are normal. Peritoneal cavity contains 500 c.c. clear fluid. Thoracic trachea contains foam, its mucosa being normal. Left lung shows hpyeraemia and oedema, froth in bronchi. Right lung shows slight hypostasis. Pericardial sac contains fluid and a gelatinous clot. Epicardium shows extravasations. Left ventricle contains blood-clots and shows numerous extravasations. Right ventricle contains blood-clots and shows extravasations; myocardium normal. Liver capsule covered with fibrous filaments, on section hypostasis. Gall-bladder contains 50 c.c. thick green bile. Spleen measures 42 by 13 by 3 cm., pulp firm. Left kidney hyperaemic, right kidney normal. Rumen, reticulum, and omasum are normal. Abomasum shows a few extravasations, and the duodenum intense hyperaemia and bile-staining. Jejunum and ileum are bile-stained and show slight hyperaemia. Caecum normal. Colon contents blood-stained, mucosa hæmorrhagic. Rectum contains dry dung. The mucosa of the bladder shows hyperaemia, extravasations, its wall being thickened and oedematous.

Pathological Anatomical Diagnosis: Hyperaemia and oedema of left lung. Extravasations of epicard and endocard. Hydroperitoneum. Enteritis, oedema, and extravasations of bladder.

Etiological Diagnosis: Lamsiekte.

Post-mortem Examination Heifer 4747: Age 2 years. Condition good. Rigor mortis present. Pharyngeal glands very much enlarged and hyperaemic. Mediastinal and bronchial glands injected. Cervical trachea shows the vessels of its mucosa to be injected. Larynx shows hyperaemia and thickening of its mucosa. Oesophagus paralysed, portion being plugged with food. Lungs collapsed; the left one shows hypostasis and the right diffuse hyperaemia. Thoracic trachea deeply injected. Pericardial cavity contains 20 c.c. blood-stained fluid. Epicardium shows some petechiae and injection of vessels. Right endocardium shows numerous small extravasations. In the left ventricle the blood is well coagulated, endocardium shows extravasations. The surface of the liver is mottled in appearance, on section commencing decomposition. Gall-bladder distended with bile. Spleen measures 49 by 15 by 3 cm., enlarged, on section jelly-like fluid present. The surface of the kidneys mottled in appearance, showing on section patchy hyperaemia and injection of vessels of cortex. Rumen, reticulum, and omasum nothing unusual. Abomasum shows acute hyperaemia, mucosa thickened. Duodenum has its mucosa thickened and contains catarrhal exudate. Jejunum and ileum show acute hyperaemia and contain catarrhal exudate. Caecum and colon show punctiform hæmorrhages and slaty discoloration.

Pathological Anatomical Diagnosis: Hyperaemia of lungs, trachea, and larynx. Paralysis of oesophagus. Hyperaemia of the pharyngeal glands. Hyperaemia of kidneys. Enlarged spleen. Catarrhal gastro-enteritis.

Etiological Diagnosis: Lamsiekte.

THE LAMSIEKTE TOXIN.

THE experiments recorded in the following pages (the protocols of which will be found in Appendix, page 1152) wree undertaken with the object (1) of studying the properties of the lamsiekte toxin and determining its minimum lethal dose for the various species of domestic animals; (2) of attenuating the toxin; and (3) of finding a method of immunization against lamsiekte. It may be stated at once that all three objects were achieved to some extent. The minimum lethal dose was determined for the more important domestic animals. Further, it was found possible to attenuate the toxin both by heat and by the addition of chemicals. And, thirdly, a certain degree of immunity could be conferred on cattle and goats, although unfortunately the immunity was afterwards found to be inadequate and the methods too cumbersome.

In describing the work which led to these results, the following arrangement has been followed:—

A.—METHOD OF CULTIVATING THE LAMSIEKTE ORGANISMS.

The first cultures were made from veld débris which had proved to be toxic, from rotten bones in the case of the Onderstepoort cultures, and from fly larvae in the case of the earlier Armoedsvlakte cultures. In the case of the former, the toxic material was used as inoculum into various fluid and semi-solid media, and excellent results obtained with minced raw liver in deep layer (fruit jars) heated to 70° C. after inoculation in order to suppress non-sporulating gas-producing organisms.

A subculture of this highly toxic material, containing a miscellaneous mixture of sporulating anaerobes, was sent to Armoedsvlakte and used as convenient basis of the experiments detailed in this section.

