Botulism (Parabotulism) in Equines.

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History.

In the course of the last twelve years a number of outbreaks in various parts of South Africa of a somewhat mysterious disease mainly occurring in mules came to our notice, whose aetiology at the time was uncertain and has only been cleared up within the last few years. The disease was diagnosed by us as spinal paralysis, and by others as cerebro-spinal meningitis, and declared itself in most instances as a paresis and paralysis of the locomotor organs, the animals going down suddenly and in fatal cases being unable to rise, or when placed in standing position, unable to remain standing. In the majority of cases the issue was lethal, the course lasting from a few to hardly more than 24 to 48 hours. There was never any fever recorded. A few recoveries that were noted took a very slow course, which lasted several weeks or months before the animals were again fit to work.

The main feature in all outbreaks was the sudden onset usually in animals that were employed in ordinary transport work, well cared for, properly fed and stabled. Usually a small number of animals were attacked within a few days when the disease would suddenly stop and no further cases occur.

In some instances it was noted that animals standing next to one another succumbed together. The idea of an intoxication presented itself, but no suggestion of a specific cause could be made. This cause was first looked for in the food which, however, in all instances where examination was still possible, did not show any abnormal constituents in the form of toxic plants. The idea of an infectious agency notwithstanding the absence of fever was from the beginning not lost sight of, and experiments to this effect were undertaken in the first instance.

(1) The first outbreak occurred amongst the mules of the Prison Department in Johannesburg, at the beginning of April, 1913. On the 5th April, blood of one mule (74) was sent to Onderstepoort where it was injected into one mule and two horses. On the 14.4.13 the blood of another mule (76) that had sickened the day before was sent and injected on the 15th into two mules and into two horses. (Results *vide infra*.) Subsequently, on the 16th May, three mules that had been ill for some time were sent to Onderstepoort for further observations. On arrival they were found to be but slightly ill. They recovered completely and were returned on 5.11.13.

(2) A second outbreak occurred in Johannesburg about the same time amongst the mules of the Johannesburg Municipality. On the 14.4.13 four mules went down and died of the disease which was then

diagnosed by the attending veterinarian as cerebro-spinal meningitis. Of all four mules material (blood, brain, spleen, liver, heart, kidneys), was sent to Onderstepoort for examination and experiment. Intrajugular and subcutaneous injections were made into horses and mules. On 8.5.13 another sick mule was sent to Onderstepoort. It was killed immediately after arrival and injections of blood and emulsions of brain, spinal cord, heart, lung, liver, spleen, and kidney were made into horses and donkeys. On the 14.5.13 material from four different dead mules was sent to Onderstepoort and again injected into horses and mules. Between the 20th April and the 13th May altogether ten mules sickened. Of these six were sent to Onderstepoort on the 16th May for observation. One died on the 17.5.13 and a second one on the 18.5.13. Four remained under observation until 20.8.13 and no further developments were noted. (For results of these injections *vide infra.*)

(3) A third outbreak occurred again in Johannesburg, viz. 15.12.14, amongst the mules of the Railway Cartage Department, when during the night a couple of mules went down with paralysis and both succumbed. This outbreak was investigated by one of us and post-mortems were made, but no definite diagnostic lesions were found. Three sick mules that sickened in the course of the next few days were taken to Onderstepoort for further examination. These three mules were kept under close observation. In March, 1915, they had not yet completely recovered. One was returned in 27.4.15, two on the 10th June, 1915. All recovered.

(4) In April, 1915, the officer in charge of the Laboratory, Grahamstown, reported the occurrence of spinal paralysis in two cart-horses grazing in the veld, which were found lying on their side and unable to rise, apparently quite paralysed in the hind legs. One of the horses died 40 hours later whilst the other, brought back into the stable, recovered after three months, being kept in slings all this time. The post-mortem of the one horse revealed no pathognomonic changes. The officer gave also the further information that nine similar cases were reported to him during the previous nine months, and that in Port Elizabeth, in the shed of a cartage contractor a similar outbreak had occurred.

(5) The fifth outbreak, on 8th July, 1917, was in Bloemfontein, amongst the mules of a merchant firm. Three mules were affected, and all died within the period of four days. All mules were kept in an open shed and doing daily regular light delivery work. The daily ration consisted of 7 lb. of forage, 5 lb. of lucerne hay, and 4 lb. mealies. The post-mortem was made by the Government Veterinary Officer, but no definite diagnosis could be made.

(6) The sixth outbreak was reported at the end of September, 1923, amongst mules of the Roads Department, Bloemfontein, on construction work Hoopstad-Bloemhof, when three mules died suddenly. Anthrax was excluded by examination of blood-smears. A post-mortem was made by the road inspector, who could not detect any changes. The animals had been fed on oats and lucerne hay. Lucerne hay, which was recently purchased in Bloemfontein, was suspected to be the cause. The mules began to die after they had consumed one bale. No poisonous plants were found in the remaining bales, a sample of which was fed at the Laboratory to a horse with no ill results.

(7) The seventh outbreak occurred in the neighbourhood of Bloemfontein amongst horses and also cattle of a farmer. The animals were fed on lucerne hay that had been bought on the 12th January, 1924, at the Bloemfontein market. The first horse sickened and died on the 13.1.24, showing symptoms of sudden weakness and colic. A second horse sickened on the 15th and died a week later; a third taken ill on the 15th was destroyed four days later; the fourth animal sickened on the 19th and died three weeks later. The latter two showed great muscular weakness, were unable to rise, but continued to feed. All horses were stable fed, two were never let out, three were allowed to graze.

There also died five cows fed on the same lucerne, all showed weakness and inability to rise; in some dribbling of saliva was observed. The disease in the cows resembled lamsiekte, but the farm was said not to be a lamsiekte farm. A sick bull lived for seven weeks. A week after taking ill it showed paralysis of the deglutitive organs and was unable to swallow for several days. No cases of similar nature had been noted before lucerne was fed and none after the exhaustion of the lucerne. Arsenical poisoning was thought of at the beginning, but was excluded by analysis of the lucerne. A horse fed with the material did not take ill.

(8) The eighth outbreak occurred amongst the transport mules of the Division of Veterinary Education and Research in Onderstepoort. One mule was found in lateral position on the morning of the 2.12.24 and died the same night. Its mate, next door neighbour, went down on the night of 5.12.24 and died on the 6.12.24. This incidence gave the starting point for research that led to the discovery of the cause and understanding of the nature of the disease. A dead, decomposed rat was found in the hayrack, just above the manger from which the mules had been feeding. The second mule was utilized for blood transfusion into two horses (for results *vide infra*). No outbreaks of a similar nature had been observed in the stables of the laboratories during the twenty years preceding this occurrence. The death of the rat was the result of the campaign against rats by means of poisons.

(9) The ninth outbreak occurred amongst the mules of a cartage contractor in Krugersdorp. It affected three mules. The first went down at 11.20 on the 16th April, 1925, and died at 4 p.m. the same day. The second mule sickened and went down on the 17th at 7.30 a.m. It died soon afterwards. The third mule was taken ill and went down on the 19th. It died at 4 p.m. on the 21st of April. Anthrax was excluded by microscopical examination of blood-smears.

Since at this time the aetiology of the disease had been cleared up, the suggestion was made that probably some dead animals, such as rats, had been decomposing in the forage. On inquiry it was stated that a dead rat, much decomposed, had been seen passing the chaff cutter, was cut up and became mixed with the feed that was consumed by the mules.

Aetiology.

A.—Results of inoculation experiments with blood of sick and of material of dead mules that had been suffering from the disease.

