discharge. Greenish discharge from the nostrils, containing mucus and foodstuffs (lucerne). Floor of stable soiled with this discharge and froth. Pulse strong, 32 per minute; temperature, 97° F. (2 p.m.) Slight improvement. Temperature, 97.8° F. (5 p.m.) Feeding very slowly. Looks dull.

15.5.20: (7 a.m.) Looks much better, feeding normally.

19.5.20: (5.30 p.m.) Injected 15 c.c. filtered toxin subcutaneously. No reaction.

20.5.20: (5 p.m.) Looks dull and feeds very slowly.

21.5.20: (7 a.m.) Looks dull, stands on same spot, feeds slowly. (5 p.m.) Not feeding.

22.5.20: (7 a.m.) Condition unchanged. Pulse very slow (28 per minute), hardly perceptible.

23.5.20: (7 a.m.) No change. (5 p.m.) Feeding a little. Animal is very weak and dull, and lies down most of the time.

24.5.20: (7 a.m.) Found lying on side with head and legs stretched out. The animal is entirely paralysed and is unable to lift its head or to remain in sterno-costal position. Foodstuffs in nostrils (regurgitation). Pulse almost imperceptible and slow (28 to 32 per minute).

25.5.20: (7 a.m.) Animal still alive. Stretched out on side. Dirty green discharge from nostrils. Pulse imperceptible. Breathing accelerated, 54 to 60 times per minute; respiration deep, of abdominal type, the whole body is moved and the anus is protruded at every movement. Urine flows continually. (3.30) Dies of lamsiekte.

SECTION 5.—ISOLATION AND DESCRIPTION OF THE TOXICOGENIC SAPROPHYTE.

THE article on " Cause and Prevention of Lamsiekte " published in the *Journal of the Department of Agriculture*, June, 1920, by Theiler and co-workers contains notes on the toxin producing lamsiekte and upon impure cultures in which three sporulating anaerobes were constantly present (page 9). Spasmodic further work at different dates by Du Toit, Green, Meyer, Andrews, and Fourie, carried the problem of isolation a little further, but it was left to the present writer (E. M. Robinson) to effect the final isolation and identification of the single causal organism as a member of the *parabotulinus* group.

The actual final separation was made from mixed cultures of anaerobes in meat mash media [Robertson (2)], supplied by Fourie, Officer in Charge of the Experiment Station at Vryburg.

These cultures had been obtained from colonies in glucose shake media, and had proved toxic for rabbits. They, however, like previous partially purified cultures, were found on further examination to contain at least three types of bacteria, and the actual isolation of the real toxicogenic saprophyte was only accomplished after several months of uninterrupted painstaking work. The present paper offers a preliminary description of the organism and of the symptoms produced in animals by the toxin in pure cultures in various media; a more detailed study being left over for a separate later article.

From analogy of similar studies in the existing literature, a botulinus group of organism was suspected and innumerable glucose agar shake cultures were, therefore, made from those impure toxic cultures in which any organism of any of the botulinus types appeared to be dominant over the other species. The first colonies did not produce any toxin in meat mash medium. After making a further series of dilution experiments with the toxic cultures, rediluting from the highest dilution in which growth still occurred when sown in

meat mash medium, it was, however, finally possible to obtain an apparently pure toxic colony in a glucose agar shake culture. From this colony subcultures were made in liquid media, and from these media glucose agar shake cultures again poured. This procedure was repeated through several generations, and as far as it is possible to determine, without actually carrying out isolation by the " single cell " technique, the culture which will be described is a pure one. Both Burri's and Barber's methods for the isolation of a single organism have been attempted, but so far without success. The former method will be attempted again as soon as the weather becomes cool enough for using gelatine plates.

To facilitate description the name *Clostridium parabotulinum bovis* is provisionally assigned to the organism on account of its close resemblance to the *Bac. parabotulinus* described by Seddon in Australia. Before proceeding with the description of the organism and its toxin as obtained from a pure culture, a short review may be given of the articles recently published on closely related bacteria isolated in America and Australia.

