable. One of the sheep (Group 1), developed a bilateral bronchopneumonia and died. This symptom has often been noticed in the field in sheep which died after immunization with bluetongue vaccine. The direct cause responsible for the bronchopneumonia is not known, but the fact that this phenomenon occurred under experimental conditions is of interest.

Experiment 3. February, 1943.

The weather conditions during this experiment were very severe, as can be seen from the figures in Table I. Cloudiness prevented exposure after the 7th day. The clinical symptoms of bluetongue were definitely more pronounced and the condition of the sheep much worse than in either of the previous two experiments. The mildest reactions were seen in the control sheep kept in the stable, and the severest in the photosensitized- or nonphotosensitized sheep (Group 2 and 4) which had been exposed for seven Bronchopneumonia developed in three of the exposed sheep treated davs: with methyleneblue. Death due to this condition occurred already 60 hours after the first exposure. A marked weakness was observed in all the exposed sheep already on the second day of the reaction, before the coronitis was evident. Six sheep in this experiment died and only four recovered. Besides the bluetongue lesions, intramuscular haemorrhages were observed in several muscles of three of the exposed sheep. In one of these sheep which died from bronchopneumonia during the incubation period (108 hours after the artificial bluetongue infection) haemorrhages in several groups of muscles were noticed at autopsy. It is not quite clear whether the lesions in the muscles in this case were produced by the bluetongue virus, or whether this condition was brought about by solar radiation in the photosensitized sheep. Further tests will have to be carried out in order to clear up this point.

Experiment 4. June, 1943.

The weather conditions during the period of this experiment were distinctly different from those prevailing during the previous three experiments. The air temperatures, maximum as well as minimum, were markedly lower; the intensity of the radiation at noon was only two-thirds of what it had been in February, 1943. The clinical symptoms in the exposed sheep were much milder than in any of the exposed sheep in the previous experiments. There was no difference in the clinical symptoms between the photosensitized and the non-photosensitized exposed sheep. The control sheep in the stable also showed very mild symptoms of bluetongue. In one slaughtered sheep intramuscular haemorrhages were observed.

Meteorological Conditions during Experiments 1-4.

A summary of the various environmental factors influencing the sheep during the periods of exposure in the previous four experiments is given in Table 1. The figures can shortly be summarized as tollows:—

- (a) The mean maximum air temperature was comparatively similar during the first three experiments, but distinctly lower during the June test.
- (b) The mean minimum temperature increased from the April experiment (41°) to the September (49°) and February (57°) test. During the June test, it was, however, 24° lower than during the February experiment.
- (c) The cooling ball temperature was similar during the first two experiments (April and September) but very distinctly higher during the February test and just as distinctly lower during the June test.
- (d) The average amount of radiation impinging daily during the periods of exposure was similar during the first two experiments, but very distinctly higher during the February test. During the June experiment it was again lower.

Relationship between Meteorological Factors and the Clinical Symptoms.

Some very interesting information can be gained from the clinical observations made during each experiment in connection with the meteorological data (see Table 1).

Generally speaking one can say that the clinical symptoms observed in the April experiment were fairly severe and a little more pronounced than The environmental conditions those during the September experiment. during these two tests were more or less similar. In February by far the severest reactions were experienced, so that when two sheep died at the beginning of the experiment, the programme of exposure had frequently to be altered for fear of losing even more sheep before obtaining the desired information. The environmental factors during the period of this experiment differed markedly from those during the previous experiments in two essential points, namely solar radiation and cooling ball temperature. The amount of solar radiation was much greater and its intensity higher (1.5 gr. cals. at noon as compared with 1.25 in April and September, and 1.0 in The cooling ball temperature was extremely high during the June). February test. During the June experiment the clinical symptoms were fairly mild. The sheep were influenced by distinctly smaller intensities of solar radiation than during the previous three experiments. Air and cooling ball temperatures were very low during this test.

Besides the indicated relations between the amount of solar radiation and the severity of the reactions in bluetongue; the following points should be mentioned in connection with unfavourable reports from the field.

TABLE 1.

| | 1-141 | hote od. | | Ball. | 104° | | 104° | | N-44 | 112° | | | 080 | | - | vister . |
|---------------|------------|---------------------|-----------------------------|------------------------|-------------------------------|------------------------|---|--------------------------------------|--|--|----------------|-------------------------|-------------------------|-------------------------|----------------|------------------|
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| Nos. | A. | Values | Air T | Max. | 81° | | 86. 80 | | | 88° | | | 640 | 8 | | |
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| of E | Meteòf | tes for ital Per | emp. | Min. | 46° 41° 37° | 41° | 42° 49° | 56° | 55° | 61° 57° | 1 | 33° | 33° | 33° | 1 | |
| Data | | an Valu perimer | Air T | Mąx. | 86° 81° 76° | 81° | 88 88 9 | 85° | 86° | 86° | 1 | 66° | . 66° | 66° | 1 | |
| ical 1 | u Saile | MetEx | Radia- | Gram- cals/ Day. | $1,180 \\ 2,350 \\ 1,170$ | 2,350 | 1,030 1,330 1,330 Nil | $1,150 \\ 1,890$ | 2,400 | 3,200 | IIN | 4,300 | 4,600 | 4,600 | Nil | a ke was gave |
| olog | | lo sy | of Day | Exposure Number | 5 12 6 | 12 | 0000 | en 14 | 9 | .4 | 1 | 5 | 18 | 18 | 1 | * |
| nd the Meteor | | | Post Mortem Examination. | | | | Bronchopneumonia [*] | Bronchopneumonia Bronchopneumonia | Intramuscuaar haemorrhages Bronchopneumonia Intramuscular | haemorrhages * Intramuscular haemorrhages | (1 sheep)* | Intramuscular | | 1 | 1 | |
| ns a | | | | Mortality | 110 | 0 | -000 | | | 501 | 0 | Kil- | 0 | 0 | 0 | |
| ervatio | | toms. | Clinical | Observ. | Typical Severe. Typical | Nil | Typical Severe. Typical Mild | Died | Died | Typical Severe. Severe. | Typical | Mild | Mild | .1 | Mild | |
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| f th | | ·də | of She | Number | ~ ~ ~ ~ | 1 | ରାରାଭୁରା | 67 | 53 | 61 61 | 50 | 1 | T. | 1 | 1 | |
| Summary of | | | Feriod of Exposure. | | Incubation | Normal Control exposed | Incubation and reaction Incubation and reaction Incubation and reaction Control stable | Incubation | Incubation and reaction | Reaction | Control stable | Incubation and reaction | Incubation and reaction | Normal control, exposed | Control stable | |
|]] | | | | · · · | APRIL. | | SEPT. | | .YAA | FEBRU | | | ·9 | NOC | | 1 |