The first outbreak in Johannesburg involving a considerable number of mules and occurring about the same time at two different localities and amongst mules of two different owners suggested the

presence of a contagious disease, which had been diagnosed by the attending veterinarian as cerebro-spinal meningitis. Accordingly it was thought advisable to undertake transmission experiments by means of material taken from sick and dead animals and a series of injections were carried out : —

5.4.13. Mule 74, Prisons Department, 111. It was bled on 5.4.13 and 20 c.c. blood was injected intrajugularly on 10.4.13 into mule DOB 355. This mule was kept under observation until 10.6.13. No abnormalities were noted.

Horses DOB 7930 and 7932 each received intrajugularly 20 c.c. of the same blood. They were kept under observation for 81 days. No changes were noted.

14.4.13. Mule 76, Prisons Department, was bled on this date and the blood injected on 15.4.13 into two mules, DOB 479 and 5253, and two horses, DOB 8021 and 8022, each subcutaneously 10 c.c. and intrajugularly 20 c.c. The mules were kept under observation for 74 days and the horses for 76 days. No illness was noted during this period.

14.4.13. Inoculation experiments with blood and other material from the mules of the municipality. (1) Blood of municipality mule 710 was injected on 15.4.13 into DOB mule 5339, both subcutaneously (10 c.c.) and intrajugularly (20 c.c.). This mule died on the following day. No definite diagnosis was made, there was no pathognomonic changes found and the non-committal diagnosis "toxaemia" was made. (2) Of the same blood horse DOB 8010 was injected, both subcutaneously and intrajugularly, as above. The horse died three days later. There was likewise no definite diagnosis made. No typical changes were found and the non-committal diagnosis "toxaemia" was again made.

Blood of municipality mule 2388 was injected intrajugularly in the quantity of 20 c.c. and subcutaneously 10 c.c. into DOB mule 5544. This mule was kept under obsevation for 52 days. Horse DOB 7985 was injected in the same way and kept under observation for 20 days. No illness was recorded in the mule and the horse.

Blood of municipality mule 1849 was injected in the quantity of 20 c.c. intrajugularly and 10 c.c. subcutaneously into DOB mule 5212 It died on the second day. No diagnosis was made. No typical changes were found, and the diagnosis "toxaemia" was given. Horse DOB 7988 that had been injected with the same blood and in the same way went down on the second day and was killed *in extremis*. No diagnosis was made and no typical changes were found. The term "toxaemia" was again suggested for the disease. Brain substance of municipality mule 140 was injected intrajugularly 5 c.c. and subcutaneously 10 c.c. into horse DOB 7860. It died on the fourth day. This horse showed at the autopsy gelatinous exudate on neck and chest. Death was probably due to a secondary infection due to the injections (malignant oedema). Mule DOB 3987 was injected with the same material in the same way. It was kept under observation for 98 days. Mule DOB 5482 injected as above was kept under observation for 73 days. Neither of these two mules showed any illness during the time of observation. Municipality mule 140. Emulsions of spleen, liver, heart, kidneys were injected intrajugularly 5 c.c. and subcutaneously 10 c.c. into horse DOB 8025. The horse was kept under observation for 76 days. Mule DOB 4031 injected in the same way

was kept under observation for 98 days. Mule DOB 5498, also injected in the same way, was kept under observation for 98 days. None of these animals showed any illness.

8.5.13. Municipality mule 8101 was received on this day, and the following injections were made:—

Donkeys DOB 2926 and 3574 received subcutaneously 20 c.c. blood. They were kept under observation for 51 days.

Donkeys DOB 2550 and 5712 received intrajugularly 17 c.c. They were kept under observation for 51 days.

Horses DOB 8069 and 8075 each were infused and received 2,500 c.c. They were kept under observation for 53 days.

Mule 8101 was then killed and the material of the brain and spinal cord was emulsified and injected into horse DOB 8074 subcutaneously 20 c.c. and horse DOB 8072 intrajugularly 20 c.c. Both horses were kept under observation for 53 days and neither was noted to contract any illness. An emulsion of heart, lung, liver, kidney, spleen was injected into horse DOB 8079 subcutaneously 20 c.c. The horse was kept under observation for 42 days.

Horse DOB 8078 received intrajugularly 20 c.c. of the same material. It was kept under observation for 29 days. None of these animals sickened subsequently.

Of the third outbreak amongst the Railway Cartage Transport, three mules, Nos. 8987, 8988, and 8989, were sent to Onderstepoort in December, 1914, and transfusions were made in the following mules:—

From railway cartage mule 8987 infused into mule DOB 8915 = 1,000 c.c.; into mule DOB 8689 = 1,000 c.c.; into mule DOB 8691 = 2,500 c.c.

From railway cartage mule 8988 into mule DOB 8456 = 2,500 c.c.

From railway cartage mule 8989 into mule DOB 8692 = 2,500 c.c.

These mules were kept under observation for 46 days, during which time they were not observed to be ill.

Injections with the blood from the Onderstepoort mule DOB 15328. Two horses, DOB 16602 and 16603, were injected each with 250 c.c. taken *intra vitam*. Both horses were kept under observation for 52 days and no illness was recorded.

Discussion of Results.—It is of interest to note that the injection of blood from two municipality mules (1849 and 710) produced in the mules DOB 5339 and 5212 and DOB horses (8010 and 7988) a very acute disease that lasted but a very short time and ended with death without producing any characteristic lesions. Accordingly no definite opinion was expressed at the time, since the nature of the disease was not understood and the non-committal term "toxaemia" was employed to name the disease. From the two positive cases (8010 and 7988) subinoculations were made with blood into a horse and a mule respectively. The blood had been taken at the autopsy and was accordingly still fresh. The injected animals remained healthy. One mule subsequently died of a gangrenous pneumonia, which may or may not have been connected with the previous intrajugular blood injection.

The outstanding feature of these experiments is the fact that where fresh material was utilized for injections, no disease developed. This only occurred in the case of blood from two dead mules that was collected the day previous to the injection. The disease so produced

was diagnosed as "toxaemia." We have now no doubt that this "toxaemia" is identical with what we call "parabotulism," although not understood at that time. There is no difficulty to explain the fact that only the blood of two mules proved toxic. Animals that contract parabotulismus naturally consume with the food not only parabotulismus toxin, but also the organisms that produce it. These develop rapidly in the dead animal whose organs they invade, and naturally begin again to produce toxin. The quantity of spores ingested with the food probably determines the rapidity of the toxin production in the dead animal. The blood of living animals did not produce the disease because the organisms do not enter the blood-stream *intra vitam*. It is evident that in the contaminated blood after removal from the carcass and before the injection, the growth of the organisms continued and increased the quantity of toxin *in vitro*. Such did not take place in the blood that did not produce the disease, because at the time of its removal the circulating blood was not contaminated.

B.—*Bacteriological Investigation. Circumstances that led to the discovery of the cause.*

In the course of time as a result of clinical and pathological examination and particularly on account of the negative results from the earlier transmission experiments, the idea of contagious disease was given up. Meanwhile, however, the important discovery had been made (1919) that lamsiekte in cattle was caused by the ingestion of putrid carcass material, which contained a bacillus, producing a highly potent toxin, causing the disease lamsiekte in as small a quantity as 0.0001 c.c. per 1 kilo live weight. This bacillus was subsequently isolated and described as *Clostridium parabotulinum bovis* (Robinson).

The symptomatology of the disease in mules and the negative results of the autopsies resembled in certain forms, particularly the peracute and acute one, lamsiekte in cattle so much that from analogy with it a similar cause had been suspected for some time. This surmise verified itself almost in a dramatic manner. When the Onderstepoort outbreak occurred and when the two mules standing together and eating from the same manger succumbed, the idea of contaminated food at once occurred and the first suggestion was that probably a dead rat would be found in the forage. And, indeed, on examination, a dead rat, half decomposed, was found in the hay rack, just above the place where the mules were feeding. The rat was buried in the hay of the rack, which showed by its discoloration that it had been soiled with the putrefactive material emanating from the rat carcass. This rat now formed the starting material for further investigation.

