the fibrosa stripped easily. The left kidney was light brown in colour, the right one was larger and darker and rich in blood; the consistence was firm. The rumen contained normal ingesta. The reticulum contained soft ingesta. Small pieces of bone and grains of sand were present. The omasum contained fairly moist ingesta, whilst the abomasum contained liquid and semi-liquid ingesta. The mucosa of the fundus was brownish-discoloured. The duodenum contained a considerable quantity of viscous mucus. The mucosa of the small intestines was brownish-discoloured; faint red patches and cross stripes were present. The mucosa of the caecum and of the first portion of the colon was also brownish-discoloured; that of the rectum was somewhat reddened. The faeces were soft. The mesentery was rich in fat; some of the mesenteric lymph nodes contained gas. A small quantity of normal urine was present; the mucosa showed no changes. The parenchyma of the superficial lymph nodes was conspicuously reddened. The brain showed no changes.

Pathological Anatomical Diagnosis: Gastro-enteritis catarrhalis. Foreign bodies in the reticulum. Pleuritis fibrosa adhaesiva. Oedema pulmonum. Bronchitis catarrhalis traumatica. Slight tumor splenis. Hyperaemia of the superficial cervical lymph nodes. Necrosis of a lymph node.

Diagnosis of Disease: Lamsiekte.

Epicrisis: The case represented one of acute lamsiekte, lasting two days, and affecting the locomotor system. On post-mortem examination, the presence of a catarrhal gastro-enteritis was diagnosed. Bones were found in the reticulum. The animal was a bone-eater and was picked out as such on the veld and confirmed on three different occasions by testing with sterilized bones. It is possible that the bones found in the reticulum dated back to the last test, which was carried out only three days before the animal took ill. The animal was a recent arrival on the farm and took ill about five weeks after it had been turned out on the veld.

SECTION 3.—EXPERIMENTS OUTSIDE THE RECOGNIZED LAMSIEKTE AREA.

Plan of Contents.

The results of our earlier experiments at Armoedsvlakte in the lamsiekte area having clearly shown that decomposing animal matter contained a highly potent toxin which could be held responsible for the causation of lamsiekte, it was thought advisable to repeat the experiments on an area where this disease was unknown.

It was hoped that by a carefully planned series of experiments many obscure points might be elucidated, among these being—

(1) Whether toxin production in decomposing animal matter was confined to lamsiekte areas or occurred in any part of the country;

(2) whether in the latter case the toxines were the same as or different from those occurring in the lamsiekte areas;
(3) if different, what train of symptoms would be set up in experimental animals?
(4) the conditions under which toxin production would occur;
(5) the distribution of bacterial infection, giving rise to specific toxin production, in various parts of country.

The farm Onderstepoort was considered eminently suitable for these experiments because natural cases of lamsiekte had never been known to occur there and all laboratory and other facilities for the work were available. At the beginning we absolutely failed to produce any symptoms of disease in cattle by drenching with comparatively large quantities of putrid animal matter, and, since for this reason no progress could be made it was decided to obtain toxic material from Armoedsvlakte for the further experiments.

Later on material was also obtained from parts of Natal where the existence of lamsiekte had been suspected but where the disease had never been diagnosed with certainty. The main sources of the material used in the drenching experiments were, therefore—

(1) Onderstepoort, a known non-lamsiekte farm;
(2) Armoedsvlakte, a known lamsiekte farm;
(3) farms in Natal where the existence of the disease had been suspected.

Most of the experiments to be discussed here fall under three groups, arranged according to the sources of the material referred to above.

In the following pages will be given a short summary of the different experiments with the results in each case.

Series A.

DRENCHING WITH MATERIAL ORIGINATING FROM ONDERSTEPOORT.

The object of this series of experiments is as previously indicated.

For the collection of material a special paddock was made about a mile from the laboratory, and in this paddock the carcasses were placed to undergo putrefaction under natural veld conditions.

Experiment I.

Drenching of Cattle with Pycnosoma Larvae Reared on Carcasses of Cattle.—Pycnosoma larvae were collected from decomposing animal carcasses on different dates between 15th February and 15th December, 1919, and used in quantities varying from 25 to 480 grammes for drenching cattle.

Results: Two out of the fifteen animals used in these experiments contracted and died from lamsiekte.

These positive results, however, were only obtained towards the end of the experiment, namely, on the 8th December. Up to this period no toxic substance seemed to develop in the putrefying animal carcasses.

EXPERIMENT II.