* 34

* Presence of typical bluetongue lesions at autopsy.

† Slight symptoms of photosensitization.

11 Marked symptoms of photosensitization.

Several cases of bronchopneumonia were noticed in the photosensitized sheep used in these experiments. An outstanding feature was the rapidity with which it developed in some cases. The presence of bronchopneumonia has frequently been observed at autopsy in the field in natural and artificial cases of bluetongue. The occurrence of this lesion under experimental conditions suggests that a similar predisposing cause may be responsible for the development of bronchopneumonia in the field. From the accounts of Steyn (1934) the occurrence of photosensitization in sheep is not unusual in many parts of South Africa during the months of December, January and February. The above information naturally prompts the consideration that instances may occur where the period of natural photosensitization coincides with the occurrence of bluetongue. Severer reactions, complicated with bronchopneumonia may result from such coincidence and be partly responsible for the higher mortality.

Intramuscular haemorrhages were observed in these experiments. The frequency with which this lesion occurred and the fact that it had also been observed in other infectious diseases of man and animal, made it desirable to ascertain its nature microscopically. These studies were carried out by our colleague, Dr. A. D. Thomas of this Institute, who was able to demonstrate various stages of muscular degeneration, not only in those muscles in which haemorrhages were noticed but also in several others in which no macroscopical lesions were present. This preliminary information was of interest and some importance, because it gave an explanation for symptoms such as the general weakness, muscular wasting, torticollis, etc. In the February test the weakness was already noticed several days before the coronitis was observed. This fact gives one a new conception as to the cause of the general weakness, peculiar gait and attitude of the sick sheep.

3: The Influence of Solar Radiation on 120 Sheep undergoing Bluetongue Vaccine Immunization.

Once the influence of solar radiation on the course of bluetongue had been established, an experiment on a large scale seemed necessary and justified. For this purpose 120 sheep, used for testing the vaccine, were employed. The object of the tests, carried out during September and October, 1943, was to investigate whether it was possible to produce at Onderstepoort under the influence of solar radiation as severe reactions as had been occasionally observed in the field. The "Theiler" strain of bluetongue virus, which is used for the preparation of the vaccine, was employed. This strain, is known to produce as a rule milder clinical symptoms than those caused by the "Bekker" strain.

Experiments (yet unpublished) carried out by Elder and Riemerschmid of the South African Solar Radiation Survey, had shown that the reactions of closely shorn sheep to their thermal environment differed materially from those of sheep with long wool. Since the severe reactions in the field had frequently been observed in shorn sheep, it seemed quite possible that the nature of the reactions to bluetongue may be different in shorn and unshorn sheep. It was consequently considered advisable to investigate this problem as well, in order to find out whether any importance should be attached to shearing with regard to immunization.

For the following two experiments sheep were used which were more or less of the same age (2-4 tooth), which were in good condition and had been fed on the same ration. Half the sheep were kept in the stable, whilst the

other half were freely exposed in a camp. The stable had a galvanized roof, brick walls and good ventilation; the camp did not offer any shade or shelter at all. In each of the two localities half the sheep were shorn, whilst the other half with 5-6 months' growth of wool were left unshorn. The following observations were carried out during the two experiments:—

- (1) The air temperature was recorded on thermographs, one being erected in the stable, another one in a Stevenson screen next to the camp. The intensity of the solar radiation and the cooling ball temperature was registered over the whole period of three weeks.
- (2) The rectal temperatures of the sheep were measured three times a day, at 7 a.m., 2 p.m. and 4 p.m. for a period of three weeks. The observations at 2 p.m. were included in order to get information on the body temperature at that time of the day when the solar radiation is nearly as intense as at midday and the air temperature is near its daily maximum.
- (3) The following clinical symptoms were recorded daily: hyperaemia of the buccal mucosa, oedema of the lips, superficial erosions on the upper lip and around the nostrils, nasal discharge and coronitis.
- (4) Out of each group of animals two were killed, one during, and one after the reaction period in order to ascertain to what extent the muscles and other organs were affected.

For each experiment 60 sheep were used. They received 1.0 c.c. vaccine ("Theiler" strain) subcutaneously and were divided into the following groups of 15 each:—

Group 1. Unshorn sheep, kept in the stable.

Group 2. Shorn sheep, kept in the stable.

Group 3. Unshorn sheep, kept in the camp.

Group 4. Shorn sheep, kept in the camp.

Experiment 5. September, 1943.

1. Weather Conditions.—During the period of this test the weather conditions were very favourable for the purpose of this experiment. During the first four days it was fairly cool and the sky was overcast; then the weather cleared up and remained cloudless for practically the whole period of the experiment. The wind during the hottest hours of the day was frequently fairly strong. The maximum air temperature was on an average 79° F. (when excluding the first 4 days with 55-67° F. maximum). The minimum temperature was on an average 46° F., and varied between 36° (3rd day) and 51° F. (10th day).