(1) *Preliminary investigations with toxin from impure cultures made from the dead rat.*—The carcass was partially dried up. It was cut to pieces and ground with pestle and mortar into an emulsion with normal saline solution. Two rabbits were inoculated subcutaneously 5 c.c. of this emulsion. Both rabbits sickened. They showed symptoms akin to those noted in rabbits that are injected with parabotulismus toxin of lamsiekte. Both died.

A.—*Toxin prepared in raw liver medium.*—The rat emulsion was further utilized to initiate anaerobic cultures. For this purpose the raw liver medium as primarily utilized for the production of

lamsiekte toxin was utilized. The medium after inoculation was heated to 70° C. for one hour and then incubated for one week. On the eighth day a Berkefeld filtrate was made and two horses were injected:—

(1) 17.12.24. Horse D0B 15633 received subcutaneously 10 c.c. filtrate. In the morning of the 29th, the horse was found in a lateral position and unable to rise. It died the following day. The disease was diagnosed as spinal paralysis. The incubation period in this case was 12 days, the illness lasted 24 hours.

(2) Horse D0B 15266 received subcutaneously 5 c.c. filtrate. The horse was kept under observation for three weeks and remained healthy. Apparently the 5 c.c. filtrate did not contain the minimal lethal dose of toxin.

B.—Toxin prepared in chopped meat medium.—The test was first carried out on rabbits and guinea-pigs which all showed the typical symptoms of parabotulism. Accordingly experiments were planned with horses, and the following injections were carried out:—

(3) 27.2.25. At 2.30 p.m. horse D0B 16414 was injected intrajugularly 35 c.c. filtrate. At 9 p.m. the horse was down and unable to rise. Profuse sweating was present. Occasional kicking movements of the hind leg were noted. There was no loss of sensation in the skin. The horse died the same night. The incubation period lasted about 7 hours and the illness also but a few hours.

(4) 21.3.25. Horse D0B 14746 received intrajugularly an injection of 1 c.c. filtrate. 22.3.25, at 5 p.m. the horse went down and was subsequently unable to rise. It was soon comatose and the skin lost all sensitiveness. It died 1 hour later. The incubative period lasted over 24 hours and the illness a few hours.

(5) *Enteral Application.*—21.3.25. Horse D0B 16363 received 5 c.c. filtrate per os, diluted in 200 c.c. water. With the exception of a slight anorexia registered during the following days no abnormalities were noted. Apparently the toxin contained in 5 c.c. of the culture filtrate was not sufficient to act when applied through the digestive channel.

(6) 11.3.25. Horse D0B 16555 received 5 c.c. of a filtrate intrajugularly. At noon of the 12.3.25 the horse went down, unable to rise. The cutaneous sensation was present. The respiration became laboured. It died at 12.45. The incubation period lasted about 12 hours, the illness about 1 hour.

(7) *Enteral Application.*—11.3.25. Horse D0B 15710 received a whole culture per os. On the 13.3.25 at 3 p.m. the horse went down and lay stretched out on the left side. The cutaneous sensation was partly suppressed, weak movements of the legs were occasionally observed. Towards 4 p.m. marked dyspnoea appeared, and the horse died at 4.15 p.m. The incubation period lasted about two days and the illness little more than an hour.

(8) 31.3.25. Horse D0B 16289 received 0.3 c.c. filtrate intravenously. 2.4.26, 9 a.m., the horse was down, unable to rise. The respiration was dyspnoeic from the onset, the cutaneous sensation was absent. Occasional kicking movements of the hind legs were noted. It died at 10 a.m. The incubative period lasted 2 days and the illness 13 hours.

(9) *Enteral Application.*—Horse D0B 16352 received 20 c.c. of the filtrate per os. No illness was noted. The horse was later again used and for an intrajugular injection in (10).

(10) Horse D0B 16352. On 6.4.25 this horse received an intrajugular injection of 0.1 c.c. filtrate. 8.4.25, the horse was not eating well, mastication appeared somewhat difficult, on 12.3.25 the horse was very weak. It was in recumbent position, unable to rise, but when lifted on its legs it was able to stand. The incubation period lasted 2 days and the illness was light and the horse recovered.

(11) Horse D0B 16363. 18.4.25, injected 0.2 c.c. filtrate intrajugularly. In the afternoon the same day perspiration was noticed and again in the morning of the 19.4.25. Subsequently no further changes occurred.

(12) Horse D0B 16363. 2.5.25, injected subcutaneously 0.5 c.c. filtrate. On the 7.5.25 the horse was inclined to lie down. On the 8.5.25 it fell down to the ground. sweating profusely and showing marked dyspnoea. Five minutes later it rose; the pulse was about 80. This horse recovered. The incubation period lasted 5 days, the disease lasted about 24 hours, but the horse recovered.

Discussion of Results.—The preliminary toxicity trials of the impure toxin obtained from cultures in raw liver and chopped beef media produced an acute attack of the disease when injected intrajugularly in quantities 10 c.c. filtrate in the case of raw liver medium and in quantities of 0.3 c.c., 1 c.c., 5 c.c., and 35 c.c., in the case of chopped beef medium. Less than 0.3 c.c. of the latter (viz.: .1, .2, c.c.) produced a slight illness that was characterized by sweating. In one instance a subcutaneous injection of 0.5 c.c. also gave rise to but a slight illness with marked dyspnoea.

Positive results by the application per os were not obtained when the quantity of filtrate was 20 c.c. and less, but death resulted when a whole culture was given.

It was thus evident that the impure toxin was fatal to horses when applied in rather small quantities after intrajugular injection, but less so when given per os.

The disease produced showed a definite course, in all fatal cases characterized by an identical sequence of symptoms which were those of an acute paralysis of the locomotor system. In the case of the large quantity injected, the disease appeared after a few hours; when smaller quantities were given the disease appeared in from 24 to 48 hours; likewise in 24 hours in the horse that was dosed. A remarkable observation is the incubation period in the case of one horse that lasted 12 days. The symptoms after subcutaneous injection were of a passing nature in two horses and consisted in temporary sweating and anorexia and muscular weakness.

The Isolation of the Toxogenetic Organism.—The isolation of organisms of the parabotulismus type presented many technical difficulties, since the original material contained a number of sporulating bacteria. Since, however, the small animals present suitable subjects for testing the toxicity, the colonies could be tested in all stages as to their pathogenic effect.

At an early stage it was recognized that the spores of the toxogenetic bacterium were very heat resistant. They would stand boiling (in chopped meat culture) for 60 but not for 75 minutes. In this way the number of organisms was reduced to about 5 or 6 resistant types. Isolation of cultures was then attempted through the method of deep shake cultures in tubes containing glucose agar or liver agar, or in surface cultures on horse blood agar in anaerobic

jars. Isolated cultures were enriched in tubes of meat medium and tested after 3 days' incubation at 37° C. by giving 2 c.c. of the fluid to guinea-pigs per os. Subcutaneous injections were also carried out in some instances. The methods mentioned, however, did not lead to the isolation of the organism, no toxic colonies being obtained in this way.

A toxic substance was obtained from mixed cultures grown in exhaust media made from filtrates of non-toxic bacteria. It was thought that in such a medium the toxogenetic organism might become dominant and thus easier to be separated by colonization. In this manner a few colonies were obtained in a glucose agar shake culture, which when transplanted into chopped beef medium produced a toxin that given per os to guinea-pigs produced the disease. These cultures were, however, not yet pure. Three different types of organisms were still present. Microscopic examination, however, did show that a gram positive organism corresponding to the parabotulismus type was present.