Drenching of Cattle with Pycnosoma Pupae Hatched out in Carcasses of Cattle.—The pupae were collected from the same carcasses as the larvae used in Experiment I. Sufficient material was difficult to obtain owing to the fact that pupation usually occurred under the surface layers of a rather porous soil. Since the Armoedsvlakte experiments had proved the larvae to be at least as toxic as the pupae, no special effort was made to collect this material.

Only one animal was used in the experiment, 70 grammes of pupae being administered per os on the 25th February, 1919, and again 30 grammes on 6th March.

Result: This was entirely negative.

EXPERIMENT III.

Drenching of Cattle with Pycnosoma Larvae Reared on Carcasses of Horses.—With the exception of one, these carcasses were placed together in one paddock. Larvae were collected on different dates during the period between 15th February, 1919, and 16th January, 1920, and used, in quantities varying from 170 to 480 grammes, for drenching cattle.

Result: Here again no positive result was obtained until December and January, when three out of the seven animals contracted lamsiekte. It was thought the infection might have been derived from the cattle carcasses lying close to the horse carcasses, and to verify this point the carcass of a horse which died on the 12th January was placed by itself some half-mile away. However, larvae collected from it and used for drenching a beast four days later also promptly produced lamsiekte.

EXPERIMENT IV.

Drenching of Cattle with Pycnosoma Larvae obtained from Carcasses which had been carefully kept away from the ordinary sources of infection.—Owing to the fact that practically all carcasses seemed to contain toxic substances during the later stages of the preceding experiments, namely, in December and January, it was thought that the infection might have been carried from one carcass to another by means of flies, etc. To determine whether this was so or whether specific carcass infection had become general by this time, carcass material was prepared in the following way:—

Horse 27 died in our stables from experimental horse-sickness on the 9th February, and, in order to avoid all sources of infection, its carcass was removed from the stable straight to a small, specially-constructed, wire enclosure, about $1\frac{1}{2}$ mile away from the nearest lamsiekte carcass. On arrival here the abdominal wall was slit open with a sterile knife to allow of putrefaction setting in more quickly and of collecting larvae more easily.

Four days later larvae from this source were used for drenching two heifers.

Result: Both contracted lamsiekte very promptly, thus showing that the causal organism of lamsiekte was fairly widely distributed at that time.

The chances of this carcass having become infected from the lamsiekte carcasses appeared to be very remote, and hence the indications were that the lamsiekte organism is widely distributed in this country, but that, owing to climatic and other conditions, which may

influence its growth, toxin production in animal carcasses only takes place at certain times of the year. The absence of natural cases of the disease could be explained by the fact that well-marked osteophagia is not seen in cattle at Onderstepoort.

EXPERIMENT V.

Drenching of a Horse with Fly Larvae Reared on Carcasses of Cattle.—The object was to see whether fly larvae would set up symptoms of a toxaemia in animal species other than cattle.

One horse was drenched on the 20th February, 1919, with 200 grammes fly larvae.

Result: No ill-effects could be observed.

During the same period, fly larvae appeared harmless to cattle also.

EXPERIMENT VI.

Drenching of Goats with Fly Larvae Reared on Carcasses of Horses, Goats, and Dogs.—Carcasses from these species of animals were placed in the same paddock, and larvae collected during the period from 6th to 13th March, 1919.

Four goats received per os quantities of larvae varying from 25 to 150 grammes.

Results: Although goats are susceptible to lamsiekte, no ill-effects were produced. These results, therefore, tend to confirm those of experiments carried out in cattle during the same period, namely, that at that time no toxic substances were developing in carcasses of Onderstepoort animals.

EXPERIMENT VII.

Drenching of Cattle with Putrid Bones obtained from Carcasses of various Animals.—In this case putrid bone material was used instead of fly larvae and pupae, not because the former had been proved to be more toxic, but simply to ascertain by all possible methods whether or not toxic substances were present.

The material consisted mostly of ribs and vertebrae, which were rendered suitable for drenching by being chopped or crushed into small pieces.

During the period between 27th March, 1919, to 30th January, 1920, twelve animals were drenched with the above material in doses varying from 70 to 480 grammes.

Results: Only one animal contracted lamsiekte, and that was the one which was submitted to treatment on the 30th January. The last animal drenched prior to this date was done on the 9th December, and in this case the results were still negative. In the experiments with fly larvae the first positive cases occurred on the 8th December, but the bone material used in the above experiment on the 9th was obtained from older carcasses, in which, apparently, up to that date no toxic substances had developed. The results of this experiment, therefore, agree with those obtained from drenching with larvae, namely, that up to the 8th December, 1919, lamsiekte toxin was not present in decomposing carcasses of animals that had died at Onderstepoort.

EXPERIMENT VIII.