The same type of medium was used for preparing further quantities of "crude toxin." A fresh liver was taken, cut into strips, and finely minced in a Latapie mincing machine. The liver pulp was then filled into ordinary fruit-jars to about two-thirds. In order to get the best results, it was found necessary to add some liquid so that the medium had the consistency of porridge. If the medium was too hard and dry, or if it was too liquid, the toxin produced was less potent than in a medium of the correct consistency. The liquid used was either sterile physiological saline solution or liver bouillon.

In exceptional cases *brain* substance or *muscle* was used as a medium, but these substances were found to be less favourable for the development of the organisms.

After filling the minced medium into the jars, the latter were *heated* in a water-bath for periods varying from half an hour to twelve hours. The jars were generally submitted to a heat of 80° to 85° C. Sometimes the jars were kept at this temperature for a few hours on successive days. In cases where the heating extended over several hours a large proportion of the liquid evaporated and the medium became too dry; the best results were obtained with jars heated for a short period only (half an hour).

This heating process did not, of course, destroy all the organisms in the medium used. Even after prolonged pasteurization it was found that on incubation various gas-producing anaerobes grew in the jars, but it is of importance to note that such "control jars" were always non-toxic.

After pasteurization the jars were *inoculated* with the mixed culture containing lamsiekte organisms. It was found necessary to inoculate fairly heavily, and, as a general routine, quantities of culture up to 100 c.c. were added to each of the jars containing about 1 litre of medium.

These were then placed in an incubator at 37° C., where they were kept for a few days. After that they were kept at room-temperature in fly-proof cupboards.

Growth commenced after about eight to ten hours, and was very profuse after twenty-four hours. By this time a considerable amount of gas had generally been formed. In jars that had not been pasteurized the gas production was generally much more vigorous, and in such cases the covers of the jars might be blown off and the medium overflow. Normally, however, gas production was not excessive and ceased after about two or three day's incubation.

In the pasteurized jars the liver medium has a reddish-grey colour. As soon as growth starts, this gradually changes first into a dirty purple, and then through greenish-brown to greenish-black. At the same time the consistency of the tissue alters. At first it seems to swell, and then gradually *liquefaction* sets in. When growth is completed the contents of the jar, which after pasteurization was fairly firm, again has the consistency of a fairly thin porridge.

These cultures have a most offensive smell. Neither odour, proteolysis, nor gas formation, however, are necessarily characteristic of the activities of the lamsiekte organism itself, but may well be wholly due to the miscellaneous putrefactive flora of the mixed culture.

The jars were tested at various periods, and it was found that a high degree of toxicity was manifested after six days. The toxin remained unaltered in the jars for a considerable period, and in two instances was found to be of the same virulence almost two years after inoculation.

It was found that the toxicity of the jars varied within wide limits. Some of the factors that influence the production of toxin have been mentioned above, others remain unknown. Every jar was tested on small animals, and only those that proved to be most toxic were used for further experiments. The toxin used for the experiments detailed below was obtained from a number of jars of different generations, which showed approximately the same degree of toxicity. This was used as the standard for fixing the minimum lethal dosis of the "crude toxin" for the various species of animals.

B.—PREPARATION OF THE TOXIN.

The technique employed for the preparation of lamsiekte toxin was as follows:—The quantity of culture which was necessary for the particular experiment was drawn up into a wide-mouthed pipette and added to the requisite amount of normal saline solution to give the desired dilution. Most of the cultures were just liquid enough to be drawn up into a pipette. The culture had invariably to be diluted to enable the liquid portion (i.e. the "toxin") to pass through a filter. When large quantities of toxin were required, one part of culture was mixed with one part of saline giving a dilution of 50 per cent.

The mixture was then well shaken so as to break up all the particles of the medium. It was then *filtered* first through ordinary filter-paper. This process was generally (i.e. in cases where a high concentration was used) very slow, and filtration was, as a rule, continued over-night. The filter-paper filtrate was then passed through a sterilized Berkefeld candle, which process again was generally very slow. The filtrate invariably remained sterile.

All experiments described in this section were carried out with a sterile Berkefeld filtrate prepared in the manner described above. Whenever in the following pages reference is made to "filtered toxin," a Berkefeld filtrate is invariably meant, and whatever dilution was actually used, figures are recalculated on the common basis of "jar contents."

C.—DETERMINING THE MINIMUM LETHAL DOSE.