A paper entitled " Bulbar Paralysis in Cattle due to the Action of a Toxicogenic Saprophyte," by H. R. Seddon, published after the 1920 article on lamsiekte had appeared, gives some very interesting data in connexion with a bacterium isolated by him from a decomposed bone from a farm in Australia where bulbar paralysis in cattle occurred, a disease resembling and probably the same as lamsiekte. He suggested the name *B. parabotulinus* for the organism. It is not necessary here to go into the details of the description of the bacterium and its toxin, as they are essentially similar to those of *Cl. parabotulinum bovis* now to be described. The symptoms caused by the toxin of Seddon's organism are very similar to those set up by the toxin of *Cl. parabotulinum bovis* and come into the class of symptoms seen as a result of the inoculation or ingestion of toxins of the *botulinus* type.

Two further articles from the United States of America describing organisms corresponding very closely to the *Cl. parabotulinum bovis* have recently appeared. One is on " A toxin-producing anaerobe isolated from the larvae of *Lucilia caesar*," by I. Bengtson (4), and the other is " *Clostridium botulinum*, type C," by Graham and Boughton (5). The former article describes an organism isolated from fly larvae preserved in glycerin, sent from a poultry yard where the disease " limberneck " occurred. The organism is essentially similar to the *parabotulinus* ones and the toxin it produces causes similar symptoms. Bengtson classified her organism as *Cl. botulinum*, type C, on account of its resemblance to the *botulinus* A and B types as regards its toxic effects, though the cultural characteristics were very different, and antitoxin against A and B types did not neutralize C toxin. The article by Graham and Boughton describes a bacterium, isolated from outbreaks of " limberneck " in chickens in Illinois. The *Cl. botulinum*, type C, described by them is certainly closely related to the *parabotulinus* organisms. An important point of interest and difference, however, is that the toxin produced is not very toxic for cattle and horses. It needed 50 grams per 1,000 lb. body-weight in horses and 100 to 400 grams per 1,000 lb. in cattle of culture medium containing toxin to kill these animals. In fowls at least 3 to 5 c.c. of toxin were needed to produce symptoms, much less being

required of either *botulinus* A or B types. This would be a point of resemblance to *Cl. parabutulinum bovis* which is not very toxic for fowls, though so highly toxic for cattle.

Two articles have appeared recently in the *Journal of Infectious Diseases on Cl. botulinum*, C type, and Seddon's *parabolutinus*. The first by Pfennirger (6) deals with the toxico-immunological relationship of the C type and *parabotulinus* and their serological relationships. It was found that the C type antitoxin will neutralize the *parabotulinus* of Seddon toxin, but that *parabotulinus* antitoxin will not neutralize type C toxin. The agglutinating serum against type C does not agglutinate the *parabotulinus* organism, but the serum against the *parabotulinus* does agglutinate the type C in low dilutions. This would tend to show that the organisms are related but not identical.

The other paper by Wagner (7) deals with the biochemical activities of *Cl. botulinum*, C, and the *parabotulinus* type of Seddon, and it is pointed out that it is very probable that the original *Cl. botulinium* of Van Ermengen was probably not an A or B type at all, but a C.

Morphology and Staining Reactions.

Cl. parabotulinum bovis is a rod-shaped organism 3μ to 7μ in length by 0.8μ in width. The spore is so close to the end of the organism as to be practically terminal. Some are actually so, but many show a darkening at the end of the rod at which they are situated, so must be considered subterminal. The spores are oval, 1.5μ to 2μ in length by 1μ in width, and they distend the rod. They are not always frequent in culture media and are rarely seen free. The rods are often seen in pairs and short chains in young cultures and may show filaments of 6 to 10 organisms in old cultures. Spores do not appear in culture media, where they are found, until 36 to 48 hours after sowing.

Motility.—No motility has been observed even in young cultures of the organism.

Staining Reactions.—The organism is gram positive in young cultures, but after 4 to 5 days' incubation, gram-negative forms become frequent, and one sees numerous forms in the process of losing the violet of the gram stain. These appear granular. After 10 to 14 days one sees very few gram-positive forms. Staining by any of the ordinary basic aniline dyes gives good results.

Optimum Temperature for Growth.—Growth is more rapid at 37° C. than at lower temperatures, but also occurs at lower temperatures and is quite good at the summer temperature in this Laboratory (Pretoria) which varies from 20° C. to 28° C. during the normal day.

Toxin is formed at room temperature, at 30° C. and at 37° C., and pure cultures incubated at these temperatures are highly toxic after three days' incubation.