Drenching of Cattle with Mixed Putrid Bones obtained from the Carcasses of various Animals.—In the previous experiment the bone material obtained from each carcass was collected and used separately, whereas in this experiment bone material was obtained from carcasses of different animals (including horses, cattle, and dogs) mixed together, and the mixture so obtained used for drenching animals.

It was thought that by using such a mixture the chances of demonstrating any toxic substance that might be present would be much greater than if material from only one carcass were used.

Twelve cattle were drenched during the period between 8th and 24th April, 1919, with the above material, the doses employed varying from 250 to 540 grammes.

Results: No ill-effects were produced in any of the animals, thus showing that during the period mentioned no toxic substances were being elaborated in the carcasses of animals at Onderstepoort. These results were, therefore, in entire agreement with those of the preceding experiments.

EXPERIMENT IX.

Drenching of Catte with Putrid Bones obtained from the Carcasses of different Animals.—Fly larvae that have been reared on carcasses in lamsiekte areas are toxic by virtue of the toxic substances contained in the decomposing flesh on which they have been living. In this experiment, therefore, the meat itself was used to ascertain definitely whether or not toxic substances could be demonstrated. After weighing, the meat was passed through a mincing-machine and then mixed with just sufficient water to make drenching easy. During the period 30th April to 7th November, 1919, four animals were drenched with this material, the quantity in each case being 480 grammes.

Results: These were negative, thus agreeing with those obtained from concurrent experiments.

Only four animals were employed in this experiment, the reason being that fly larvae were found to be much easier to collect and cleaner to work with.

EXPERIMENT X.

Drenching of Cattle with material from an Unopened Carcass of a Horse placed within a short distance of a number of Lamsiekte Carcasses.—The object was to find out whether toxic substances would develop in the unopened carcass of a healthy animal placed within a short distance of infected carcasses, i.e. assuming the toxogenic organism to be absent from its gastro-intestinal tract, whether the carcass would become infected from the soil or by blow-flies feeding on the near-by lamsiekte carcasses. A healthy horse was killed on the 17th December, 1919, its carcass being left untouched, and placed about twenty yards from some highly toxic lamsiekte carcasses.

Between the periods 22nd December, 1919, to 4th February, 1920, seven cattle were drenched with material consisting of either fly larvae or putrid bones, the dose administered being 480 grammes in each case.

Results: No symptoms of illness were observed in any of the experimental animals.

This is rather remarkable, in view of the fact that during this period infection on the farm appeared to be rather widespread and a few weeks later was shown to be present in carcasses far removed from all sources of infection . (See Experiment IV.)

The only difference in the case under discussion was that the material was obtained from an animal whose skin was left intact and in which disintegration through the assistance of blow-fly larvae could not take place so rapidly. It is obvious that while the skin covering the carcass remained intact, depositing of eggs and hatching of fly larvae could go on only at the natural openings. With our scanty knowledge of the putrefactive processes which animal carcasses undergo and of the organisms concerned in the process, it is difficult to place a definite interpretation on these results.

Discussion of Results.

1. In this series of experiments no fewer than sixty-four animals, comprising fifty-nine head of cattle, one horse, and four goats, were drenched with putrefying material obtained from the carcasses of animals which had died at Onderstepoort from causes other than lamsiekte. The material consisted of blow-fly pupae, larvae, flesh, and bones collected from these carcasses.

2. The results obtained show very definitely that during the period between 15th February and 7th December, 1919, no toxic substances were present in decomposing carcasses at Onderstepoort. As will be seen later, during the same period a number of animals were killed by drenching with toxic carcass material obtained from the lamsiekte farm, Armoedsvlakte. Most of these animals died during May, when their carcasses were removed to and left lying in a special open paddock about half a mile distant from the non-toxic animal carcasses.

3. From the 8th December onwards toxic substances could be demonstrated in nearly all animal carcasses exposed under natural veld conditions, and it would seem that during this time the toxogenic saprophytes were widely distributed, as instanced by the results recorded in Experiments III and IV.

On the other hand, the results of Experiment X show that the specific organisms were not present everywhere, or, at any rate, that their presence did not always lead to toxin production.

4. The absence of toxic substances in the Onderstepoort carcasses during the period ending 7th December, 1920, may be explained as follows:—

(*a*) The toxogenic saprophytes may have been present at Onderstepoort only in very small numbers, and, owing to the careful destruction of all animal carcasses in the past, hardly ever obtained suitable media to grow on. With the large number of carcasses made available during the course of these experiments, conditions for its growth may have become more favourable. There is also a possibility that the climatic and other conditions may have influenced its growth. It is a well-established fact that lamsiekte is a disease of dry regions, so that it is quite conceivable that the organisms may not thrive so well in

a more humid atmosphere such as obtains at Onderstepoort. Lamsiekte does not occur naturally at Onderstepoort, but this fact cannot be used as an argument against the presence there of the organism which is responsible for toxin production, because craving for rotten bones is absent from the Onderstepoort cattle.