The cooling ball temperature represents the above-mentioned conditions very clearly: the average of the readings recorded between 9 a.m. and 3 p.m. every day was 95° F. after the first four days (with an average of 70° F.)

The amount of radiation impinging from sunrise to sunset was very small during the first three days, after which it remained mostly above 500 gram calories per square centimetre per day. The total during the period of 19 days was about 8,700 gram calories per square centimetre. 2. Body Temperatures (Experiment 5).—Mean body temperatures for each group of 15 sheep were calculated for 7 a.m. (Fig. 1), 2 p.m. (Fig. 2), and 4 p.m. The air temperatures measured in the stable and the camp are also represented on these graphs. It can be seen from Figs. 1 and 2 that the body temperatures of the sheep in the camp was usually lower at 7 a.m., and at 2 and 4 p.m. distinctly higher than those measured in the stable. The lower body temperatures in the camp at 7 a.m. may be ascribed to the distinctly lower (up to 10° F.) air temperature in the camp at that time of the day, when the intensity of solar radiation was as yet not high. In the afternoon, however, the air temperatures in the camp and the stable were similar and the distinctly higher body temperatures in the camp can consequently only be ascribed to the influence of solar radiation.



Fig. 1.—Body Temperatures of Bluetongue Sheep and Air Temperatures, at 7 a.m. (Expt. 5).





A comparison of the daily fluctuations of body temperatures as measured in the camp and in the stable is represented in Fig. 3 (unshorn sheep) and Fig. 4 (shorn sheep). The graphs represent morning and afternoon readings of body temperatures, air temperatures, wind force and amount of clouds as well as the amount of incident radiation during each day. The range of body temperature between morning and afternoon of each day is distinctly greater in the sheep in the camp than in those in the stable. This phenomenon is more pronounced in the shorn than in the unshorn sheep. It is interesting to compare the daily variation of body temperature with that of the air temperature as shown in Figs. 3 and 4.



Fig. 3.—Comparison of Body and Air Temperatures between Camp and Stable, Unshorn Sheep (Expt. 5).

In order to verify the conclusions drawn from the charts and also in order to distinguish between significant and non-significant differences, the body temperature observations were subjected to a statistical analysis. This included an analysis of variance and a determination of the significance of the differences between group mean values. The following are the results of this analysis:—

- (a) If all the sheep were taken together, regardless of whether they were shorn or not or whether their body temperatures were measured in the morning or in the afternoon, it was found that on 13 out of the 18 days of the experiment, the body temperatures were very significantly higher in the camp than in the stable.
- (b) The next step was to ascertain whether the body temperatures were, at all three observation times higher in the camp than in the stable. The statistical analysis showed the following:—

At 7 a.m.—The body temperatures in the camp were significantly *lower* on 10 days out of the 18, and equal on 7 days. The equal temperatures in camp and stable were observed during the beginning of the experiment only, i.e., during the incubation period and during the height of the febrile reaction, when the fever reaction overshadowed the fact that the air temperature was much lower in the camp than in the stable.

At 2 p.m.—On 15 of out 18 days the body temperatures measured in the camp were very significantly higher than those in the stable.

At 4 p.m.—The same as at 2 p.m. was observed on 13 out of the 18 days. The rest of the days showed equal temperatures in the camp and in the stable at both afternoon observation times.



Fig. 4.—Comparison of Body and Air Temperatures between Camp and Stable, Shorn Sheep (Expt. 5).

(c) A comparison between the shorn and the unshorn sheep showed the following statistical results:--

In the Stable.—Only occasionally was a significant difference between shorn and unshorn sheep observed. This refers to all the observation times.

In the Camp.—At 7 a.m. the shorn and unshorn sheep showed mostly equal body temperatures. At 2 p.m. a striking difference between the two groups was observed; on 10 out of the 18 days the shorn sheep showed a very significantly higher body temperature, on 5 days the temperatures in both groups were equal and only during the first three overcast days the shorn sheep showed lower body temperatures than the unshorn. At 4 p.m. when the radiation is already less intense, the body temperatures of the shorn sheep were mostly equal to that of the unshorn sheep.

The statistical analysis of the body temperatures in combination with the measurements of air temperature, wind velocity, cloudiness and solar radiation gives naturally much more valuable information which, however, does not lie within the scope of this article. In order to facilitate the study on the reaction of the shorn and unshorn sheep to their environment under various meteorological conditions, the statistical analysis is given in the form of a table (Table 2) which can easily be compared with the meteorological conditions demonstrated in Figs. 3 and 4.

3. Clinical Observations (Experiment 5).—The results of the clinical observations are demonstrated in Fig. 5, where the height of the columns indicates the number of sheep which showed the respective symptom, and the figures at the bottom give the number of days after the injection of the bluetongue vaccine.



Figures on left side of blocks allude to number of sheep. Figures below blocks indicate day after infection. A indicates that no observations were made.

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The mildest clinical symptoms were observed in the unshorn sheep in the stable. The shorn sheep in the stable showed slightly more severe reactions, a fact for which no satisfactory explanation could be given. The clinical symptoms in both the exposed groups were more severe than those observed in the stable, but were most pronounced in the shorn group.

The clinical observations were made between 11 and 12 o'clock every day. By observing the animals at other times of the day it was noticed that in the stable there was no obvious change in the intensity of the clinical symptoms during the course of a day. In the camp, however, the clinical symptoms, particularly in the shorn sheep, were distinctly more pronounced at 11 a.m. and 2 p.m. than in the morning.

The respirations of the sheep in the stable did not vary much from the normal during the whole period of the observations. In the exposed sheep, however, an acceleration was noticed during the greater part of the day and was particularly marked during the hottest hours. This acceleration was most pronounced during the reaction period, when the slightest exertion, as for instance driving the sheep slowly into a paddock in the camp, resulted in many of the sheep opening their mouths and gasping.

Signs of general weakness and stiff gait in the sheep were distinctly more pronounced and frequent in the camp than in the stable.