Toxin may naturally be formed after 24 hours both in pure cultures and in impure. It would seem that in the latter, the toxin is more slowly formed, but this is uncertain, as it is at present impossible to determine the number of *parabotulinus* organisms in a mixture with putrefactive organisms. Frequently in examining highly toxic mixed cultures from carcass material, it is practically impossible to determine miscoscopically whether the *Cl. parabotulinum bovis* is present or not.

In testing cultures for toxin production on the 1st and 2nd days after inoculation, the seed material was not heated to kill the toxin, since heating apparently delays the growth of the organism for 24 to 48 hours. The amount of toxin from the inoculum remaining in the subcultures, however, was not more than 0.001 c.c., and in the 0.5 c.c. used for injecting the test animals it was less than 0.00005 c.c., a dose considerably below the minimum lethal for rabbits.

The range of H ion concentration most suitable for growth has not been definitely determined, but cultures grow well and produce toxin in large amount at any range from Ph. 6 to 8.

Cultural Characteristics.

Cl. parabotulinum bovis is a strict anaerobe and grows fairly well in the usual media in use for the cultivation of anaerobic bacteria. No growth has so far been obtained on the surface of solid media incubated anaerobically, but it is good in deep cultures in a medium such as glucose agar. The most useful anaerobic methods used in this study are the meat mash medium recommended by Robertson (2) and Hibler's brain medium, in the class of liquid media which can be incubated anaerobically; and of other methods, the anaerobic jar used by McIntosh and Fildes, and various devices using pyrogallic acid and caustic potash for reducing oxygen. Deep glucose agar tubes of the vignal type have been used on a very large scale and have proved exceedingly useful. In these tubes, one end has always been left open, since otherwise, if gas forms in the tubes, the medium may appear sound on examination after incubation, but bursts into fragments as soon as the tube is broken to get at the colonies. Fragmentation of the medium is one of the great drawbacks to the use of glucose agar and other media in shake cultures. When isolation is attempted from a mixed collection of sporulating anaerobes, it is only in the highest dilutions that the tubes remain free from fragmentation. It may be mentioned here that in the isolation of such an organism as *Cl. parabotulinum bovis*, present in small numbers in the midst of a great variety of other sporulating anaerobes, one almost invariably finds that highly diluted shake cultures do not contain it at all. At present one has to depend on a chance isolation.

Glucose Broth.—In this medium the growth is fairly good. The medium does not become turbid, but fine flakes appear, which settle after a few days, some sticking to the sides of the tube.

Plain Broth.—Growth is very poor, and the medium, as with glucose broth, does not become turbid.

Serum Broth.—Growth is good, more profuse than in glucose broth, but of a similar type. A powerful toxin is formed in this medium.

Meat Mash Medium.—Most of the cultural work in liquid media was done in this. Both on account of good growth and good toxin formation, the medium was found very suitable for the sub-cultivation of the strain, and for growing organisms from colonies in shake cultures during attempts at isolation. Gas is always produced in this medium, in some cases on an extreme scale. Gas bubbles are usually seen on the day after showing and continue to be formed for 4 or 5 days, being visible between the meat particles even if they are not present at the surface of the liquid portion. Apart from gas there is very little evidence of growth at all, the liquid portion of the

medium remaining practically clear except for fine flakes which subsequently settle out. No blackening has been noticed, however long the medium has been incubated, nor has any distinct reddening been seen. A powerful toxin is formed and is present in large amount by the third day of incubation. A sweetish smell emanates from the medium during growth, and the odour never becomes putrid or offensive.

Hibler's Medium.—The growth is similar to that in meat mash medium, and no blackening or reddening occurs. A peculiarity, mentioned later in discussing toxin formation, is that in this medium little or no toxin is formed. Another feature is that spores are not seen, and if formed must be in exceedingly small numbers.

One-tenth per cent. Agar.—Colonies of a fluffy type were formed in this medium, but tended to run together. In cultures incubated aerobically the growth started about a centimetre below the surface, but in anaerobic jars (McIntosh and Fildes) growth extended to the surface of the medium.