(*b*) On the other hand, it is also possible that the toxicogenic organism was completely absent from Onderstepoort, and that it was only introduced with material sent from Armoedsvlakte. From March, 1919, onwards, a large number of animals were killed in lamsiekte experiments, and the carcasses of these animals were deposited in a camp some distance away from the paddock in which the carcasses of non-lamsiekte animals were exposed. It is possible that the infection was spread from the carcass site all over the farm by various agencies, such as blow-flies, dogs, rain-water, etc.

SERIES B.

DRENCHING WITH MATERIAL ORIGINATING FROM ARMOEDSVLAKTE.

The object of these experiments was—

(1) to control the results of drenching experiments with decomposing animal matter which had been carried out at Armoedsvlakte;

(2) to determine whether lamsiekte could be produced on a non-lamsiekte farm by the use of material obained from a lamsiekte farm, and in this way to exclude such factors as climatic, telluric, and other conditions prevailing in lamsiekte areas;

(3) to provide material for experimental work at Onderstepoort.

EXPERIMENT I.

Drenching of Cattle with Fly Pupae Collected from Carcasses of Cattle that Died of Lamsiekte.—The pupae were placed in glass jars containing alcohol as a preservative.

Three cattle were drenched with pupae suspended in water, the amount varying from 90 to 300 grammes.

Results: The results were negative, showing that the toxin had in all probability been rendered inactive by preservation in alcohol. This experiment was carried out at the time when very little was known regarding the lamsiekte toxin.

EXPERIMENT II.

Drenching Cattle with Blow-flies Hatched out from Pupae Collected on Carcasses of Cattle that Died of Lamsiekte.—The pupae were dispatched from Armoedsvlakte for experimental work at Onderstepoort, but on arrival at the latter place it was found that most of the flies had hatched out, leaving only the empty dry shells, which were useless for the work. It was then decided to utilize the flies for drenching experiments with the object of finding out whether the toxin was still present in the newly hatched flies.

The flies were weighed, then crushed and mixed with the necessary water for drenching. During April, 1919, four animals

were used, each receiving from 45 to 300 grammes of the fly suspension.

Results: These were negative, showing that this kind of material is not very toxic. One cannot say that no toxic substances were present, since the experiments were not carried out on a sufficiently large scale and since the individual doses of material may not have been big enough to produce definite symptoms in cattle.

EXPERIMENT III.

Drenching of Cattle with Decomposed Flesh Obtained from Carcasses of Cattle that Died of Lamsiekte.—The material consisted of half dried, stale smelling portions of hide, muscle and stomach wall, collected from a carcass which had been proved to contain highly toxic substances. The material was chopped up in small pieces which, after weighing, were suspended in water for drenching.

On the 9th May, 1919, one animal was drenched with 100 grammes and the other with 500 grammes of this material.

Results: The animal which received the smaller dose showed no ill-effects, while the other developed typical symptoms of lamsiekte five days later and died the following day. This showed clearly that lamsiekte could be produced in a non-lamsiekte area by drenching an animal with toxic material obtained from a lamsiekte farm.

EXPERIMENT IV.

Drenching Cattle with Material from an Animal sent Alive from Armoedsvlakte and Killed at Onderstepoort.—This animal had been running on lamsiekte veld for some considerable time. It was dispatched by rail from Vryburg to Onderstepoort, where it arrived on the 13th May, 1919, and was killed the same day. The carcass was placed in a paddock to undergo decomposition and the necessary material for drenching collected when required. The particular object was to find out whether the lamsiekte organism was a normal inhabitant of the gut of animals running on a lamsiekte farm, and whether, if such an animal was slaughtered and allowed to undergo decomposition in a non-lamsiekte area (where the specific organisms were presumably not present), toxin production would take place. During the period between 23rd May and 15th July, 1919, a total of fourteen animals were drenched with material (larvae, flesh and bones) from this carcass, the quantities used varying from 300 to 480 grammes.

Result: No definite symptoms of lamsiekte developed in any of the animals; one animal showed signs of slight illness, but this was probably not lamsiekte. This animal received only 240 grammes of material, while another on the same day received 400 grammes of the same material, without any ill-effects developing.

Although the results of this experiment were negative, it cannot be accepted as having been proved that the lamsiekte organisms cannot be carried in this way from one farm to another.