4. Post-mortem Examinations (Experiment 5).—The observations made at autopsy are given in Table 5 in the Appendix. From this table it will be seen that the usual bluetongue lesions were present in the sheep killed during the early stages of the reaction (5th day), whereas these lesions were partially or completely absent in the animals killed during the later stages of the disease (12th day of reaction). The bluetongue lesions were more pronounced in the sheep of the exposed group than in those of the stabled group.

Oestrus ovis larvae were found in only a very small number of animals.

Intramuscular haemorrhages were observed in one or both of the sheep of each group.

Experiment 6. October, 1943.

This experiment was conducted in exactly the same way as the previous one with the only difference that there were 6 normal sheep included in each group for the purpose of obtaining comparative measurements of body temperature in both sick and healthy animals. The observations of body temperatures and clinical symptoms were taken an hour earlier than in the previous experiment on account of the fact that South African Summer Time had been introduced in the meantime. For technical reasons the time of taking the observations could not be altered. In order to indicate the difference of the observation times from the previous experiments, the South African Standard Time will be used as before.

1. Weather Conditions (Experiment 6).—The weather conditions during this experiment were, generally speaking, again very favourable for the purpose of the experiment, because the sky was mostly cloudless with only short intervals of cloudy weather. The total amount of radiation impinging from sunrise to sunset over a period of 21 days was 11,520 gram calories per square centimetre, i.e., an average of about 550 gr. cals. per day.

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The maximum air temperature was on an average 82° F., ranging from 69° to 92° F. The minimum air temperature was on an average 52° F. with a range from 40° to 63° F.

The wind was on an average slightly stronger than during the previous experiment.

The cooling ball temperature during the hours from 9 a.m. to 3 p.m. was on an average the same as during the previous test, namely 95° F.

2. Body Temperatures (Experiment 6).—The group mean values of body temperatures for 6 a.m. and 1 p.m. are represented in Figs. 6 and 7.



Fig 6.—Body Temperatures of Bluetongue Sheep and Air Temperatures, at 6 a.m. (Expt. 6).



Fig. 7.-Body Temperatures of Bluetongue Sheep and Air Temperatures, at 1 p.m. (Expt. 6).

At 6 a.m. (sunrise 5.50 a.m.) the body temperatures during the first three days were lowest in both groups of shorn sheep. During the height of the febrile reaction, all four groups of sheep showed very similar body temperatures at that time of the day, notwithstanding a comparatively big difference (up to 12° F.) of the air temperature in the camp and in the stable.

At 1 p.m. the two groups of sheep in the camp showed distinctly higher temperatures during the whole period of the experiment (Fig. 7). It can again be seen that this was not due to a difference in air temperature which was similar in both places, but that it must be ascribed to the influence of solar radiation. This was also proved by the fact that on cloudy days the temperatures of the sheep in the camp approached those of the sheep in the stable, whilst on cloudless days the divergence of the temperatures was more distinct. Another interesting feature of Fig. 7 is that in the camp and in the stable the shorn sheep showed higher temperatures than the unshorn during the height of the febrile reaction.

At 3 p.m. the body temperatures of both groups in the camp were always higher than those in the stable.

The daily fluctuation of body temperatures were, as in the previous experiment, much larger in the exposed than in the stabled sheep (see Figs. 8 and 9). This fluctuation was distinctly more pronounced in the shorn than in the unshorn sheep.



Fig. 8.—Comparison of Body and Air Temperatures between Camp and Stable, unshorn sheep. (Expt. 6).

Comparison of the Body Temperatures in Normal Sheep and in Sheep Reacting to Bluetongue.

A comparison of the body temperature of the sick and the healthy sheep in each group gives particularly interesting information.

(a) In the Stable.

At 6 a.m. the body temperatures in the normal sheep varied very little from those infected with bluetongue. This refers to both the unshorn and the shorn groups.

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At 1 p.m. the bluetongue sheep showed higher temperatures than the healthy sheep during the first eleven days after the infection. (Fig. 10, unshorn, and Fig. 11, shorn.) This difference was very pronounced in the shorn sheep, for which no satisfactory explanation can be given.

At 3 p.m. the difference between normal and bluetongue sheep (shorn and unshorn) was very small, the latter showing, only during the febrile reaction, slightly higher temperatures than the normal sheep.



Fig. 9.—Comparison of Body and Air Temperatures between Camp and Stable, shorn sheep. (Expt. 6).







Fig. 11.—Comparison of Body Temperatures between Healthy Sheep and those reacting to Bluetongue, in the Stable. Shorn sheep at 1 p.m. (Expt. 6).

(b) In the camp.

At 6 a.m. the body temperatures in the normal sheep varied very little from those in the bluetongue sheep.

At 1 p.m., however, a distinct difference was found. A remarkable fact is, that this difference could be noticed right from the first day after the artificial infection, long before any sign of a bluetongue reaction had developed. The difference was more pronounced in the shorn (Fig. 13) than in the unshorn (Fig. 12) sheep.

At 3 p.m. the shorn bluetongue sheep showed during the first 14 days a higher body temperature than the normal ones; in the unshorn sheep this was the case during the height of the febrile reaction only.





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3. Clinical Observations. (Experiment 6.)—The observations on the clinical symptoms are illustrated in Fig. 14. The variation in the different groups was similar to that observed in the previous experiment. In the exposed sheep, particularly in the shorn group, the symptoms were even more pronounced than they had been in the same groups during the previous test.

The rate of respiration remained normal in the stabled sheep, but in the camp it showed a similar increase as in the previous experiment. In the two groups of normal sheep in the camp the frequency of respiration was not quite as high as in the immunized animals.



Fig. 13.—Comparison of Body Temperatures between Healthy Sheep and those reacting to Bluetongue, in the Camp. Shorn sheep at 1 p.m. (Expt. 6).