Glucose Agar.—This was the medium used for preference in the making of deep shake cultures in long, narrow tubes of the vignal type. When these tubes are used, colonies, if not too frequent, are easily seen and can be very easily got at for isolation. The type of colony produced by *Cl. parabotulinum bovis* is of the fine fluffy type, and very closely resembles a piece of frayed out cotton wool. The colonies do not usually show a dense centre, but may do so when large. In big colonies the central portion sometimes appears darker, but there is never a definite dark nucleus. Colonies appear in the medium on the day after inoculation, but are then usually very small, and seldom visible to the naked eye until the second day. By the fourth or fifth day they attain the size of a small pea with a diameter of 3 to 4 mm. The colour of the colonies is greyish white. Only when well separated do they form the large colonies already mentioned. When numerous they remain small, but however thickly the tube is crowded, no gas is formed, and the medium does not fragment. In preparations made from colonies and stained by Gram's method only gram-positive filaments without spores were seen, although gram-negative portions were visible in some of the filaments.

Ordinary Agar.—Colonies similar to those in glucose agar were formed, but they developed very slowly, always remaining small and like very fine fluffy fragments of cotton wool.

Liver Agar.—In this medium colonies of a larger and denser type were formed. The centres were dense, and thick woolly growths projected from them into the medium. The colonies attained a diameter of 3 to 4 mm. in two or three days and were greyish white in colour. The medium was always fragmented when more than a few colonies were present, and in consequence liver agar was not used in routine isolation of the organism.

Glucose Gelatin.—A uniform turbidity occurs but growth is poor. In stab cultures growth occurs along the needle track as a faint greyish line with some lateral outgrowths in the form of fine threads. No liquefaction occurs in periods up to a fortnight.

Toxin Production of "Cl. Parabotulinum Bovis."

A large amount of work, carried out on the toxin obtained from impure cultures, appears in another section of the report of which

the present article is a portion. The work to be described here was all done with toxin obtained from pure cultures and is valuable by way of comparison.

The organism produces a very powerful toxin in pure culture, and the filtered media produce symptoms of lamsiekte in susceptible animals whether given by the mouth, or by subcutaneous intraperitoneal, or intravenous incubation. As was anticipated, a much larger dose is required to produce symptoms per *os* than by inoculation. Most of the tests of virulence were carried out on rabbits, but a fair number on guinea-pigs as well, the former being more readily obtainable locally and eminently satisfactory as test animals, since they show very typical symptoms. A powerful toxin was developed in meat mash medium by the third day after inoculation, as also in serum broth and other liquid media under anaerobic conditions. In Hibler's brain medium, however, very little toxin appears, in spite of good growth of the organism. The sterilized nerve tissue is probably able to fix the toxin, although small amounts remain in the fluid part of the medium, as demonstrated by the development of symptoms in rabbits inoculated with it. The symptoms, however, are very much delayed and may take ten days to appear.

On account of the feeble toxicity in Hibler cultures, meat mash medium is now used exclusively. It is probable that in the earlier work done in this institution, colonies of the *Cl. parabotulinum bovis* were actually isolated in Hibler medium, but discarded on account of lack of toxicity.

Filtered cultures of the organism have proved toxic for rabbits in quantities as small as 0.001 c.c., subcutaneously. This corresponds roughly with the toxicity elsewhere recorded for filtrate from mixed cultures. Less than 1 c.c. of filtrate per *os* will usually not produce symptoms, and even 1 c.c. often produces chronic cases, which recover after showing symptoms for several days. The best chronic cases in rabbits have all been produced by dosing per *os*, and it is noteworthy that by injection methods the minimum lethal dose may not produce the chronic form of intoxication, though the appearance of symptoms is always delayed for a few days. In testing the toxicity of various materials, preliminary doses of 0.5 c.c. to 1 c.c. have usually been tried subcutaneously. Such a quantity usually kills rabits in 8 to 12 hours, death taking place very quickly after symptoms are first shown. The symptoms shown by other animals will be considered presently.

Berkefeld filtrate of toxic media is also being kept under toluol in a refrigerator at 2° C., and experiments are in progress to determine how long the toxicity is maintained.

A further point of considerable interest concerns factors which destroy the toxin or inhibit its development in miscellaneous media. At the moment, however, it is only necessary to refer to an observation which was made during the earlier work carried out on the organism. It was found difficult to carry the strain of the lamsiekte organism on to fresh media by subinoculation from the very mixed cultures in which it occurred, unless the media were heated to between 60° and 70° C. for about an hour subsequent to inoculation. It would therefore appear that in the mixed cultures some alien organism was present which was capable of hindering toxin production. This organism apparently did not destroy the toxin in the old

mixed cultures, but in unheated subcultures could hinder its formation, perhaps by mere numerical domination. After heating subinoculated media for a few successive generations, the procedure was no longer necessary, as the subcultures developed a high toxicity on simple subinoculation. The interfering putrefactive organism had apparently been sufficiently suppressed.