Finally an attempt was made to colonize the toxogenetic organism in a medium containing a non-sporulating organism. A shake culture was made with a coccus. This coccus grew in lenticular-shaped colonies that on microscopical examination were shown to include a few gram positive bacilli. These colonies were transplanted and incubated for three days to allow time for sporulation. The coccus was then killed by heating for one hour at 75° C. and sub-cultures now yielded the toxogenetic organism in pure culture. Sub-cultures into glucose agar shakes gave, however, more often than not, no result. In spite of very heavy sowing and incubation at various temperatures and in different media, only rare colonies developed. These colonies were of the fluffy type met with in parabotulismus types. Filtrates of these cultures proved toxic for laboratory animals and were tested on the domesticated animals.

Description of the Organism.

Morphology.—The organism is a bacillus 4 to 8 μ in length by .8 to 1 μ in width. It is non-motile. Chains of varying length but often containing 8 to 10 organisms are formed. Pairs are frequent.

Staining Reactions.—It stains with all the usual basic aniline dyes and is gram positive. Many gram negative bacilli are seen even after two or three days, and in cultures a week old most of the bacteria are gram negative. There is a tendency for certain organisms in a chain not to take the gram stain and some are partially stained.

Sporulation.—This occurs in about 36 hours in chopped meat medium. Spores are not produced in great numbers and often are only found after a prolonged search. In Hibler's medium they do not appear to be formed except in rare cases. Hibler cultures have in isolated instances given a growth in subcultures heated to 70° C. for one hour. The spores are 1.5 μ in length by 1 μ in width. They are terminal and slightly distend the organism.

Cultural Characteristics.—These are briefly as follows :—The organism is a strict anaerobe.

Chopped Meat Medium.—Marked gas production, but no turbidity. There is no change in the meat, which never becomes red or black. If it were not for the gas production no growth would be noticed with the naked eye.

Hibler's Brain Medium.—Marked gas production with slight turbidity of the fluid occurs. The latter clears up subsequently and the bacteria settle on the top of the brain material. No change in the brain substance itself is observed.

Glucose Liver Broth and Ordinary Liver Broth.—Marked turbidity occurs which clears up, leaving a whitish deposit at the bottom of the tube, rather fluffy in character. If the tubes are examined before the growth settles out, it will be seen that it consists of a fine flocculi only visible with a hand lens.

Serum Broth.—A slight turbidity occurs which clears up leaving a whitish fluffy deposit at the bottom of the tube. Some of the growth may adhere to the sides of the tubes.

Ordinary Broth and Glucose Broth.—Very poor growth, if any at all, takes place. A slight turbidity occurs which settles out.

Surface Growth.—So far no growth has been observed on the surface of any medium in anaerobic jars. Contrary to what is seen in *Cl. botulinum A* and the other parabotulinus types so far described, it is difficult to obtain colonies of *Cl. parabotulinum equi* in deep shake cultures. Colonies have frequently failed to develop in glucose agar shake cultures (Vignal tubes) inoculated with three platinum loopfuls of a good growth in chopped meat medium. In glucose liver agar a few colonies usually develop, but they are never numerous. In *agar* and *glucose agar* shake cultures, colonies when they occur are of the fine fluffy type, resembling pieces of cotton wool, and they do not appear dense in the centre. They develop to a diameter of 3 to 4 mm., but then usually break up and fade.

In glucose liver agar colonies are much more dense in appearance than in glucose agar and may have dense centres with fluffy outgrowths from them. Fragmentation of the medium occurs if there are a number of colonies.

Subcultures have been made from single colonies into chopped meat medium and have given a typical growth, the medium proving highly toxic for small animals and producing typical symptoms of parabotulism.

Sugar reactions were done with the organism using ordinary liver broth as a basis for the media. Difficulty was experienced at first in finding a suitable medium in which the organism would grow fairly well after the sugar present had been removed by the growth of another organism. Eventually it was found that liver broth in which *B. coli* have grown for 38 hours would, after sterilization and filtration, give a good growth of *Cl. parabotulinum equi*. To this exhausted medium the various sugars, etc., were added and Andrade's indicator was used.

Growth with gas production occurred in all the sugars and other substances tried. Liver broth tubes were used to which were added glucose, lactose, maltose, laevulose, galactose, starch mannite, dulcite, inulin, saccharose, salicin, and glycerine.

The production of acid in any of the media was not very marked, but occurred in glucose, lactose, maltose, laevulose, and starch.

It is doubtful whether the sugar reactions will be of great use in the differentiation of the botulinus and parabotulinus types as comparative tests carried out with them have not shown any marked difference between them.

The Toxin.—This is formed in culture media incubated at 37° C., but also at summer room temperature (20° C. to 26° C.). As long as the cultures actually grow there seems to be little difference between the amounts formed at temperatures suitable for growth. After three days' incubation at 37° C., the toxin produced in chopped meat medium is highly potent and further incubation does not materially increase the potency. A Berkefeld filtrate of a toxic culture is highly toxic, and a dose of .0001 c.c. inoculated subcutaneously into a guinea-pig is frequently fatal. By the mouth in this animal .1 to 1 c.c. of the filtrate are required to produce the symptoms of the disease. It has been noted that the fluid portion of a chopped meat culture appears to lose some of its toxicity by passing through the filter as it is less toxic after filtration than before. It has been observed in addition that symptoms of parabotulism could be produced in a horse by dosing it per os with 20 c.c. of a chopped meat toxic culture, whereas toxic cultures from the same batch when filtered through a Berkefeld filter gave a filtrate of which 100 c.c. was required to produce the disease in a horse per os.

A few experiments carried out to determine the resistance of the toxin to heat, light, etc., show that it has considerable stability. The tests were carried out with a Berkefeld filtrate with a minimum lethal dose for guinea-pigs of .001 c.c. and in testing the filtrate after exposing the various conditions, 100 M.L.D. were given to a guinea-pig subcutaneously.

Exposure to direct sunlight for 36 hours had no appreciable effect on the virulence of the toxin. Heating to 60° C. for two hours reduced the virulence slightly as judged by the incubation period of the toxin in guinea-pigs. 60° C. for one hour had no effect on the toxin. Heating to 70° C. for two hours or longer or heating to 80° C. or higher for even half an hour rendered the toxin inactive. In the preparation of cultures for inoculation of rabbits for serological tests, it has been found that the organisms washed three times in normal saline had subsequently to be heated to 70° C. for one hour to render the little remaining toxin inactive. A temperature of 60° C. for one hour has failed to inactivate the toxin and the rabbits undergoing inoculation with the bacteria died from typical parabotulism.

Tests of filtrates cannot be compared to tests of the toxin as it occurs naturally in decomposing carcasses, but they give some idea of the resistance under artificial conditions.

Experimental Parabotulism produced by Berkefeld Filtrates of Pure Cultures.

The symptoms produced in small animals do not differ in any way from those seen after inoculation or dosing per os with unfiltrated toxin. They are also identical with those produced by the true botulinus toxin or any of the parabotulinus types. The train of symptoms is as follows:—

Rabbits.—Inco-ordination of movement is the first symptom noted and the eyes have a staring appearance. There is usually difficulty in eating and salivation is well marked. Great muscular weakness sets in and the head is rested on the floor of the cage, often turned to one side. At first the head can be lifted if the animal is disturbed, but later it cannot. In the later stages there is complete paralysis,

the animal lying stretched out on its side, all the muscles being completely relaxed. Death occurs within 24 hours with large doses of toxin such as 100 to 1,000 M.L.D., but may be delayed for as long as 9 to 10 days with doses close to the minimum lethal one, symptoms only appearing after 4 or 5 days. Chronic cases with ultimate recovery sometimes occur with very small doses or in toxin anti-toxin experiments. Several rabbits have shown symptoms with recovery when inoculated with toxin heated to between 60° and 70° for varying periods in attempts at attenuation.