4. Post-mortem Examinations. (Experiment 6.)—The details of the post-mortem examinations are given in Table 6 in the Appendix. The nature of the bluetongue lesions in the various groups were similar to those observed in the previous experiment.

Oestrus ovis larvae were again observed in only a very small number of sheep.

Intramuscular haemorrhages were present in one or two sheep of each group.

In the normal sheep which were killed at the end of the experiment, Oestrus ovis larvae could be demonstrated in two out of the four sheep. Otherwise no pathological changes were present.

DISCUSSION.

The tests on 120 sheep undergoing the bluetongue vaccine immunization process showed that the body temperature reactions as well as the occurrence and severity of the clinical symptoms were much more pronounced in the exposed sheep than in those kept in a stable.

On considering the difference in the environmental factors which influence the sheep in the stable and in the camp, not only solar radiation but also air temperature, wind and rain had to be taken into account. The influence of rain could be excluded in the present experiments, because fine weather prevailed and only once during each test 0.45 inch of rain was recorded.

The air temperature during day-time showed very little difference in the two localities; after sunset, however, the temperature in the camp dropped below that of the stable and reached the greatest difference (up to 14° F.) in the early hours of the morning. Whether this difference in air temperature during the night only had an influence on the course of the disease can not be decided until an adequate experiment has been conducted. During daytime, however, when the air temperature in the stable and in the camp was practically equal, the clinical symptoms in the sheep remained constant in the stabled group, whilst the severity in the camp increased towards midday and during the afternoon, when the solar radiation was very intense and the exposure had already lasted for several hours.



Our knowledge of the influence of wind on sick animals is, unfortunately, very limited. During the two experiments it was observed that the increase in the severity of the clinical symptoms during the course of the day also took place on calm days, when the influence of wind could be excluded.

This observation and the fact that the air temperature was practically equal during day-time in the two localities prove that the deterioration of the condition of the sheep during the course of the day could only be ascribed to the influence of solar radiation. It seems consequently fully justified to accept that not only the deterioration during the course of the day but also the distinctly severer reactions in the exposed sheep during the whole period of the disease is predominantly caused by the influence of solar radiation. A proof which further supports this view is the fact that on overcast days the clinical symptoms, body temperatures and the behaviour of the sheep in the camp and in the stable showed less difference than on clear days.

Whether the influence of solar radiation on the course of bluetongue reactions is mainly a heating effect or whether it is due to the actinic rays can only be proved by experiments, which are under progress at Onderstepoort at present. It is suggested, however, that the heating effect of the sun's rays plays a predominant rôle. This opinion is based on the fact that unshorn sheep, which have the greater part of their bodies protected against the direct influence of actinic rays, also showed distinctly severer reactions in the sun than in the stable. The lesions on the upper lips and nose, on the other hand, may be aggravated by the direct influence of actinic rays.

The above discussion enables one to express an opinion about the adverse results observed in immunized sheep in the field. Although the environmental conditions in our experiments can, strictly speaking, not be compared with those prevailing in the field, the relative influences of the various meteorological factors might be similar. An effect of solar radiation on the course of bluetongue in the field can consequently not be excluded. Extreme air temperatures, high or low, combined with strong wind or calm air and also rain may certainly add in one way or another, to the effect of the solar radiation, but the effect of the direct irradiation on the relative importance of the meteorological factors is gained, stress must be laid on the exposure of sheep to solar radiation during bluetongue reactions. The provision of shade for such animals should, therefore, wherever possible, be promoted in order to exclude the harmful influence of solar radiation on the course of bluetongue.

SUMMARY.

1. Experiments which clearly indicate the detrimental influence of solar radiation on the course of bluetongue are described.

2. Preliminary experiments were carried out during different seasons of the year in a limited number of sheep. Some of them were photosensitized against light with methyleneblue. During the course of the disease the sheep were exposed to solar radiation daily for several hours.

3. The result was, that the exposed sheep showed distinctly severer reactions than those kept in the stable. The strongest and most fatal reactions (6 out of 10 sheep died) were observed during a test conducted in February, when the solar radiation was very intense. Exposure during winter (June) had practically no influence on the course of the disease.

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4. In order to verify these observations, two experiments on a large scale (60 sheep each) were undertaken on sheep subjected to the immunization process. Half the sheep were kept in the stable and the other half freely exposed to solar radiation. In each locality half of the sheep were shorn, the others remained unshorn.

5. The body temperatures and the nature of the clinical symptoms showed that the reactions were much more pronounced in the camp than in the stable. The symptoms of bluetongue were most pronounced in the shorn animals in the sun.

6. Since a distinct influence on the severity of bluetongue reactions was noticed in sheep photosensitized with methyleneblue, it is believed that sheep in the field may react very severely to this disease if they become photosensitized naturally.

7. Bronchopneumonia in sheep photosensitized with methyleneblue occurred on several occasions and developed sometimes very rapidly. This observation suggests that natural photosensitization in the field may partly be responsible for the bronchopneumonia which is frequently observed in sheep in the field reacting to bluetongue.

8. During the course of the above investigations, multiple haemorrhages and muscular degeneration, a lesion which had not been previously associated with bluetongue, were observed in the stabled as well as in the exposed sheep. This observation gives an explanation to the general weakness, torticollis and stiffness of the sheep reacting to bluetongue.

9. The unthriftiness frequently observed in sheep after immunization or natural infection can also be attributed to the muscular degeneration.

10. Since the influence of solar radiation on the course of bluetongue has definitely been established, particular attention should be paid to the time of the year when the immunization is carried out. On account of the fact that the severest reactions were observed in shorn sheep, immunization should preferably be undertaken on unshorn sheep.

11. The provision of shade for sheep which have been vaccinated or which contracted the disease naturally should be promoted wherever possible in order to exclude the harmful influence of solar radiation on the course of bluetongue.