EXPERIMENT V.

Drenching Cattle with Putrid Bone Material Obtained from Armoedsvlakte.—The material was divided up by crushing or grinding into small pieces, so as to make drenching possible. During the period between 3rd April and 2nd May, 1919, five animals were drenched with quantities varying from 130 to 480 grammes.

Results: Both animals which received 480 grammes developed typical lamsiekte, while those which received less, from 360 to 120 grammes, showed no ill-effects.

This shows clearly that comparatively large quantities of material—depending on its toxicity—may be required to set up lamsiekte.

EXPERIMENT VI.

Drenching of Cattle with Fly Pupae Collected on Carcasses at Armoedsvlakte.—After collection from the carcass the pupae were packed in wooden boxes and dispatched by post to Onderstepoort. Owing to the fact that blow-flies hatch out within a few days, some of the material which arrived was found to consist of flies, empty shells, and some intact pupae. In every case, however, careful notes were made of the condition of the material used. Fourteen head of cattle were placed in the experiment, the amount of material used in each case varying from 30 to 480 grammes. Two kinds of pupae were used, namely, (*a*) those which still remained intact and (*b*) those of which practically only the dry shells remained, the flies having hatched out in many cases.

Results: With the first class of pupae ten animals were drenched, the result being that six died of lamsiekte, these having been drenched with quantities of pupae varying from 120 to 480 grammes. Of the remaining four animals, two received rather small doses, while the other two where drenched with 120 and 240 grammes, respectively. Both these were done on the same day with the same consignment of material *ex* Vryburg. It would appear, therefore, as if this particular sample of pupae was not very toxic. Four animals were drenched with the second class of pupae, the quantities varying from 162 to 480 grammes per animal. The results were entirely negative, thus showing that the toxic substances do not remain behind in any appreciable quantity in the empty shells of pupae from which the flies have hatched.

DISCUSSION AND SUMMARY.

The results obtained from this series of experiments may be summarized as follows:—

(1) Three animals were drenched with pycnosoma pupae collected on Armoedsvlakte carcasses and preserved in alcohol. The results were negative, showing that the toxic substances are probably killed by alcohol.

(2) Four animals received per os different quantities of flies that hatched out from pupae collected on Armoedsvlakte carcasses, and of these none showed any ill-effects.

(3) Of the two animals drenched with putrid meat collected from Armoedsvlakte carcasses the one which received 500 grammes died, while the other which only got 100 grammes remained healthy.

(4) Fourteen animals were drenched with material collected from the carcass of bull 4780, which was sent from the Armoedsvlakte veld to be killed on arrival at Onderstepoort. None of these died from lamsiekte, thus showing that the carcass did not contain any toxic substances. Further experiments to prove whether or not toxogenic saprophytes are normally present in the intestinal flora

of animals running on a lamsiekte farm, and whether the organisms can be carried from one farm to another in the faeces of such animals, are called for.

(5) Of five animals drenched with putrid bones collected from Armoedsvlakte carcasses, the two which received 480 grammes both died of lamsiekte, whereas the others that received 360 grammes and less remained healthy.

(6) Fourteen cattle were drenched with fly pupae that were reared on Armoedsvlakte carcasses, and of these six died from lamsiekte, the quantities which produced fatal results varying from 120 grammes upwards. Four of the remaining animals received mostly shells from which the flies had already escaped and in none of these could any ill effects be observed.

(7) The results of these experiments showed that (*a*) material collected from carcasses on a lamsiekte farm contain toxic substances which can be used to produce lamsiekte in parts of the country where the disease is normally absent; (*b*) a fairly definite quantity of the particular material used is required to produce fatal results, i.e. the toxic substances do not increase after introduction into the stomach of the living animal.

Series C.

DRENCHING WITH MATERIAL OBTAINED AT ONDERSTEPOORT BUT ORIGINATING FROM ARMOEDSVLAKTE.

The first experiments discussed under Series A having indicated that toxic substances do not in normal circumstances develop in carcasses of animals that have died on a non-lamsiekte farm, it was considered necessary to determine the extent to which the infection introduced into carcasses through the experiments in Series B, will spread under the climatic and other conditions obtaining at Onderstepoort.

Experiment I.

Drenching Animals with Putrid Bones Collected from Heifer 4670.—This animal died of lamsiekte on the 8th May, 1919, as a result of drenching with bones received from Armoedsvlakte. During the period between 26th June and 15th July each of five animals received 480 grammes of crushed putrid bones collected from heifer 4670.

The results were negative, showing that no toxic substances had developed in this carcass.