Guinea-pigs.—The symptoms are essentially the same as in rabbits. Salivation is usually marked and muscular relaxation very noticeable. When the symptoms are well developed, the animal frequently lies with its legs spread out in different directions while still in the normal sitting position, though the head cannot be raised.

White Rats, House Rats, and White Mice.—Symptoms of paralysis are seen. Smaller doses than .1 c.c. did not kill rats, and 1 c.c. per os had no effect.

Cats and *dogs* are insusceptible as are fowls. In each case 5 c.c. subcutaneously produced no symptoms, a dose equal to 250 M.L.D. for the horse.

Pigs are insusceptible, 5 c.c. subcutaneously producing no effect.

Toxin Anti-toxin Experiments.—At present an efficient anti-toxin against the toxin of *Cl. parabotulinum equi* has not been obtained, but experiments in immunization against it are being carried out. An efficient anti-toxin has been prepared against the very similar organism producing lamsiekte in cattle, which has provisionally been called *Cl. parabotulinum bovis.* Polyvalent anti-toxin against *Cl. botulinum, A* and *B* types, has been obtained for comparison purposes. In the toxin anti-toxin tests so far carried out it has been found that the anti-toxin against the *Cl. parabotulinum bovis* protected guinea-pigs only against the toxin of lamsiekte, the toxic effect of the equine organism not being influenced. With the polyvalent botulinus anti-toxin, only botulinus toxin was neutralized.

TABLE 1.

Results with Cl. Parabot. Bovis Anti-toxin.

Guinea-pig.	Toxin.	Anti-toxin.	Result.
1	*Cl.parabot. bovis*, 10 M.L.D.	*Cl. parabot. bovis*, 5 c.c.	Survived.
2	,, ,, ,,	,, ,, 1 c.c.	,,
3	,, ,, ,,	None.	Died in 36 hours.
4	*Cl. parabot. equi*, 2 M.L.D.	Anti-toxin, 5 c.c.	,, 72 ,,
5	,, ,, ,,	None.	,, 72 ,,
6	*B. parabotulinus* (Seddon), 2 M.L.D.	5 c.c.	,, 60 ,,
7	,, ,, ,,	None.	,, 72 ,,
8	*Cl. botulinus* C, 2 M.L.D.	5 c.c.	,, 90 ,,
9	,, ,, ,,	None.	,, 90 ,,
10	*Cl. botulinum* A, 2 M.L.D.	5 c.c.	,, 72 ,,
11	,, ,, ,,	None.	,, 96 ,,

Table 2.

Results with Polyvalent Botulinus A and B Anti-toxin.

Guinea-pig.	Toxin.	Anti-toxin.	Result.
1	*Botulinus* A, 2 M.L.D.	1 c.c.	Survived.
2	,, ,, ,,	None.	Died in 90 hours.
3	*Cl. parabot. equi*, 2 M.L.D.	1 c.c.	,, 90 ,,
4	,, ,, ,,	None.	,, 72 ,,
5	*B. parabotulinus* (Seddon), 2 M.L.D.	1 c.c.	,, 60 ,,
6	,, ,, ,,	None.	,, 72 ,,
7	*Cl. botulinum* C, 2 M.L.D.	1 c.c.	,, 72 ,,
8	,, ,, ,,	None.	,, 80 ,,
9	*Cl. parabot. bovis*, 2 M.L.D.	1 c.c.	,, 72 ,,
10	,, ,, ,,	None.	,, 60 ,,

The minimum lethal doses of the toxins were previously determined by guinea-pig inoculation and are approximate.

It has not yet been determined whether the organisms provisionally called *Cl. parabotulinum equi* is distinct from *Cl. botulinum C.* or the parabotulinus organism of Seddon, but as soon as anti-toxins against the latter two organisms are available, further comparative tests will be carried out.

Judging from extreme toxicity of the equine toxin and the clinical symptoms produced, it would seem to be different from both the above toxins, but toxin anti-toxin tests are the only conclusive comparative ones.

Experiments with Toxin of Pure Cultures in Domesticated Animals.

I.—*Parenteral Application.*

(1) 9.3.26: Horse DOB 17000 was injected subcutaneously 1 c.c. toxin 302. 10.3.26: The horse was found down on the next morning and unable to rise; it showed dyspnoea and symptoms of cyanosis in the mucous membranes of the head. At 10 a.m. the horse was dead. The incubation period and course of the disease in this case lasted 24 hours.

(2) 9.3.26: Donkey DOB 16083 was injected subcutaneously 1 c.c. toxin 302. 10.3.26: It was found next morning in a lateral position, unable to rise and remained in this position until death. The animal died at 8.30 a.m. Incubation period and course of disease lasted less than 24 hours and were even shorter than in the horse.

(3) 12.3.26: Donkey DOB 16086. Injected subcutaneously 0.2 c.c. toxin 302. 14.3.26: The donkey was found dead next morning. Incubation period and illness lasted less than 48 hours.

(4) 12.3.26: Horse DOB 17001. Injected subcutaneously 0.3 c.c. toxin 302. 14.3.26: The next morning the horse was found in lateral position, stretched out, unable to rise. It showed complete paralysis. The temperature was normal during the period of observation. It died during the course of the day. In this case the incubation period was less than 48 hours and the symptoms lasted less than 12 hours.

(5) 16.3.26: Horse DOB 17002. Injected subcutaneously 0.05 c.c. toxin 302. 18.3.26: 2 p.m., the horse was in a lateral position stretched out, unable to rise. At intervals it made but feeble kicking movements of the hind legs. The sensitiveness of the skin over the back and neck was diminished; it appeared to be normal over the legs. The eye reflex was normal. 4 p.m.: The cutaneous sensation was much reduced. The horse had difficulty in moving the legs. There was marked perspiration present. Dyspnoea was pronounced. The animal subsequently became comatose and died at 11 p.m. The incubation period lasted 48 hours, the illness less than 12 hours.

(6) 16.3.26: Donkey DOB 16157 was injected subcutaneously 0.03 toxin 302. 18.3.26: 8.30, the donkey was found in lateral position, unable to rise, but feeble movements were carried out with the legs. The sensitiveness of the skin of the body was absent, on the legs and nose it was still present. There was marked dyspnoea; the pulse was 60, it was full and strong. The donkey died two hours later at 10.30 a.m. Incubation period and course of disease lasted about 48 hours, the illness only a few hours.

(7) 24.3.26: Horse DOB 16313. Injected 0.005 c.c. toxin. The horse remained healthy. It was subsequently injected with a larger quantity of toxin, *vide* Sub. (10).

(8) 24.3.26: Horse DOB 17003. Injected subcutaneously 0.01 c.c. toxin. The horse remained healthy. Subsequently injected with an increased quantity of toxin, *vide* under (9).

(9) 30.3.26: Horse DOB 17003. Injected subcutaneously 0.025 c.c. toxin 302. 1.4.26: 9.30 a.m., the horse was found lying in lateral position, unable to rise. It remained in this position all day long. 2.4.26: 7 a.m., the horse was found in the same position. There was marked dyspnoea and muscular tremor was noticed. At 4.30 p.m. the horse was in the same position. The pulse was 120. The reflexes were still present over the whole body. Profuse perspiration was noted. Every few minutes short spasmodic tremors occurred. The temperature during this period was never above 101° F. The horse died 9 p.m. The incubation period in this case was less than 48 hours, the illness lasted about 12 hours.