(*a*) Cattle.

(1) *By Drenching.*

Only two cattle were used in this experiment.

Bull 189 (see Appendix, page 1152) was drenched with 20 c.c. of a toxic culture diluted in 200 c.c. water. The first symptoms of paralysis manifested themselves about fifty-one hours after drenching, and the animal was dead about six or eight hours later.

The course of the disease corresponded with that seen in peracute cases of natural lamsiekte. At the onset of the symptoms the animal showed a rapidly progressing paralysis of the body muscles and of the tongue with profuse salivation. Death followed shortly afterwards.

Heifer 4727 received 5 c.c. of the culture diluted with 100 c.c. water. Three days later it showed the first signs of paralysis. During the same day it was treated with magnesium sulphate, and died as a result of the treatment.

In this case, in accordance with the smaller dose of toxin, the incubation period was much longer and the symptoms less violent than in the case of bull 189. The same point will be illustrated more fully in the next section, where it will be seen that the duration of the incubation period, the duration of the disease, and the severity of the symptoms stand in direct relation to the quantity of toxin administered.

Conclusion.—The amount of toxin contained in 5 c.c. of ordinary culture material was sufficient to set up a typical and probably fatal case of lamsiekte when given per os to a beast weighing 412 lb. The dose in this case was approximately 0.025 c.c. crude toxin per kg. body-weight. It is probable that the actual minimum lethal dose is much smaller.

(2) *By Subcutaneous Inoculation.*

Experiment No. 1.—As a preliminary test two cattle were injected with 1 c.c. and ½ c.c. respectively of the filtered toxin. Both animals died as a result of the injection.

Bull 196 (see Appendix, page 1155), which received 1 c.c. developed the first symptoms about thirty-six hours after injection, and bull 4834, which received half the amount, about forty-four hours after injection. The former animal showed very severe symptoms and died shortly after the onset of the disease. The latter—again corresponding with the smaller dose of toxin—contracted the disease in a somewhat milder form after a longer incubation period. The disease lasted about eighteen hours, the animal dying about sixty-three hours after injection.

Conclusion.—The amounts of toxin used in this experiment for subcutaneous injection, viz., 1 c.c. and 0.5 c.c. seemed to be considerably above the minimum lethal dose.

In the next experiment these quantities were therefore reduced.

Experiment No. 2.—Two animals were injected with 0.1 and 0.01 c.c. of filtered toxin.

Bull 188 received the larger quantity and showed almost complete paralysis of all the muscles sixty-seven hours later (see Appendix, page 1157). Bull 4361, which received 0.01 c.c., showed no symptoms at all.

Conclusion.—It appeared from this experiment that the minimum lethal dose lay between the two quantities used. In the case of bull 188, weighing 417 lb., the dose injected corresponded to 0.00052 c.c. per kg. body-weight, and in the case of 4361 to 0.000058 c.c.

In the following experiment the dose was therefore calculated accurately according to the weight of the animals.

Experiment No. 3.—Four animals were used. Bull 190 received 0.0004 c.c. toxin per kg.; bull 194, 0.00015 c.c., heifer 3932, 0.0001 c.c.; and bull 4361 the same amount.

The results were both interesting and instructive. Bull 190, which received the largest quantity, showed the first symptoms of lamsiekte about seventy-two hours after the injection; it was then treated and died. Bull 194 also showed its first symptoms after seventy-two hours. The disease made comparatively slow progress, the animal dying five and a half days after injection. Of the two animals which received 0.0001 c.c., one (4361) developed the disease after an incubation period of four and a half days and died nearly ten days after the injection; the other (3932) showed the first symptoms only after five and a half days, the disease took a mild course, and recovery took place about a week later.

Conclusion.—From this experiment it was concluded that the *minimum lethal dose of lamsiekte toxin for cattle was* 0.0001 *c.c. per kilogram body-weight.* The dose did not prove fatal invariably, but was found to be accurate enough for future purposes.

The following table summarizes the results of the experiments in Section C (*a*):—

TABLE No. 1.