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TABLES 1 TO 4.-

| | | | 8 | | | |
|--------|---------------------------|------------------|-----------------|------------------|----------------------------|----------------------|
| Group. | Period of Exposure. | No. of Sheep. | No. of Days. | Hours, Daily. | Calories per sq. cm. | Meth. Bl. Inject. |
| 1 | Incubation | 61,493 | 6 | ± 3 | 1,175 | 3 |
| | Control in a presentation | 62,062 | 6 | ± 3 | 1,175 | 3 |
| 2 | Incubation and reaction | 62,222 | 11 | ± 3 | 2,140 | 6 |
| | | 62,080 | 12 | ± 3 | 2,350 | 7 |
| 3 | Reaction | 60,587 | 6 | ± 3 | 1,160 | 3 |
| | and the second second | 62,233 | 6 | ± 3 | 1,160 | 3 |
| 4 | Normal control exposed | - | 12 | ± 3 | 2,350 | Nil |

TABLE 1.-Experiment 1 (No. S. 7327),

TABLE 2.- Experiment 2 (No. S. 7389), Sept.

| | | | | - | - | | |
|--------|-------------------------|------------------|-----------------|------------------|----------------------------|----------------------|------|
| Group. | Period of Exposure. | No. of Sheep. | No. of Days. | Hours, Daily. | Calories per sq. cm. | Meth, Bl. Inject. | |
| 1 | Incubation | 62,311 | 6 | 2-3 | 1,000 | 3 | |
| - | | 62,053 | 6 | 2-3 | 1,000 | 3 | - |
| 2 | Incubation and reaction | 64,213 | 8 | 2-3 | 1,330 | 4 | Î |
| | | 60,978 | 1 8 | 2-3 | 1,330 | 4 | - |
| 3 | Incubation | 62,330 | 8 | 2-3 | 1,330 | Nil | - |
| | Incubation and reaction | 62,173 | 8 | 2-3 | 1,330 | Nil | To . |
| 4 | Control not exposed | 62,148 | 0 | 0 | 0 | Nil | - |
| 4.1 | | 60,430 | 0 | 0 | 0 | Nil | - |

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APPENDIX.

TABLES 1 TO 4.—The Treatment, Exposure, Clinical Symptoms and Post-mortem Examinations of 1 (No. S. 7327), April, 1942 (22.4.42 to 3.5.42). Injection of 2.0 c.c. subcutaneously "Bekker"

| Results. | | DURATION OF- | | CLINICAL SYMPTOMS. | | | | | | |
|----------|-----------|--------------|-----------|--------------------|------------|------------|---------|-------------|--|--|
| Respi- | Flinching | Incu- | Discourse | Hyperaem. | Lij | ps. | Nasal | Commitia | | |
| ration. | r menng. | bation. | Disease. | Mue. | Swelling. | Ulcers. | charge. | COTOILITIS. | | |
| Accel | †† | 6 | 12 | †† | †† | 0 | ttt · | †† | | |
| Accel | † | 6 | 9 | †† | <u>††</u> | †† | †† | †† | | |
| Accel | †† | 6 | 6 | †† | ††† | ††† | . *** | ttt | | |
| Accel | † | 5 | 9 | +++ | ttt (| <u>†††</u> | ††† | †††* | | |
| Accel | † / | . 6 | 14 | . ††† | ttt * | †† | - †† | †† | | |
| Accel | † | 5 | 23 | †† | ††† | 0 | † | +++ | | |
| Accel | - | anu-T | m | | | - | - | - | | |

o. S. 7389), September, 1942 (5.9.42.to 21.9.42). Injection of 2.0 c.c. subcutaneously "Bekker"

| Rest | ULTS. | DURATI | ON OF | CLINICAL SYMPTOMS. | | | | | | |
|---------|-----------|---------|----------|--------------------|------------|---------|---------|------------|--|--|
| Respi- | THE L | Incu- | | Hyperaem. | Lips. | | Nasal | | | |
| ration. | rinching. | bation. | Disease. | Muc. | Swelling. | Ulcers. | charge. | Coronitis. | | |
| Accel | † | 5 | 1 | † | 0 | 0 | + | 0 | | |
| Accel | † | 5 | 7 | †† | ††† | +† | †† | †† | | |
| Accel | † | 5 | 7 | | <u>†††</u> | t† | + | ttt | | |
| Accel | † | 6 | 7 | * ** | <u>††</u> | ††† | †† . | †† | | |
| Áccel | 0 | 6 | 6 | 11 | +tt | †† | + | .† | | |
| Accel | 0 | 5 | 7 | †† | † | 0 | †† | 0 | | |
| - | - | 6 | 5 | †† | 0 | 0 | † ' | 0 | | |
| - | - | 6 | 5 | †† | 0 | - \ 0 | † | 0 | | |

 $\dagger =$ Symptom mild. \dagger

 $\dagger \dagger =$ Symptom marked.

 $\dagger\dagger\dagger$ = Symptom severe.

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11.

em Examinations of Experiments 1 to 4.

ecously "Bekker" virus of sheep 60523 on the 22nd April, 1942.