(10) 30.3.26: Horse DOB 16313. Injected 0.01 c.c. toxin 302 subcutaneously. 2.4.26: 7 a.m., the horse was found stretched out in lateral position, it was unable to rise, struggling and kicking movements with all legs were noted. At 4.30 p.m. the horse was in the same position. Short spasmodic tremors were seen. Profuse sweating and marked dyspnoea were present. All reflexes were present. The pulse was 88, the number of respirations 28. The horse died during the night. The incubation period was a little less than 3 days; the illness lasted over 12 hours.

(11) 9.4.26: Mule DOB 3421. Injected 0.0175 c.c. toxin subcutaneously. 13.4.26: 8.30 a.m., the mule was found in sternocostal position, unable to rise. The nose rested on the ground, the head was raised when the animal was aroused. The pulse rate was 52, the respiration 18. At 4 p.m. the animal was found in lateral position and comatose. The sensitiveness of the skin was slightly reduced. The respiration was not dyspnoeic. The mule died at 8.30 p.m. The incubation period lasted 4 days, the illness about 12 hours.

(12) 23.4.26: Mule DOB 9025. Injected 0.015 c.c. toxin 302 subcutaneously. 28.4.26: 8.30 a.m., the mule was found in lateral

position, unable to rise on to the legs, but can assume the sterno-costal position and in this position is noted to nibble at its food. The respiration was accelerated. 29.4.26: At 8.30 the animal was found in lateral position and unable to take up the sterno-costal position. At times it carried out kicking movements of the legs. The respiration was dyspnoeic, the pulse was accelerated and weak. There was but slight cutaneous sensation. Coma was progressing during the morning and the mule died at 11 a.m. The incubation period lasted about 5 days, the disease less then 48 hours.

II.—Enteral Application.

(1) 9.4.26: Mule DOB 9025. 2 c.c. toxin given per os. The mule remained healthy.

(2) 21.4.26: Horse DOB 15002. Drenched 4 c.c. filtrate 493. The horse remained healthy. Subsequently again used with larger quantity of toxin, *vide* Sub. (3).

(3) 10.5.26: Horse DOB 15002. Drenched 10 c.c. filtrate 493. The horse remained healthy.

(4) 30.5.26: Horse DOB 17130. Drenched with 30 c.c. filtrate of toxin 493. The horse remained healthy. Subsequently again used Sub. (5).

(5) 16.6.26: Horse DOB 17130. Drenched 30 c.c. of culture 828 at 11 a.m. 17.6.26: At 4 p.m., slight salivation was noted. At 9 p.m., muscular tremor. 18.6.26: 8.30 a.m., the horse was found prostrated, showing marked dyspnoea. It was already comatose and died an hour later. The incubation period lasted about 17 hours, the illness over 24 hours.

(6) 25.6.26: Horse DOB 17357 received 30 c.c. toxin per os. There was no result. 23.7.26: The horse received 100 c.c. filtrate per os. 5.8.26: The horse was noted to be weak and went down. It was able to rise when assisted and was then moved into a loose-box, being still able to walk. 6.8.26: The horse was prostrate on one side and unable to rise; it was conscious and moved neck and limbs from time to time. 7.8.26: There was no change. The horse when raised was unable to stand. The reflexes were still present. The pulse was rapid and weak, 84 per minute. Respiration was 24 per minute and shallow. 8.8.26: No change in the condition was noted. 9.8.26: The pulse rate was increased to 90; it was very weak. Respiration, 30 per minute. Reflexes still present. 10.8.26: The horse died 4 p.m. The incubative period in this case was 13 days; the illness lasted about 5 days. This case represents the subacute form of parabotulism.

Discussion of Results.—The experiments were carried out on horses, mules, and donkeys, and the toxin was applied by subcutaneous injection or by ingestion (dosing, drenching). In the case of horses the minimum effective quantity subcutaneously injected was 0.01 c.c., in the mule 0.15 c.c., and in the donkey 0.03 c.c. The dose of 0.01 c.c. did not affect one horse that subsequently succumbed to the 0.025 c.c. dose. Quantities of 0.05, 0.3, 0.1 c.c. were in all instances effective. A mule injected 0.0175 c.c. also succumbed. Quantities of 0.2 and 1 c.c. toxin killed the injected donkeys. In the enteral application quantities of less than 30 c.c. did not prove toxic, 30 c.c. proved toxic in one of three cases. In this positive case, however, both culture and toxin were given, whereas the filtered toxin was ineffective; the toxin, however, was that of a different culture. This observation

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would correspond to what happens under natural conditions, when of course both organisms and toxin would be ingested. After the quantity of filtered toxin had been increased to 100 c.c. a positive result was obtained.

The disease observed after subcutaneous injection was in all cases a rapid one and after a short incubation period, lasting from less than 24 to 24 and 48 hours in horses, in the case of a mule it lasted 4 days. In a donkey the incubation period lasted 24 to 48 hours. The onset of the disease was a sudden one, the animals without any warning went down and died within a few to 24 and 48 hours, so that the course may be described as a peracute and acute one.

The disease noted after drenching had in one instance an incubation period of about 24 hours and lasted about the same time. In a second case the incubation period was 13 days and the disease itself lasted five days. This then represents a subacute case. Similar observations have been made on parabotulismus bovis toxin, a longer incubative period being usually succeeded by a longer course of the disease.

Ruminants.

(1) 9.3.26: Bull DOB 2041. Subcutaneous injection of 1 c.c. toxin 302. 11.3.26: 8 a.m., the ox was found standing and very dull, salivating profusely and the tongue protruding. It was unable to eat. It died during the night. The incubation period lasted 2 days and the illness a little more than 12 hours.

(2) 12.3.26: Ox DOB 2042 injected subcutaneously 0.3 c.c. toxin 302. 14.3.26: The ox was noted to be salivating a lot; it appeared to be very weak and was most frequently in the sterno-costal position. There was no rise in temperature. The minimum recorded was 102.4° F. 15.3.26: The ox died at 8.30 a.m. The incubation period lasted about 48 hours, the illness about 24 hours.

(3) 16.3.26: Ox DOB 2053. Injected subcutaneously 0.03 c.c. toxin 302. The ox remained healthy. It was subsequently again used, *vide* Sub. (4).

(4) 30.3.26: Ox DOB 2053 injected subcutaneously 0.08 c.c. toxin 302. 3.4.26: The animal was found in costo-lateral position and slightly salivating. When forced it rose with difficulty. 4.4.26: 8.30 a.m., the ox was lying in lateral position, stretched out. It was able to raise the head slightly. The pulse was 70, the respiration 60, but not laboured. The eye reflexes were present. The cutaneous sensation was noticeable all over the body. The ox died during the night. The incubative period lasted 4 days, the illness less than 24 hours.

Discussion of Results.—The quantities that proved toxic were 0.08 c.c., 0.3 c.c., and 1 c.c. The incubation periods lasted two days in two instances, 5 days in the third one. The disease lasted from 12 to 48 hours. The symptoms resembled those of lamsiekte very closely, particularly those that pointed to a paralysis of the tongue and of the deglutitive organs.

Goats.

(1) 9.3.26: Goat DOB 13996 was injected subcutaneously 0.1 c.c. toxin 302. 11.3.26: It was found dead in the morning. Incubation period of illness lasted less than 48 hours.

(2) 12.3.26: Goat DOB 14378 was injected subcutaneously 0.02 c.c. toxin 302. 15.3.26: The animal appeared to be weak.

There was slight salivation present. 16.3.26: The goat died at 8.30 a.m. The incubation period lasted about 3 days and the illness about 24 hours.

(3) 16.3.26: Goat DOB 14387 injected subcutaneously 0.004 c.c. toxin 302. The goat remained healthy.

(4) 30.3.26: Goat DOB 14387 injected subcutaneously 0.004 c.c. toxin 302. The goat remained healthy.