Cattle No.	Mode of Administration.	Amount of Toxin	Dose in c.c. per kg. Body-weight.	Result.		Remarks.
				Incubation Period.	Death after Administration.	
189	Drenching	20 c.c.	0·074	51 hours	58 hours	Considerably above lethal dose.
4727	Drenching	5 c.c.	0·025	3 days	Killed	Considerably above lethal dose.
196	Subcutaneous injection	1 c.c.	0·0039	36 hours	Killed	Considerably above lethal dose.
4834	Subcutaneous injection	0·5 c.c.	0·0027	44 hours	63 hours	Considerably above lethal dose.
188	Subcutaneous injection	0·1 c.c.	0·0005	67 hours	Killed	Above lethal dose.
4361	Subcutaneous injection	0·01 c.c.	0·00006	No reaction		Below lethal dose.
190	Subcutaneous injection	0·098 c.c.	0·0004	72 hours	Killed	Above lethal dose.
194	Subcutaneous injection	0·034 c.c.	0·00015	72 hours	5½ days	Apparently very near minimum lethal dose.
3932	Subcutaneous injection	0·0138 c.c.	0·0001	5½ days	Recovered	Considered to be the minimum lethal dose.
4361	Subcutaneous injection	0·01715 c.c.	0·0001	4½ days	10 days	Considered to be the minimum lethal dose.

(*b*) In Horses.

Lamsiekte is not a natural disease of horses. Nevertheless it was found that this species of animal was affected by the toxin in much the same way as cattle. A few experiments were carried out, not so much with the object of determining accurately the minimum lethal dose as for the sake of trying to immunize horses against lamsiekte, and thus obtaining an immune horse serum which would be used again for immunization experiments in cattle. This aspect of the problem will be discussed in Section E (*b*). At the present stage it is merely necessary to detail the preliminary experiment, which gives an indication of the extent to which horses are susceptible to lamsiekte when the toxin is administered artificially.

Experiment No. 1.—Horse 11996, weighing just over 400 kg., received 20 c.c. toxin subcutaneously, i.e. 0.05 c.c. per kg. body-weight. Twelve hours later the animal appeared dull, and after twenty-four hours it showed definite symptoms of disease (see Appendix, page 1161). Twenty-six hours after the injection the horse was paralysed in the front legs. After a further twelve hours it presented a typical picture of lamsiekte, and died forty-three hours after the injection.

Horse 12963, weighing 354 kg., was injected subcutaneously with 5 c.c. toxin, i.e. about 0.014 c.c. per kg. The first symptoms of paralysis (protrusion of tongue and ptosis) manifested themselves two days after the injection. The condition then seemed to improve (see page 1162), so that the tongue could be withdrawn completely six days after injection. However, the same day the horse became much weaker, the temperature sank below normal, the tongue was again protruded. On the seventh day the animal was in a comatose condition and had to be destroyed.

Horse 13330 received 1 c.c. toxin (about 0.003 c.c. per kg.) subcutaneously. Three days later it showed the first slight symptoms, and on the fourth day after injection definite signs of lamsiekte (protrusion of tongue, ptosis) were noticed. The next day these symptoms had disappeared.

Eight days after the first injection horse 13330 received a second injection of 1 c.c. toxin subcutaneously. Three and a half days later the horse was dead.

Summary of Experiment No. 1.

Table No. 2.

Horse No.	Amount of Toxin Injected Subcutaneously.	Dose in c.c. per kg. Body-weight.	Result.		Remarks.
			Incubation.	Death after Injection.	
11996	20 c.c.	0·05	12 hours	43 hours	Considerably above lethal dose.
12963	5 c.c.	0·014	2 days	7 days	Slight improvement on fifth and sixth days.
13330	1 c.c.	0·003	3 days	Recovered on fifth day	Apparently this was near the minimum lethal dose.
	1 c.c.	0·003	—	3 days	After first injection, recovery; after second, death.

Conclusion.—This experiment proves that horses are affected by lamsiekte toxin and develop the disease in a form similar to that in cattle. The minimum lethal dose is considerably larger for horses

than for cattle. The dose given to horse 13330 at the first injection, viz., 0.003 c.c. per kg. body-weight, was probably quite near the lethal dose. It seems safe to assume that a dose of 0.004 or 0.005 c.c. per kg. would kill a horse. The minimum lethal dose for horses is therefore about fifty times larger than the dose for cattle.

(*c*) In Donkeys.

Only one donkey (13789) was used for these experiments; 10 c.c. of filtered toxin were injected subcutaneously into this animal. Four days later it looked dull and refused its food (see Appendix, page 1164). Seven days after the injection the donkey was paralysed and died the next day.