Experiment II.

Drenching with Putrid Bones from Heifer 4695.—Heifer 4695 died of lamsiekte on the 7th May, 1919, as a result of drenching with putrid bones received from Armoedsvlakte. During the period between 23rd May and 15th July four animals were drenched with putrid crushed bones collected from the carcass of the above animal.

The results were also negative.

Experiment III.

Drenching with Material from Heifer 4765.—This heifer died of lamsiekte on the 4th May, 1919, as the result of drenching with fly pupae received from Armoedsvlakte.

During the period 6th May to 15th July seventeen animals passed through this experiment and of these four were drenched with fly larvae, seven with putrid bones, and six with muscle material from the carcass of the above animal.

Results: Out of the seventeen animals used in this experiment nine contracted and died from lamsiekte, positive cases occurring in animals drenched with all the three different kinds of material (putrid flesh, bones, and larvae). This clearly showed that carcasses of animals which had been killed with lamsiekte material on a non-lamsiekte farm can on their part become infected and provide material for the development of toxic substances.

Heifer 4765 died on the 4th May and the first case of lamsiekte produced by drenching animals with material collected from it, occurred on the 10th, so that in this case it took 6 days for toxic substances to develop in sufficiently large amounts to be detected by our tests. That the carcass material cannot be considered highly toxic is shown by the fact that it required at least 240 grammes of fly larvae to produce positive results.

The last drenching experiment with this carcass material took place on the 15th July, nearly 2½ months after heifer 4765 died, and the result was negative, showing that toxic substances had disappeared or become reduced considerably in amount.

EXPERIMENT IV.

Drenching with Material from Heifer 4691.—This heifer died of lamsiekte on the 19th May, 1919, as a result of drenching with fly larvae collected from the carcass of heifer 4765 (see previous experiment). The main object of the experiment was to find out through how many generations the infection could be passed.

During the period between 3rd June and 15th July five animals were drenched with material from the above carcass, consisting of putrid flesh, fly larvae, and bones, the quantity given varying from 300 to 480 grammes.

Results: These were negative, showing that no toxic substances developed in this carcass.

EXPERIMENT V.

Drenching with Material from Heifer 4707.—This animal died from lamsiekte on the 14th May, 1919, as a result of drenching with fly larvae collected from the carcass of heifer 4765 (see previous experiment). Two animals were drenched on the 17th June with 480 grammes each of putrid crushed bones collected from the above carcass.

Results: These were also negative.

EXPERIMENT VI.

Drenching with Material from Carcass of Heifer 4770.—This animal died of lamsiekte on the 18th May, 1919, as a result of drenching with putrid bone from the carcass of heifer 4765 (see previous experiments). During the period from 23rd May to 10th June six animals were drenched with putrid flesh, fly larvae, and bones collected from the above carcass, the quantity used for each animal varying from 240 to 480 grammes.

Results: These were again negative, showing that in this carcass also no toxic substances had developed.

EXPERIMENT VII.

Drenching with Material from Heifer 4738.—This animal died from lamsiekte on the 17th May, 1919, as a result of drenching with putrid flesh collected from the carcass of heifer 4765 (see previous experiments). Between the 17th June and 15th July three animals were given per os quantities of putrid bones and flesh from the above carcass, varying from 215 to 480 grammes.

Results: These were negative, showing that here also no toxic substances could be demonstrated.

EXPERIMENT VIII.

Drenching with Material from Heifer 4742.—This animal was killed on the 19th May by drenching with putrid flesh collected from the carcass of heifer 4765 (see preceeding experiments).

During the period between 28th May and 15th July four animals were drenched with fly larvae and putrid bones collected from the above carcass, the quantity given to each animal varying from 240 to 480 grammes.

Results: These were again negative.

DISCUSSION OF RESULTS.

The results obtained from this series of experiments may be summarized very briefly, as follows:—

(1) No fewer than forty-five animals were used in the drenching experiments.

(2) Of this number twenty-five were employed in the first three experiments of the series. The drenching material employed was derived from carcasses of three animals which died as the result of drenching with carcass material obtained from Armoedsvlakte. The carcass material of only one out of the three animals proved to be toxic, thus showing that infection of carcasses, with toxin production, on a non-lamsiekte farm, does not invariably take place.

(3) Material from carcasses of five animals that were killed by toxic material referred to under (2) was used for drenching twenty further animals. None of these animals developed lamsiekte, showing that, under the conditions prevailing on a non-lamsiekte farm, the infection cannot be passed through the carcass of one animal to another. During the same period, May to July, 1919, toxic substances were entirely absent from local (Onderstepoort) carcasses, and this may have been due to unfavourable conditions for the growth of the organisms.