(5) 26.4.26: Goat DOB 14387 injected subcutaneously 0.1 c.c. toxin. 29.4.26: The animal showed weakness, but was feeding. 30.4.26: The condition the same as on the day before. 1.5.26: The animal was in a lateral position, unable to rise. 2.5.26: The goat died during the night. The incubation period lasted about 3 days, the illness lasted slightly more than 2 days.

(6) 26.4.26: Goat DOB 10295 injected subcutaneously 0.005 c.c. toxin. No changes noted during the succeeding days. Subsequently was injected under (7).

(7) 3.5.26: Goat DOB 10295 injected subcutaneously 0.01 c.c. toxin. 6.5.26: The goat was down, unable to rise. It died 4 p.m. The incubation period lasted about 3 days, the disease about 12 hours.

(8) 3.5.26: Goat DOB 12213 injected subcutaneously 0.005 c.c. toxin. 9.5.26: Polypnoea present. The goat was feeding. 10.5.26: Polypnoea was still present. The goat was lying, but rose on its own accord. It was not eating. 11.5.26: The goat died at 8.30. The incubation period lasted 6 days, the illness about 48 hours.

Discussion.—The toxic doses for goats were 0.005 c.c. and 0.1 c.c. respectively in two cases and 0.02 in one instance. The quantity of 0.004 c.c. in two instances did not prove toxic. The incubative periods varied from 24 hours to 6 days and the disease from less then 24 to 48 hours. The symptoms resembled that of lamsiekte in goats very closely.

Sheep.

(1) 9.3.26: Sheep DOB 11401 injected subcutaneously 0.1 c.c. toxin 302. Found dead in the morning of the 10.3.26. Incubation period and illness lasted less than 24 hours.

(2) 16.3.26: Sheep DOB 13059 injected subcutaneously 0.002 c.c. toxin. The sheep remained healthy. 30.3.26: Sheep 13059 injected subcutaneously 0.008 c.c. toxin 302. The sheep remained healthy.

(3) 12.3.26: Sheep DOB 9582 injected subcutaneously 0.02 c.c. toxin 302. 14.3.26: The sheep appeared weak. There was slight salivation. 15.3.26: The sheep was found dead in the morning. The incubation period was about 24 hours and the illness lasted about 2 days.

Discussion of Results.—The toxic doses were 0.02 c.c. and 0.1 c.c. 0.002 and 0.008 did not prove toxic. The incubative period lasted less than 24 hours and the disease from a few to 48 hours.

EXPERIMENT TO NOTE WHETHER THE DECOMPOSITION OF ANY RAT CARCASS WILL PRODUCE TOXINS.

Experiments on Horses with Contaminated Food.

For this purpose a number of rat carcasses were placed between bales of lucerne hay where they underwent decomposition. When this was complete, the lucerne hay of the lower bales was fed to horse DOB 14988 from 6.3.25 until 25.5.25. The hay was mixed with other food and readily consumed. The horse went down on two occasions,

viz. 9.4.25 and 13.4.25, but was able to rise and no other symptoms were noted. The results were not considered conclusive, but they at least showed the fact that decomposition of a rat does not necessarily mean contamination of the foodstuffs.

Experiments on Guinea-pigs.

Rats were caught in traps in the various stables and stores on the Station. The carcasses were allowed to decompose in ordinary fruit jars at room temperature. The tops of the jars were not screwed down sufficiently tight to prevent the access of air, but flies were effectively kept out.

Experiment No. 1.—18.3.26: Six rats were killed, two of them were previously fed on food contaminated with cultures of *Cl. parabotulinum equi.* After decomposing for 10 days, 2 c.c. of the liquefaction products of each rat were administered to a guinea-pig per os. None of the guinea-pigs subsequently showed symptoms. Cultures in chopped meat, heated to 70° C. for 1 hour, made from material in each jar, did not produce the toxin.

Experiment No. 2.—6.5.26: Six rats were killed, two of which had been eating food contaminated with cultures of *Cl. parabotulinum equi,* 24 hours previously. Decomposed material taken from both rats that had been feeding on the contaminated food was toxic for guinea-pigs after two weeks' decomposition at room temperature. Material taken from the other four proved non-toxic.

Experiment No. 3.—Two rats which died in the cage after being caught were allowed to decompose. Both showed heavy infections with Tr. Lewisi which was the probable cause of death. After two weeks' decomposition the material from each rat was given in a dose of 2 c.c. to a guinea-pig per os. No toxic effect was noted. Cultures were made from each rat into chopped meat medium which was then heated to 70° C. for 1 hour. The cultures did not develop toxin.

Experiment No. 4.—19.7.26: Ten rats were killed and allowed to decompose for 2 weeks. The material from them did not prove toxic for guinea-pigs.

Preliminary Conclusions.—Rats caught in the stables at Onderstepoort and allowed to decompose and contaminate food did not produce toxin. Rats fed on food contaminated with *Cl. parabotulinum equi* cultures developed toxin when undergoing decomposition.

Symptomatology of the Parabotulismus in Equines.

Two distinct forms of disease are observed; an acute and a chronic one; a few cases may be described as subacute.

The Acute Form.—It has very often a peracute course only lasting a few hours. The animal is usually found lying in a lateral position. Frequently in such cases no attempts to rise are made, the animal soon becomes comatose and dies. In other cases attempts to rise are observed, a fierce struggling both with front and hind legs is observed, and the animal gives the impression of being in pain. In some instances the first symptoms noted were interpreted to be those of colic. Usually from the beginning polypnoea is present which later goes over into marked dyspnoea and is usually succeeded by distinct asphyxia. Consciousness in the dyspnoeic stage may or may not be present. For some time after it has gone down, the animal can often be seen turning ears and eyes in the direction of an approaching person, thus still taking notice of what happens around it. Attempts

to raise the head are frequently made, but it usually falls back to the ground. Profuse perspiration can be seen in some animals in various parts of the body and covering sometimes a large surface. In other cases perspiration may be absent. In cases of a less acute course, lasting up to 24 hours, the impossibility of rising is equally marked and when such animals are placed on their feet, they are unable to support themselves. Also in these cases dyspnoea is usually present from the beginning and becomes more and more marked in the course of the succeeding few hours and is finally superseded by asphyxia. More often such horses retain consciousness for a considerable time, judging from the movements of ears and eyes, when they are approached. Violent movements of legs, both fore and hind, more particularly the latter, are carried out, more frequent in the beginning, less so towards the end, and may completely disappear when coma supervenes. In some cases, however, there were practically no or but few movements during the course of the disease, the animal soon becoming comatose. Sensitiveness of the skin is sometimes absent or reduced in the more acute cases, but is present in the less acute ones, even hyper-sensitiveness being noted in some animals. When the skin is pricked, the animal reacts sometimes with violent kicking movements of the hind legs. The reflexes are usually present; in particular the pupil and cornea reflex, as well as the anus and tail reflex. Also the patella reflex could be evoked in a few cases that were examined in this direction. The animals whilst lying have been seen to defaecate and also to urinate. Faeces and urine were of normal appearance. In some cases the sensitiveness of the skin was reduced or absent on the dorsal portion of the body and present on the ventral side. Also a reduced tail reflex was observed, the animal making no attempt to resist when the tail was lifted. The temperature remained normal, and this having practically been found in all cases is considered to be characteristic of the disease. The pulse rate is in the beginning quite normal for a while, subsequently it is increased. In the course of a few hours the quality may change from a pulse with a good excursion and good pressure to that of low excursion and small pressure.