All we can conclude from this experiment is that donkeys are probably still less susceptible to lamsiekte than horses. The long incubation period (four days), and the compartaively long duration of the disease after the injection of a very large dose (10 c.c.), indicate a high degree of natural resistance against lamsiekte in donkeys.

(*d*) In Goats.

Three goats were used to determine the minimum lethal dose for this class of animal.

Goat 1 received 0.005 c.c. per kg. body-weight; goat 2, 0.0005 c.c.; and goat 3, 0.0001 c.c. per kg. subcutaneously. The first animal was completely paralysed twenty-four hours after the injection and died twenty-nine hours after injection (see Appendix, page 1164). The second goat showed the first signs of paresis forty-four hours after injection. The symptoms progressed rapidly, and about fifty-two hours after injection the animal was unable to stand about twelve hours later it died. In the third goat, the incubation period lasted five days. The first symptoms were general weakness and a stiff gait. In the course of the sixth day the weakness of the limbs became more pronounced, so that the goat could only walk a few yards after having been lifted (see page 1165). This condition persisted for several days. From the ninth to the thirteenth day it could only stand for a few seconds when lifted and then collapsed. On the fourteenth day a slight improvement was noticed, and on the next day the goat could again walk a few yards. On the eighteenth day it could get up by itself, but the weakness still persisted for several days. Complete recovery had taken place on the twenty-first day after injection.

Conclusion.—The minimum lethal dose for goats is approximately the same as for cattle, namely, 0.0001 c.c. toxin per kg. body-weight. The goat which received this quantity developed typical symptoms of lamsiekte and was expected to die; however, it recovered after a protracted illness. The goat which received five times this amount died after an acute attack of lamsiekte.

The same observations were made in goats as have already been recorded in cattle: that the length of the incubation period, the duration of the disease, and the severity of the symptoms stand in direct relation with the size of the dose of toxin injected. In other words, it is possible to produce almost any clinical form of the disease at will by adjusting the dose of toxin.

The results of this experiment can be tabulated as follows:—

TABLE No. 3.

Goat No.	Weight in kg.	Amount of Toxin Injected.	Dose in c.c. per kg. Body-weight.	Result.	
				Incubation Period.	Death after Injection.
1	48	0·24 c.c.	0·005	24 hours	29 hours
2	54	0·027 c.c.	0·0005	44 hours	64 hours
3	41	0·004 c.c.	0·0001	5 days	Almost completely paralysed on ninth day: recovered on twenty-first day.

(*e*) IN SHEEP.

Sheep 1 was injected subcutaneously with 0.0005 c.c. toxin, sheep 2 with 0.0002, and sheep 3 with 0.0001 c.c. per kg. body-weight. These small quantities were chosen on the assumption that the minimum lethal dose would be about the same as for goats and cattle, an assumption which proved to be correct.

Sheep 1 showed the first signs of illness about forty-two hours after the injection, it was completely paralysed forty-eight hours, and died about sixty hours after the injection (see Appendix, page 1167). In sheep 2 the incubation period lasted three days, paralysis had set in after about four days, and death took place about five and a half days after injection (see Appendix, page 1168). Sheep 3, which received the smallest dose, had an incubation period of four days; on the fifth day it showed weakness in the hind-quarters and a stiff gait; the next day it was completely paralysed and died about six and a half days after the injection (see Appendix, page 1169).

Conclusion.—The quantity of toxin injected into sheep 3, viz., 0.0001 c.c. per kg. body-weight, is probably just about the minimum lethal dose. This is the same as the minimum lethal dose for cattle and goats.

Table No. 4 summarizes the results of this experiment:—

TABLE No. 4.

Sheep No.	Weight in kg.	Amount of Toxin Injected.	Dose in c.c. per kg. Body-weight.	Result.	
				Incubation Period.	Death after Injection.
1	52	0·026 c.c.	0·0005	42 hours	About 60 hours.
2	48	0·0096 c.c.	0·0002	3 days	5½ days.
3	48	0·0048 c.c.	0·0001	4 days	6½ days.

(*f*) IN DOGS.

Experiment No. 1.—Dog 1 was injected subcutaneously with 1 c.c. toxin, but showed no symptoms. Five days later it received

5 c.c. toxin, and again showed no reaction. Ten days later 10 c.c. toxin were injected without result. After another five days it received 20 c.c. toxin, but still no reaction. It was then injected with 40 c.c. toxin and remained in perfect health. The dog weighed 20 kg., so that the last dose amounted to 2 c.c. toxin per kg. body-weight.