SERIES D.

DRENCHING WITH MATERIAL OBTAINED AT ONDERSTEPOORT AS THE RESULT OF CULTIVATING ANAEROBIC ORGANISMS CONTAINED IN TOXIC BONES ORIGINATING FROM ARMOEDSVLAKTE.

The first and main object was to determine whether toxin production would take place under laboratory conditions in tissue media inoculated with bone material which was known to be toxic when drenched to cattle. (Details regarding the methods of cultivation will be found elsewhere in this report.)

Secondly, if toxin production could be induced under laboratory conditions, it was thought that by using concentrated toxin, the further experimental study of the disease could be carried out much more easily and more expeditiously.

Thirdly, by utilizing lamsiekte cultures endeavours were made to find out through how many successive generations of animal carcasses the infective material could be passed. It was thought that the infection was more likely to be passed successfully through several generations of animals than in the preceding series of experiments where only moderately toxic material was used to commence with.

EXPERIMENT I.

Drenching with Culture No. 1, which consisted of mixed brain tissue inoculated with original bone material of low toxicity, received from Armoedsvlakte.

Only one animal was drenched with this material, during November, 1919, the dose employed being 400 grammes.

Result: It was found dead three days later, showing that the culture was highly toxic.

EXPERIMENT II.

Drenching with contents of Culture Jar No. 2, which consisted of liver tissue inoculated with original bone material of low toxicity, received from Armoedsvlakte.

During November, 1919, one animal was drenched with 400 grammes of this culture.

Result: This was negative, showing that no toxin development had taken place in this culture.

EXPERIMENT III.

Drenching with Contents of Culture Jar No. 3, which consisted of muscle tissue inoculated with original bone material of low toxicity, received from Armoedsvlakte. In November, 1919, one animal was drenched with 400 grammes.

Result: Negative, showing the absence of toxic substances in this culture.

EXPERIMENT IV.

Drenching with Culture No. 4, which consisted of mixed brain tissue inoculated with original bone material of low toxicity received from Armoedsvlakte, and pasteurized after inoculation. One animal received 300 grammes per os.

Result: Death took place after only two days, showing that the culture contained highly toxic substances.

EXPERIMENT V.

Drenching with Culture No. 5, which consisted of liver tissue inoculated with original bone material of low toxicity received from Armoedsvlakte, and then pasteurized. During the period between 3rd November and 5th December, 1919, four cattle were drenched with this culture material, the dose per animal varying from 3 to 400 grammes.

Result: This was positive in all cases, showing that the culture was so highly toxic that such a small quantity as 3 grammes proved fatal.

EXPERIMENT VI.

Drenching with Sub-culture No. 12, which consisted of liver tissue inoculated with material from Culture No. 5 (see above) and then pasteurized.

On the 5th December one heifer received 100 grammes of this culture.

Result: This proved positive, showing that toxic sub-cultures can be obtained quite easily.

EXPERIMENT VII.

Drenching with Sub-culture No. 20, which consisted of liver tissue inoculated with material from Culture No. 12 (see above).

On the 19th December, 1919, one heifer received per os 30 grammes of this culture.

Result: Lamsiekte developed within twenty-four hours, showing the culture to be highly toxic.

EXPERIMENT VIII.

Drenching with Material from Carcass of Heifer 4805, which had been killed with toxic culture No. 5.

On the 5th and 8th December, 1919, two heifers were drenched with 480 grammes each of putrid flesh and fly larvae.

Result: The animal which received fly larvae died of lamsiekte, while the other remained healthy, which appears to show that larvae are more toxic than putrid meat derived from the same source.

EXPERIMENT IX.

Drenching with Material from Carcass of Heifer 4701, which was killed with toxic culture No. 4.

On the 8th December, 1919, one heifer received per os 480 grammes of pycnosoma larvae collected from the above carcass.

Result: This was positive, showing that toxic substances were present.

EXPERIMENT X.

Drenching with Material from Carcass of Heifer 4692, which was killed with culture No. 5.

On the 9th December, 1919, one heifer was drenched with 160 grammes fly larvae collected from the above carcass.

Result: This was positive, again showing that infection was transmitted to the carcass by culture No. 5.

EXPERIMENT XI.

Drenching with Material from Carcass of Heifer 4582, which was killed with material from carcass 4805, i.e. third generation (see experiment 8).

Between the 9th and 17th December, two heifers and one sheep were drenched with material from the above carcass, the dose per heifer being 480 grammes and per sheep 250 grammes.

Result: One heifer died from lamsiekte, showing that toxic substances were still present.