The whole subject of the various symbiotic and commensal conditions under which *Cl. parabotulinum* can live, is itself an interesting study, and experiments in that field are now in progress.

Isolation of the Organism from Miscellaneous Infected Material.

As already mentioned, the isolation of the organism from material such as a portion of a decomposed carcass presents considerable difficulty. Attempts are now being made to work out a technique by which the isolation may be simplified so that there will be no need, as at present, to depend on a chance separation. *Cl. parabotulinum bovis* is not present in large numbers in decomposed carcass material and frequently it is even difficult to detect bacteria of its type in a mixed culture known to be highly toxic. By heating to 100° C. for about an hour one can get rid of all but the most resistant of the contaminating sporulating anaerobes without destroying *Cl. parabotulinum bovis* itself. Such heated cultures, however, are still dominated by clostridia of the sporogenes type, and by several species of drum-stick organisms of the *Cl. putrificum* type. *B. tetani* was also encountered in the earlier attempts at isolation, and when it occurs, the symptoms it produces in rabbits are misleading. In this animal a paralysis occurs which in some cases resembles lamsiekte, except for the unusually marked stiffness in the hind limb on the side the inoculation is made. Inoculation of guinea-pigs or dosing of rabbits per *os*, however, readily differentiates between *tetanus* and *botulism*.

A suitable " enriching method " is needed in view of the fact that it is difficult to kill off the associated sporulating anaerobes, some of which appear to be even more heat-resistant than *Cl. parabotulinum*. Various exhaust media are now being tried, and the resistance of the spores to various agents such as glycerine, antiformin, etc., is being tested. A further difficulty encountered in isolation, especially when the lamsiekte organism is not dominant, is that a medium such as glucose agar or liver agar fragments very badly. Unless very thinly seeded, in which case the *Cl. parabotulinum* is generally diluted out, it is difficult to get colonies large enough to pick out.

Still another difficulty encountered in isolation is the occurrence of a certain proportion of morphologically identical non-toxic colonies, a difficulty also experienced by other workers such as Bengtson (4). These non-toxic colonies are indistinguishable from the toxic, and the bacteria which grow from them in subcultures in meat media are in no way different from the typical toxic *Cl. parabotulinum bovis*.

Symptoms produced by " Cl. Parabotulinum bovis."

The following notes refer only to the symptoms seen in experimental animals after inoculation with pure cultures of *Cl. parabotulinum bovis*, or filtered toxin from them.

Rabbits.—When large doses are given, such as 0.1 to 1 c.c. of virulent material, death generally occurs in 8 to 12 hours if inoculation is done intravenously, intraperitoneally, or subcutaneously. Most

of the rabbits receiving these larger doses were inoculated about 3 p.m., and were found to have been dead for a few hours when examined next day at 8.30 a.m. When kept under continuous observation, symptoms were only noted about 2 to 3 hours before death, characterized by great prostration, inability to raise the head from the ground, and staggering inco-ordinated movement when the rabbits were made to walk. Before death, the animals become comatose.

When a smaller dose is given, 0.01 to 0.05 c.c., symptoms are usually noticed 24 hours later. They are the same as in acute cases but develop more slowly, and death does not usually take place until 6 to 8 hours after the first obvious symptoms are shown. It is in these less acute cases that one sees the symptoms most typical for the toxin, and most closely related to those described in the literature for *botulinus* A or B. Before marked symptoms develop, one notices that the rabbit is not able to balance itself properly when sitting up, but rocks backwards and forwards, paddling with the hind legs. Chewing movements of the jaws are usually seen although the animal is not eating. In the next stage the head is noticed to be sinking to the ground and may be actually resting on it. If touched, the animal raises its head, but rapidly sinks again, the lowering of the head being often jerky, as if the rabbit were dozing off to sleep and were trying to rouse itself. The control over the head and neck is rapidly lost, and the animal rests the head on the ground, turned to one side as if the neck muscles were out of control (see plate, fig. 4). At this stage it can still raise the head if disturbed, and if made to walk, the movements lack co-ordination. Finally, the animal may be found lying on one side quite limp, usually with the head bent towards the chest. In these acute cases no attempt is made to eat, and a greenish fluid is often seen round the mouth, deglutition being apparently interfered with.