Conclusion.—From this experiment it was clear that the dog had a remarkable degree of resistance against the lamsiekte toxin. The conclusion that any dog could resist 2 c.c. toxin per kg. body-weight would perhaps not have been justified, since dog 1 received gradually increasing doses of toxin, and it would have been quite permissible to assume that each injection increased the dog's immunity against each succeeding injection. A further experiment was therefore undertaken in which a fresh dog was used for each injection.

Experiment No. 2.—Dog 17 was injected subcutaneously with 3 c.c. toxin per kg. body-weight, dog 18 with 4 c.c., and dog 19 with 5 c.c. toxin per kg. This latter animal weighed 30 kg., so that the quantity of toxin which this dog received was 150 c.c. Since, however, the culture had to be diluted with its own quantity of physiological salt solution in order to carry out filtration (see page 1102), the actual amount of liquid that had to be injected under this dog's skin was 300 c.c. (see Appendix, page 1170).

Conclusion.—The dog seems to be completely insusceptible to lamsiekte toxin. Doses up to 50,000 times the lethal dose for cattle, goats, and sheep gave no reaction whatsoever in dogs.

Summary.

Table No. 5.

Dog No.	Weight in kg.	Amount of Toxin Injected.	Dose in c.c. per kg. Body-weight.	Result.
1	20	1 c.c. 5 c.c. 10 c.c. 20 c.c. 40 c.c.	0·05 0·25 0·5 1 2	No reaction. ,, ,, ,, ,,
17	36	108 c.c.	3	No reaction.
18	25	100 c.c.	4	No reaction.
19	30	150 c.c.	5	No reaction.

(*g*) In Pigs.

Three pigs were injected with increasing quantities of toxin. In the case of pig 345 the initial dose was 0.1 c.c. per kg. body-weight; this was increased to 1 c.c., then to 3 c.c., and finally to 6 c.c. per kg. Later on the pig again received 1 c.c. toxin per kg., but never did it show any symptoms. Pig 353 received up to 7.5 c.c. per kg. body-weight (see Appendix, page 1171, and pig 355 up to 4 c.c., without showing any sign of lamsiekte.

Conclusion.—Pigs, like dogs, seem to possess an absolute natural immunity against lamsiekte.

Summary.

Table No. 6.

Pig No.	Weight in kg.	Amount of Toxin Injected.	Dose in c.c. per kg. Body-weight.	Result.
345	25	2·5 c.c.	0·1	No reaction.
		25 c.c.	1	,,
		75 c.c.	3	,,
		150 c.c.	6	,,
		25 c.c.	1	,,
353	20	4 c.c.	0·2	No reaction.
		10 c.c.	0·5	,,
		40 c.c.	2	,,
		20 c.c.	1	,,
		50 c.c.	2·5	,,
		100 c.c.	5	,,
		100 c.c.	5	,,
		150 c.c.	7·5	,,
355	22	0·02 c.c.	0·001	No reaction.
		4·4 c.c.	0·2	,,
		22 c.c.	1	,,
		88 c.c.	4	,,
		22 c.c.	1	,,
		22 c.c.	1	,,
		44 c.c	2	,,

(*h*) In Guinea-pigs.

It will be simplest first to give a summary in tabular form of the experiments conducted with guinea-pigs, and then to draw the conclusions.

Table No. 7.

Experiment No.	Guinea-pig No.	Weight in grm.	Amount of Toxin Injected.	Dose in c.c. per kg. Body-weight.	Result of Injection.	
					Ill After.	Died After
1	9	680	0·05 c.c.	0·07	13 hours	16 hours.
	10	580	0·02 c.c.	0·04	—	30 hours.
	11	480	0·01 c.c.	0·02	—	30 hours.
	12	480	0·005 c.c.	0·01	13 hours	25 hours.
	13	300	0·001 c.c.	0·003	—	54 hours.
	14	300	0·0005 c.c.	0·0016	—	30 hours.
2	15	500	0·0005 c.c.	0·001	—	47 hours.
	16	465	0·0001 c.c.	0·0002	No reaction.	
	17	440	0·00005 c.c.	0·0001	,,	
	18	430	0·000025 c.c.	0·00006	,,	
	19	340	0·00001 c.c.	0·00003	,,	
3	20	620	0·00056 c.c.	0·0009	Nearly 7 days	7 days and 3 hours.
	21	510	0·00038 c.c.	0·00075	No reaction.	
	22	500	0·0003 c.c.	0·0006	,,	

Further details in regard to these animals can be found in Appendix, page 1171.