EXPERIMENT XII.

Drenching with Material from Carcass of Heifer 4021, which was killed with material from Carcass 4701 (see experiment No. IX), i.e. third generation.

One heifer was drenched on the 15th December, 1919, with 450 grammes putrid flesh collected from the above carcass.

Result: This heifer died of lamsiekte, showing that toxic substances were still present.

EXPERIMENT XIII.

Drenching with Material from Carcass of Heifer 4588, which was killed with material from carcass 4692 (experiment X), i.e. third generation.

One heifer was drenched on the 15th December, 1919, with 480 grammes larvae collected from the above carcass.

Result: Lamsiekte developed after four days, thus showing that toxic substances were still present.

EXPERIMENT XIV.

Drenching with Material from Carcass of Heifer 4047, which was killed with material from carcass 4582 (experiment XI), i.e. fourth generation.

On the 17th December one animal was drenched with 480 grammes fly larvae collected from the above carcass.

Result: This was negative, showing that toxic substances had not developed.

EXPERIMENT XV.

Drenching with Material from Carcass of Heifer 4767, which was killed with material from carcass 4588 (see experiment XIII), i.e. fourth generation.

On the 17th December, 1919, one heifer was drenched with 480 grammes of fly larvae collected from the above carcass.

Result: This was positive, showing that toxic substances developed even in the fourth generation.

EXPERIMENT XVI.

Drenching with Material from Carcass of Heifer 4061, which was killed with material from carcass 4767 (experiment XV), i.e. fifth generation.

On the 27th December one heifer was drenched with 480 grammes fly larvae collected from the above carcass.

Result: Negative, showing the absence of toxic substances in sufficient quantities to produce lamsiekte

DISCUSSION OF RESULTS.

(1) Of the five original cultures three, viz., jars Nos. 1, 4, and 5, proved to be highly toxic, so much so that the experimental animals died from peracute lamsiekte after exceptionally short incubation periods.

The contents of jar No. 5 were found to be particularly toxic, about three grammes given per os being sufficient to kill a large heifer.

(2) Sub-cultures made from jar No. 5 also proved toxic, 100 grammes per os proving fatal to a heifer, while a dose of twelve grammes was not sufficient to kill. Jar No. 20 was sub-inoculated from jar No. 12, and its contents were shown to produce fatal results in cattle in such small quantities as 30 grammes administered per os.

As a matter of fact, it was discovered later on that the longer the cultures were kept the more toxic they became.

(3) When it is remembered that the original bone material was not very toxic, only producing fatal results in such large doses as 480 grammes, the results obtained from the cultures are most striking.

In fact, it was shown that the culture media selected, viz., tissues such as liver, brain, etc., were very favourable for the growth of, and toxin production by, the saprophytic organisms concerned.

(4) By utilizing the carcass material of the animals which died as the result of drenching with lamsiekte cultures, it was shown that the infection, with toxin production, could be passed through several generations. Positive results were obtained up to and including the fourth generation, and possibly infection might have been established in any number of carcases provided the conditions for the growth of the organisms were favourable, and the experiments were carried out on a sufficiently large scale. The results obtained were sufficient to show that a non-lamsiekte farm could become infected with the disease, on the introduction of toxic lamsiekte material, provided always the conditions for the growth of the organism (climatic, soil, etc.), and toxin production, were favourable.

(5) It should be pointed out, however, that these experiments were carried out during a period (December, 1919) when practically all animal carcasses exposed on the Onderstepoort veld contained toxic substances (see experiments under Series A and B).

Series E.

DRENCHING WITH MATERIAL OBTAINED FROM THE CARCASSES OF ANIMALS IN NATAL.

One of us (P.R.V.) visited certain farms in Natal on which the existence of lamsiekte had been suspected by farmers, but where the disease had never been definitely diagnosed.

On many farms craving for bones was very marked in cattle, but pica was also present on some farms in the same area where lamsiekte had not been suspected.

Arrangements were made with some farmers to forward putrid bones collected from carcasses of animals which died on their farms. The drenching experiments to be reported on here were carried out with this bone material.

The object was to determine whether or not toxic substances were present in carcass material derived from Natal, and further, to ascertain whether toxin production was present only on lamsiekte farms or also on farms where the disease had never been suspected.

Experiment I.

Drenching with Putrid Bone Material collected by Mr. C., Mooi River, from the carcass of a calf. On this farm the presence of lamsiekte is suspected and cattle showed craving for bones.

On the 15th January and 13th February, 1920, two heifers were drenched with the above material, the first receiving 480 and the second 465 grammes.

Result: This was entirely negative.