With the minimum lethal subcutaneous dose, generally between 0.0005 and 0.001 c.c., symptoms do not usually appear for 48 to 72 hours and may last for 2 or 3 days. They are essentially the same as in acute cases, but develop more slowly. The animal has great difficulty in eating owing to the effect on the muscles of mastication, but usually it attempts to take food.

When less than a minimum lethal dose is given, a chronic case may be produced, but not with certainty. Indeed, any dose less than one m.l.d. is liable to produce no symptoms at all by subcutaneous inoculation. In a chronic case the symptoms are usually delayed for at least two days; but in some cases 4 or 5 days. The weakness, inco-ordination of movement, and difficulty in eating are then well marked. Symptoms last for a week or 10 days as a rule, and recovery may take place fairly rapidly. Owing to loss of condition during the attack as a sequel to difficulty in eating, many chronic cases subsequently die of cachexia. The best chronic cases observed in rabbits have been those receiving toxin per *os*. At least 1 c.c. is generally necessary to cause symptoms by this route, and such a quantity often does not kill. One very typical chronic case was produced in a rabbit inoculated subcutaneously with 0.5 c.c. of filtered toxin, which had been heated at 60° C. for 45 minutes. The filtrate before heating was extremely virulent, and fatal in doses of 0.001 c.c. subcutaneously.

Guinea-pigs.—The number of these animals used in the experimental work was very small compared with that of rabbits since the latter were much more easily obtained at the time.

The amount of virulent material necessary to produce symptoms in the guinea-pig is proportionally about the same as for rabbits. All types of cases were produced, from acute to chronic. The guinea-pig shows essentially the same type of symptoms as the rabbit, difficulty in mastication being an early and marked feature. The animal shows, in the early stages, salivation with a greenish discharge from the mouth. It lies on its chest, with the head resting on the ground. The eyes appear dull and watering, with constant blinking. If made to move, it crawls along without lifting its head. In the later stages it lies on its side, unable to move at all. According to the dosage the animal may die within 12 to 15 hours, may survive for 3 to 4 days, or may develop chronic symptoms, with subsequent recovery. Guinea-pigs, however, usually die since they do not appear to be able to stand starvation for long, and eating is difficult owing to paresis of the masticating apparatus.

White Rats.—These animals stand proportionately much larger doses than do rabbits or guinea-pigs. Of a toxin which killed rabbits in quantities of 0.001 c.c. subcutaneously, it needed at least 1 c.c. to kill a white rat. Doses of 0.1 c.c. or less had absolutely no effect. The symptoms shown are not very characteristic, but paralysis develops, and the animal is only able to crawl about slowly. With a dose of 1 c.c. subcutaneously no symptoms were shown until 48 hours after inoculation, and death did not occur until after 72 hours. With such an enormous " relative dose " it is even not quite certain that death can be wholly attributed to the specific toxin of the organism.

White Mice.—The resistance is comparatively high, and relatively large doses are necessary to kill. With 0.1 c.c. of a very virulent toxin, death occurred in 48 hours, smaller doses not producing symptoms at all. The symptoms are simply those of paralysis.

Fowls.—Even with a highly toxic filtrate symptoms have not yet been produced in these birds. 10 c.c. of a filtrate, which in a dose of 0.001 c.c. killed rabbits in less than 48 hours, had no effect at all when given subcutaneously. The fowl, therefore, resisted 10,000 minimum lethal doses for the rabbit.

Goats.—With the exception of cattle (see below), goats seem to be the most susceptible species of animal to injection with the toxin. The symptoms shown are weakness of the legs, followed by inability to rise at all. The animal lies with the head on one flank, is later stretched out flat, quite limp, and unable to move. Even in the last stages the tail and eyelids can still be moved. With large doses death may occur in twelve hours, but with doses such as 0.0002 c.c. per kilo it is usually delayed for forty-eight hours or longer. The virulence of different samples of toxin varies, but the dose 0.0002 c.c. represents approximately the minimum lethal dose for goats, of a pure culture in chopped meat medium after four days at 37° C. Doses of less than 0.0001 per kilo of this toxin produced no symptoms. With a dose of 0.00005 per kilo one goat showed stiffness, and had to be helped up when observed after symptoms had developed. This stiffness commenced two days after inoculation and continued for about a week, after which it disappeared.