÷.

| General Remarks. | Post Mortem Examination. |
|--|--|
| Inappetence, sheep recovered | |
| Inappetence, sheep recovered | |
| Inappetence, general weakness; killed in extremis on 6th day of reaction | Ulcers on the lips; subendocardial haemorrhages; oedema of the subcutaneous tissue under the shorm area of the back and intracutaneous haemorrhages in this area; tumor splenis; light degree of oesophagostomiasis. |
| Inappetence, sheep recovered | |
| Inappetence, marked general weakness and emaciation. Died on 14th day of reaction | Hydropericard; hydrothorax; subepi- and subendocardial haemorrhages; hyperaemia and oedema of the lungs; fatty degeneration of the liver; acute catarrhal abomasitis and duodenitis; oedema of the subcutaneous tissues and intracutaneous haemorrhages under the shorn area. Intramuscular haemorrhages in the pillars of the diaphragm. |
| | |
| Slight rise in temperature during time of exposure | |

neously "Bekker" virus of sheep 62309 on the 5th September, 1942.

| General Remarks. | Post Mortem Examination. | | | | | | |
|--|---|--|--|--|--|--|--|
| | And | | | | | | |
| Inappetence, general weakness; died 1st day of reaction | Subendocardial haemorrhages; hyperaemia; severe oedema of the lungs; bilatera, bronchopneumonia; organs discoloured by Methyleneblue. | | | | | | |
| Inappetence, sheep recovered | | | | | | | |
| Inappetence, sheep recovered | | | | | | | |
| Inappetence, sheep recovered | | | | | | | |
| Inappetence, sheep recovered | | | | | | | |
| Inappetence, sheep recovered | | | | | | | |
| Slight inappetence, sheep recovered | | | | | | | |
| Slight inappetence, sheep recovered | | | | | | | |

= Symptom severe.

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TABLE 3.-Experiment 3 (No. S. 7648), Febru

| | N. M. State State | 1 4 | Contraction of the | - COM A | | | |
|--------|------------------------------|------------------|--------------------|------------------|----------------------------|----------------------|---|
| Group. | Period of Exposure. | No. of Sheep. | No. of Days. | Hours, Daily. | Calories per sq. cm. | Meth. Bl. Inject. | - |
| 1 | Incubation | 66,208 | 3 | 5 | 1,150 | 2 | |
| | | 66,241 | 5 | 5 | 1,890 | 3, | |
| 2 | Incubation and reaction | 66,204 | 6 | 5 | 2,400 | 3 . | |
| | | 66,191 | 6 | 5 | 2,400 | 3 | - |
| 3 | Reaction | 66,205 | 4 | 4 | 824 | 2 | - |
| | and the second second second | 66,243 | 4 | 4 | 824 | 2 | 1 |
| 4 | Incubation and reaction | 66,283 | 10 | 4 | 3,220 | Nil | - |
| | and the | 66,226 | 10 | 4 | 3,220 | Nil | - |
| 5 | Control not exposed | 66,217 | 0 . | 0 | · 0 | Nil | - |
| | | 66,284 | 0 | 0 | 0 | Nil | - |

TABLE 4.-Experiment 4 (No. S. 7526)

| 1 | Incubation and reaction | 66,256 | 17 | 5 | 4,300 | Nil |
|---|-------------------------|--------|----|---|-------|-----|
| 2 | Incubation and reaction | 66,500 | 18 | 5 | 4,600 | 9 |
| 3 | Normal control exposed | 66,485 | 18 | 5 | 4,600 | Nil |
| 4 | Control stable | 66,482 | 0 | 0 | 0 | 0 |

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. S. 7648), February, 1943 (18.2.43 to 8.3.43). Injection of 2.0 c.c. subcutaneously "Bekke

| RES | ULTS. | DURATI | ON OF- | CLINICAL SYMPTOMS. | | | | | | |
|---------|-----------|---------|----------|--------------------|-----------|---------|------------|------------|--|--|
| Respi- | Flinching | Incu- | Disease | Hyperaem. | Li | ps. | Nasal | Coronitia | | |
| ration. | , | bation. | Disease. | Mue. | Swelling. | Ulcers. | charge. | Coronitis. | | |
| Accel | † | 1 | | 0 | 0 | 0 | · † | 0 | | |
| Accel | †† | - | - | 0 | 0 | 0 | 0 | 0 | | |
| | | 1271 | | 1. A. | | 1 | | 1 | | |
| Accel | †† | 6 | 3 | † | . † | 0 | 0 | 0 | | |
| | | alies. | | 1 | | | The second | | | |
| Accel | + | 4 | 12 | ++ | ††† | 0 | † | †† | | |
| Accel | † | 6 | 14 | †† | † | 0 | ++ | <u>†††</u> | | |
| Accel | † | 5 | 14 | †† | † | 0 | †† | ttt · | | |
| Accel | - | 5 | 11 | †† | ttt | †† | †† | ttt | | |
| Accel | | 5 | 7 | †† | <u>††</u> | 0 | t† • | 111 | | |
| | | | No. | | - max | 1. S. | , see | | | |
| Normal | | 6 | 10 | †† | + | 0 | † | * †† | | |
| Normal | 1 | 6 | 10 | /† | + | 0 | † | †† | | |

| t 4 (No. S. 7526), June, 1943 (25.5. t | to 14.6.43). Injection o | $f 2 \cdot 0 c.c.$ | subcutaneously | " Bekker |
|--|--------------------------|--------------------|----------------|----------|
|--|--------------------------|--------------------|----------------|----------|

| Accel. slightly | 0 | 6 | .12 | † | + | 0 | 0 | 0 |
|--------------------|---|---|-----|-----|----|---|---|---|
| Accel | t | 6 | . 8 | + | † | 0 | 0 | 0 |
| Accel. | | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 6 | 6 | + / | /† | 0 | 0 | 0 |

53-54a

53-54c

 $\dagger\dagger\dagger$ = Symptom severe.