  Subacute and Chronic Cases.—In less acute and in subacute cases the animals, although unable to rise, can stand when lifted on their legs and even walk. The walk, however, is unsteady; there appears to be muscular weakness present and the animals will soon lie again or even fall. The animal may succeed in standing by itself a second or third time, but the muscular weakness seems to be on the increase and finally it can no longer stand. Such animals may then lie for several days; they may or may not improve during this time. If they do improve, recovery is very slow and the case may develop into the chronic form. Such cases may last weeks and even months. Recoveries seem to be assisted when the animals are put into slings for a portion of the time. When down, these animals usually lie quite in a lateral position, the neck stretched out. When in the slings they will stand for a while. In some cases, however, they appear to tire and then hang in the slings. When standing they will eat and drink, defaecate and urinate normally. When assisted, they will walk; the gait is, however, very uncertain, the legs are not properly lifted and they will stumble over the smallest obstacle and the animals will suddenly fall. This fall is sometimes first on the knees, sometimes the animal falls over on one side. Some animals will gradually

learn to lift themselves unassisted, but it has been noted that the rising is carried out often in an unnatural way, the animal getting first on its knees, raising the hindquarters and only then lifting the forequarters, similar to the rising of an ox. They do not always succeed in rising and may get halfway up and tumble down again. Muscular weakness appears to remain in these cases for a very long time, 2 to 5 months. The animals, although able to walk, are unfit for any work. In these chronic cases, the other physiological functions appear to be normal. Consciousness is present, sensitiveness of the skin is retained, the reflexes that are usually examined show no changes; the animals defaecate and urinate normally. The visible mucous membranes are of normal colour. The pulse rate is practically always within its normal limits, the quality of the pulse shows no alteration and the respiration is not interfered with. The temperature records taken from the beginning and continued over a long period show no changes. Animals that are frequently down may develop decubitus on head, shoulder, hip, and legs. Symptoms of rare occurrence were increased salivation. They point to a difficulty in deglutition, but these cases always showed a rapid course so that it was impossible to examine them more closely. There were no such symptoms present that indicated a paralysis of the trigeminus and hypoglossus as seen sometimes in parabotulism of cattle or in horses produced with parabotulismus bovis toxins.

The Pathology of Parabotulismus equi.

There were no pathognomonic lesions found in equines that succumbed to natural or artificial intoxication with parabotulism. Negative results of the autopsy together with the symptoms described during life may be taken as characteristic. Since the course of the disease in the greater number of cases was peracute or acute, horses and mules of all conditions succumbed and accordingly the state of nutrition of the cadaver had no significance.

Lesions of decubitus in various places were often found. They were fresh in acute cases and showed inflammatory and necrotic changes in prolonged cases. In acute cases they were mostly found on one side, on that on which the animal went down in the first instance and depended naturally upon the state of agitation prior to complete paralysis and to death.

Rigor mortis was always present and set in as a rule at an early period.

The blood coagulated completely in cadavers that had been dead for some time. It did not, however, coagulate rapidly, since it was still found in liquid form in some cases even a few hours after death.

The subcutaneous tissue showed no changes except in places of decubitus, where it showed gelatinous and even haemorrhagic infiltration.

There were no alterations found in the peritoneal cavity that could be connected with this disease. In the pleural cavity in some instances the presence of slight hydrothorax was noted. This may, however, have been connected with the general poor condition of the horses approaching cachexia, that were utilized in the experimental work.

An occasional swollen lymphatic gland of an oedematous nature was ascribed to a similar cause.

The mucous membranes of the mouth, tongue, and pharynx as a rule showed no changes. Wounds caused by a trauma were occasionally present on the lips and tongue.

The mucosa of the trachea in a few cases showed a number of petechiae and haemorrhages. The lungs in practically all cases showed some changes, but these consisted mainly in hyperaemia and oedema. These two conditions were present in various degrees; the stasis as a rule was well marked; the oedema was not always so marked, although froth on the bronchi was frequently present. The pericardium sometimes contained a slightly increased amount of a clear liquid, more often probably due to the fact that the experimental horses were in low condition. In the epicardium in a number of cases haemorrhages were present, usually of small dimension, also ecchymoses and petechiae. Similar changes were met in the endocardium of both ventricles. There were no constant changes in the myocardium, degenerative processes in the nature of cloudy swelling were occasionally found. The liver in most cases showed no marked changes, slight increase in size and an increased amount of blood point to a state of stasis in this organ. Degenerative changes were rarely recorded and were probably not directly connected with the disease. The spleen as a rule showed no changes. Slight tumor splenis was recorded. It was due to an increased amount of blood. Also in the kidneys the presence of a stasis without any other alterations has been recorded.

Degenerative, indurative processes were registered, but must be interpreted to be of accidental occurrence. The stomach in acute cases still contained food, occasional hyperaemia was found in the fundus and pyloric region. The mucosa of the small intestine in most cases showed the presence of hyperaemia and even the presence of catarrhal exudate and these findings were interpreted as acute catarrhal enteritis. The large intestines were usually found unaltered; haemorrhages were, however, recorded in some instances in the caecum. The bladder and the genital organs were always found unchanged. The brain showed occasionally an increased amount of blood.

The pathological anatomical diagnosis may therefore be summarized as follows:—

Stasis and oedema of the lungs. Occasional haemorrhages and ecchymoses in both endocardiums. Stasis in the liver and occasionally in the spleen and kidneys. Occasional hyperaemia of the mucosa of the stomach and enteritis catarrhalis of the small intestine.

Pathogenesis.

The parabotulism of equines is an acute intoxication of the nervous system of the locomotor apparatus. It does not cause a complete paralysis, only a paresis of the locomotor muscles. The sensorium of the horse is not interfered with for a considerable length of time, the reflexes emanating both from the brain and spinal cord are maintained almost to the end, indicating that the reflex arc is nowhere interrupted. It seems, therefore, reasonable to accept that in the first instance the toxin more particularly affects the portion of the nerve that transmits the stimulus to the muscle, the so-called end plate, and in this respect resembles an intoxication with curare.

This view would also explain the dyspnoea practically always present from the beginning and its increase in the further course of the disease to a pronounced state of asphyxia. It is possible that later the toxin may affect the spinal cord and cerebrum. Death is probably due to suffocation in most instances, indicated by the marked symptoms of progressive dyspnoea towards the end of the disease in many cases.

The parabotulism of equines resembles in many respects the para-botulism of cattle, the so-called lamsiekte. The toxin is not the same, although very similar as shown before. In a previous publication on lamsiekte, it has been shown that lamsiekte toxin produces a disease in horses. It may be of an acute type, but more often is of a subacute and chronic type, and lesions pointing to paralysis of the cerebral nerves are often present, such as paralysis of the tongue and the pharynx, whilst the horse is still able to stand.

We have, however, no doubt that also in the aetiology of the parabotulism of equines the toxin of parabotulism bovis may play a rôle. This is definitely the case with donkeys which are known to consume the ruminal contents of cattle set free by decomposition and which may be impregnated by the toxin. An outbreak amongst horses and cattle recorded in this paper occurring in the neighbour-hood of Bloemfontein may be interpreted as such an incidence.

Prophylaxis.

Parabotulism of horses is not of common occurrence and then only under definite conditions, usually in stabled animals which contract it from foodstuffs contaminated by the decomposition of dead animals. It is evident the rat is not the only animal. On a farm where food-stuffs are stocked or baled and packed, it may even occur that wild rodents and other vermin seek shelter in the accumulations, particu-larly when ill through one or the other reason and they succumb. However, not all carcasses that undergo decomposition produce the toxin, only those that have during life been infected with the para-botulinus organism and these, as our experiments have shown, are not numerous. There is reason to suspect that in certain localities at certain times of the year, these organisms may be found more often in rats and other animals and therefore give rise to contamination of foodstuffs. It will be wise, under all conditions, to take notice of unusual mortality amongst the lower animals on a farm, sufficient reason existing then to expect that such animals may contaminate the foodstuffs in which they hide and often die.