Experiment No. 1 showed that the minimum lethal dosis for guinea-pigs was below 0.0016 c.c. per kg. body-weight (guinea-pig 14).

In *Experiment No.* 2 doses were given ranging from 0.001 c.c per kg. down to 0.00003 c.c. per kg. Only the top dose proved fatal

(guinea-pig 15). A fifth of this dose (guinea-pig 16) and the still smaller doses produced no ill-effects. It was concluded therefore that the minimum lethal dose for guinea-pigs must be 0.001 c.c. per kg. or slightly less. In order to fix this dose more accurately, three guinea-pigs were injected (*Experiment No.* 3) with 0.0009, 0.00075, and 0.0006 c.c. per kg. respectively. The animal which received the highest dose (guinea-pig 20) developed the disease after about seven days and died, thus proving this dose to be probably the smallest that would kill guinea-pigs. The other two animals (guinea-pigs 21 and 22) showed no symptoms.

Conclusion.—The minimum lethal dose for guinea-pigs is about 0.0009 c.c. per kg. body-weight. For convenience, the dose was assumed to be 0.001 c.c., i.e. about ten times the dose for cattle, goats, and sheep.

(*i*) In Rabbits.

At the time when these investigations were carried out very few rabbits were available for experimental purposes. For tests on small animals only guinea-pigs were used. There was therefore no great necessity to determine the minimum lethal dose accurately. One experiment was carried out, but unfortunately gave somewhat inconclusive results. The details may be summarized as follows:—

Table No. 8.

Rabbit No.	Weight in grm.	Amount of Toxin Injected.	Dose in c.c. per kg. Body-weight.	Result.
10	1,600	0·008 c.c.	0·005	First symptoms after 2 days. Recovered.
11	1,800	0·0054 c.c.	0·003	No reaction.
12	1,560	0·00156 c.c.	0·001	Sick after about 2 days. Recovered.
13	1,660	0·0013 c.c.	0·0009	No reaction.
14	1,440	0·001 c.c.	0·0007	No reaction.

Conclusions.—None of the rabbits used in this experiment died. The one which received 0.001 c.c. per kg. body-weight (12) showed symptoms and recovered. It would seem therefore as if this was near the minimum lethal dose. On the other hand, rabbit 11, which received three times the amount, showed no reaction, and rabbit 10, which received five times the dose, gave only a slight reaction.

In later experiments where rabbits were again used for toxin tests they proved themselves to be just as susceptible as guinea-pigs. Perhaps the unexpected results of the experiment described above must be ascribed to the particular sample of toxin used.

For the purpose of this article, the minimum lethal dose for rabbits can be assumed to be the same as that of guinea-pigs, namely, 0.001 c.c. per kg. body-weight (see Appendix, page 1173).

(*j*) In Rats.

Table No. 9 summarizes the results of the experiments carried out with rats.

Table No. 9.

Rat No.	Amount of Toxin Injected.	Result of Injection.	
		Ill after.	Died after.
3	0·01 c.c.	No re	action.
4	0·1 c.c.	No re	action.
5	1 c.c.	2 hours	Less than 18 hours.
6	5 c.c.	—	½ hour.
7	1 c.c.	15 minutes	25 minutes.
8	2 c.c.	15 minutes	1¼ hour.
9	2 c.c.	No re	action.

Conclusion.—In view of the fact that the "toxin" is a crude product formed in a putrefying medium, that a dosage such as 5 c.c. in rat 6 represents about 2 per cent. of the body-weight, and that death occurred in half an hour, it would not be safe to conclude that death was due to lamsiekte toxin. It might well be attributed to a massive dose of "putrefaction bases." Since in no other animal does death from the lamsiekte toxin occur with the astonishing rapidity manifested in cases 6, 7, and 8 of Table No. 9, it is best to draw conclusions only from cases 3, 4, 5, and 9. Rat 4, which showed no symptoms at all, weighed approximately 200 grams, so that the dosage of 0.1 c.c. means 0.5 c.c. per kg. or 5,000 times the fatal dosage for cattle and goats. Rat 5, receiving approximately 50,000 times the dosage fatal for cattle, died overnight after an injection late in the afternoon. No. 9 represents a rat injected with 2 c.c. at a different date with a different jar of toxin, the usual parallel experiment on a guinea-pig being carried out as control. The death of the guinea-pig with 0.001 c.c. per kg. and the survival of the rat with nearly 10 c.c. per kg. shows quite conclusively that the rat, like the dog, is naturally immune to the toxin (see Appendix, page 1173).