† = Symptom mild. †† = Symptom marked.

neously "Bekker" virus of sheep 66206 on the 12th February, 1943.

| | and the second |
|---|---|
| General Remarks. | Post Mortem Examination. |
| Bilateral muco-catarrhal discharge from nostrils on 2nd day. Inappetence ; died 60 hours after exposure | Hyperaemia and oedema of the lungs; bilateral bronchopneumonia; catarrhal abomasitis; pigmentation of the perijugular tissues and caecal mucous membranes with Methylene-blue. |
| Inappetence; sheep died 108 hours after the first exposure | Cyanosis; severe hyperaemia and oedema of the lungs; acute bilateral bronchopneu- monia; hydropericard; very few epicardial haemorrhages; hyperaemia of liver, kidneys, small and large intestine; superficial erosions on the tongue; hyperaemia of the subcutaneous tissues, particularly under the shorn area; multiple localized haemorrhages of the trapezius and cutaneous muscles. |
| Inappetence; coughing from the 6th day after exposure; marked general weak- ness; died 8th day after first exposure | Hyperaemia and oedema of the lungs; bilateral bronchopneumonia; hyperaemia of the mucosa of the trachea; multiple localized haemorrhages of the mucous membrane of the abomasum; hyperaemia of the small and large intestine; subcutaneous haemorrhages and oedema under the shorn area; multiple localized haemorrhages of the longissimus dorsi muscles. |
| Inappetence; general weakness; sheep killed on 15th day of reaction | Emaciation; slight anaemia; coronitis; acute haemorrhagic enteritis; pigmentation of the perijugular tissues with methyleneblue; enlargement of the lymphatic glands; petechiae on the mucous membrane of the gall bladder. |
| Inappetence ; sheep recovered | - |
| Inappetence; sheep recovered | |
| Inappetence, general weakness, killed 11th day of reaction | Emaciation; slight anaemia; slight hyperaemia and oedema of the lungs; slight hydropericard; hyperaemia of the liver; slight tumor splenis; hyperaemia of the intestinal tract and of the subcutaneous tissues under the shorn area; coronitis. |
| Inappetence, general weakness, died 7th day of the reaction | Hyperaemia and oedema of the lungs, trachea and kidneys; subendocardial haemorrhages; hyperaemia of the alimentary tract and nasal mucosa; subendacardial haemorrhages under the shorn area; haemorrhages below the peritoneum; intramuscular haemorr- hages; coronitis. |
| Inappetence ; sheep recovered | |
| Inappetence; sheep recovered | |

eously "Bekker" virus of sheep 66318 on the 25th May, 1943.

| Slight inappetence. Killed 12th day of the reaction | Multiple intramuscular haemorrhages in the cutaneous, brachio-cephalic and trapezius muscles; jejunitis, slight degree of haemonchosis and oesophagostomiasis; slight anaemia and icterus. |
|--|--|
| Slight inappetence | |
| Mild intermittent febrile reaction 15th to 18th day after first exposure. Killed on the 18th day | No abnormality apart from a very few sub-endocardial petechiae. |
| Slight inappetence, | |

= Symptom severe.

TABLE 5.

Post-mortem Examination (Experiment 5.)

| Killed on Day of Reaction. | Post Mortem Examination. |
|----------------------------------|--|
| 5th day 12th day | Hyperaemia of the buccal mucosa; moderately swollen lips; superficial erosions on nostrum; slight oedema of the lungs and tumor splenis. (Sheep No. 68901). Slight hydropericard; tumor splenis; intramuscular haemorrhages in the longissimus dorsi and lattissimus dorsi. (Sheep No. 68876). |
| 5th day 12th day | Hyperaemia of the buccal mucosa; swollen lips; superficial erosions on nostrum; tumor splenis; multiple localized haemorrhages in the longissimus dorsi. (Sheep No. 68921). Slight hydropericard; subendocardial haemorrhages; intra- muscular haemorrhages in the pillars of the diaphragm. (Sheep No. 68809). |
| 5th day 12th day | Hyperaemia of the buccal mucosa; swollen lips; superficial erosions on the nostrum. (Sheep No. 68951). Multiple localized haemorrhages of the subcutaneous tissues of the back and in the pectoral muscles. (Sheep No. 68801). |
| 5th day 12th day | Oedema of the subcutaneous tissues of the jowel region; markedly swollen lips; catarrhal rhinitis; superficial erosions on the nostrum; coronitis; a light degree of haemonchosis and bunostomiasis; Oestrus ovis larvae; intramuscular haemorrhages of the trapezius muscle. (Sheep No. 68817). Oedema of the subcutaneous tissues of the jowel region; swollen lips; slight hydrothorax; hydropericard; ascite\$; Oestrus ovis larvae. (Sheep No. 68935). |
| | Killed on Day of Reaction. 5th day 12th day 12th day 12th day 5th day 12th day 5th day |

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TABLE 6.

Post-mortem Examination (Experiment 6.)

| Group. | Killed on Day of Reaction. | Post Mortem Examination. |
|-------------------------------|----------------------------------|--|
| 1. 2 unshorn sheep, Stable | 7th day 14th day | Hyperaemia of the buccal mucosa; swollen lips; slight hydropericard; subendocardial haemorrhages: light degree of haemonchosis. (Sheep No. 68807). Slight hydropericard; slight ascites; multiple localized haemorrhages in the gluteal muscles; light degree of haemonchosis. (Sheep No. 68836). |
| 2. 2 shorn sheep, Stable | 7th day 14th day | Hyperaemia of the buccal mucosa; swollen lips; multiple localized haemorrhages in the extensor muscles of the left forelimb, longissimus dorsi, trapezius, serratus thoracalis; mild degree of haemonchosis. (Sheep No. 68893). Mild degree of haemonchosis. (Sheep No. 69005). |
| 3. 2 unshorn sheep, Camp. | 7th day 14th day | Hyperaemia of the buccal mucosa; swollen lips; sub- endocardial haemorrhages; oedema of the lungs; intra- muscular haemorrhages of the longissimus dorsi and cuta- neous muscles; mild degree of haemonchosis. (Sheep No. 68850). Slight hydrothorax; slight ascites; mild degree of haemon- chosis. (Sheep No. 68888). |
| 4. 2 shorn sheep, Camp | 7th day 14th day | Hyperaemia of the buccal mucosa; swollen lips; multiple localized haemorrhages in the serratus thoracalis, longis- simus dorsi and cutaneous muscles. (Sheep No. 69028). Subendocardial haemorrhages; hyperaemia of the lungs; intramuscular haemorrhages in the biceps femoris; mild degree of haemonchosis. (Sheep No. 69042). |