Horses.—Only two have been inoculated with filtrate from a pure culture, and in neither did death occur. Survival was due to the small dose used, but very definite symptoms developed, very similar in both cases. The horses were inoculated subcutaneously with 0.5 c.c. and 0.1 c.c. of toxin respectively. The one which received the larger dose commenced to show symptoms on the day following inoculation, the other on the second day after.

The symptoms were as follows:—At first, muscular tremors of the trunk and shoulders, restlessness, moving from one leg to the other all the time. Not eating. Difficulty in swallowing developed, and water returned by the nose. Marked salivation occurred and food dropped from the mouth during attempts at mastication, but the tongue was not protruded.

The symptoms lasted about ten days, after which recovery slowly took place.

These two cases were quite definitely due to the toxin, and there is no doubt that larger amounts would have killed, as the toxin from impure cultures is fatal to horses if given in sufficiently large dose (see section of lamsiekte report dealing with the toxin).

Cattle.—Only a few cattle have been injected with the filtrate from pure cultures of *Cl. parabotulinum bovis*, and the cases so far produced have been acute or subacute, but attempts are still being made to produce chronic cases.

Two cattle were inoculated with toxin from pure culture in chopped meat medium incubated for seven days at 37° C. The culture was toxic for rabbits in a dose of 0.001 c.c. subcutaneously. One animal received 0.5 c.c. subcutaneously and the other 0.1 c.c. The latter animal never showed any symptoms subsequently, but the former showed stiffness of the muscles of the legs on the third day after inoculation. On the fourth day it was eating very little and was unable to rise by itself. Death occurred on the fifth day after inoculation.

An ox which was given 10 c.c. per *os* of toxin, of about the same virulence as the two other cattle received, showed symptoms of a peracute type about twenty hours later. It was not showing symptoms at 10 a.m., but was found down at 11 a.m., unable to rise. The breathing was dyspnoeic, the eyes staring, and the pulse fast and very hard. Death occurred at 12 a.m..

Lesions.

Very little need be said about these, as they are fully discussed in another section of the report on lamsiekte. In the small animals no lesions at all can usually be observed in subacute or chronic cases. In the acute cases, however, one usually finds hyperaemia and oedema of the lungs, hypostasis in the liver, and often congestion of the vessels of the small intestine. The bladder is nearly always greatly distended with urine. When inoculation is done subcutaneously, only a slight injection of the vessels at the site of inoculation can be observed. It is a curious fact that in spite of inoculation with grossly contaminated material containing a great variety of putrefactive bacteria, it is unusual, particularly in the rabbit, for any local reaction to develop.

CONCLUSIONS.

1. An organism of the *Clostridium* type can be isolated from material which produces lamsiekte when given to susceptible animals per *os*, or by inoculation subcutaneously, or by other routes. This organism has tentatively been named *Cl. parabotulinum bovis*.

2. This organism produces in culture media an exotoxin which, in unfiltered material or after filtration through a Berkefeld candle, produces typical symptoms of lamsiekte in susceptible animals.

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PLATE 1.

Fig. 1.—Chains of *Cl. parabotulinum bovis* showing portions which did not take the Gram stain.
Fig. 2.—An organism showing terminal spore.
Fig. 3.—Colonies of *Cl. parabotulinum bovis* showing the loose woolly form. Glucose agar shake culture.
Fig. 4.—A rabbit showing symptoms due to the toxin. The position with the head on one side and the staring eye are very characteristic.

SECTION VI.—OSTEOPHAGIA AND PHOSPHORUS DEFICIENCY IN RELATION TO LAMSIEKTE.

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IT is a generally recognized fact that over large areas of South Africa cattle show an "abnormal craving" or "depraved appetite," particularly for old bones lying about the veld. Indeed, the fact is so well known that farmers in many districts look upon it as a natural thing and only notice it when it becomes so pronounced as to constitute a public nuisance, i.e. when the cattle congregate around the homestead or kaffir huts, devour the washing, bags, clothes, riems, skins, and miscellaneous rubbish; and persistently return when driven away. Such acute manifestations are what is understood by "craving" in the worst districts, and a man brought up in such districts will quite ignore the more subdued "abnormal appetite" manifested only for bones, which is common on so many farms throughout the country.

The term "pica" has been used in the past, by the senior author and others, to denote the depraved appetite of the lamsiekte areas, but it is now desired to modify this terminology and use a word with a more restricted significance. In the ordinary dictionary sense, pica