(*k*) In Mice.

The following experiment was carried out:—

Table No. 10.

Mouse No.	Amount of Toxin Injected.	Death after Injection.
3	0·001 c.c.	3½ days.
4	0·01 c.c.	Less than 18 hours.
5	0·1 c.c.	Less than 18 hours.
6	1 c.c.	½ hour.

Conclusion.—Mice appear to be susceptible to the toxin, but relatively resistant when compared with cattle, goats, and sheep. The smallest dose which was used, viz., 0.001 c.c., produced death only after three and a half days; it seems likely therefore that this was

just about the minimum lethal dose. Assuming the weight of the mouse to have been 50 grams, the minimum lethal dose for mice would be 0.02 c.c. per kg. body-weight, or 200 times that for cattle.

As in the case of rats, the extreme rapidity with which death follows on the injection of a massive dose of toxin (half-hour) suggests mortality from extraneous causes. It was not considered of sufficient general interest at the time to carry out further experiments with mice (see Appendix, page 1174).

(*l*) In Ostriches.

Ostriches were used for these experiments, not so much with the object of determining accurately the minimum lethal dose as for the sake of studying the symptoms set up by toxic material in these animals.

Experiment No. 1.—Ostrich 2 was dosed with 1 lb. crushed rotten bones. About four days later the bird showed signs of weakness, and it died about five and a half days after the administration of the bones (see Appendix, page 1174).

Conclusion.—This experiment showed that ostriches are susceptible to lamsiekte. Rotten bones had the same effect on this ostrich as they would have had on cattle.

Experiment No. 2.—Ostrich 3 was dosed with 1 lb. rotten meat and maggots. Less than twenty hours later it showed definite signs of muscular weakness, and within twenty-four hours the bird was partly paralysed. It died about two and a half days after the dosing (see Appendix, page 1175).

Conclusion.—The very short incubation period and the short duration of the illness showed that this ostrich received many times the minimum lethal dose.

Experiment No. 3.—Five ostriches were dosed with lamsiekte culture material. The reason why filtered toxin was not used was simply that fairly large quantities were needed, the preparation of which would have demanded much time. On the other hand, the dosing of ostriches with a pulpy material such as a lamsiekte culture is such a simple matter that there appeared to be no reason why the toxin should not be administered in this crude form.

Ostrich 1 received 20 c.c. culture per os. About six and a half days later it showed weakness in its legs. The condition improved, and eleven days after the dosing it had completely recovered.

Ostrich 5 received 100 c.c. culture. The incubation period lasted about forty hours. In less than four days it was completely paralysed and died after four and a half days.

Ostrich 6 was dosed with 200 c.c. culture. It showed the first symptoms of disease about forty hours later. The disease lasted quite a long time, death taking place about twenty and a half days after the dosing.

Ostrich 7 also received 200 c.c. culture. It was first noticed sick forty-five hours later; the paralysis progressed rapidly, and the bird died five days after the dosing.

Ostrich 8 received 250 c.c. culture. Forty-five hours later it was first noticed sick. The condition then improved so that the bird seemed to have recovered after six days. However, nineeten days after the dosing the ostrich again showed signs of general muscular weakness and inco-ordination of movement. The next day it died (see Appendix, page 1177).

Conclusion.—The above experiment does not enable us to state with any degree of accuracy what the minimum lethal dose of lamsiekte toxin (culture) for ostriches is. The results are rather divergent. Ostrich 5, which received 100 c.c. culture, died after four and a half days, whereas ostrich 6, which received double the amount, lived for twenty days, and ostrich 8 ,which got 250 c.c., also twenty days. The explanation for these discrepancies probably lies in the varying toxicity of some of the cultures used, which were not checked so systematically by controls on guinea-pigs as were those used for the more accurate work with cattle, goats, and other animals.

It may, however, be assumed that the minimum lethal dose for ostriches in the case of oral administration of the toxin lies between 20 and 50 c.c.

Experiment No. 4.—Only two ostriches were injected with lamsiekte toxin subcutaneously. Ostrich 1 received 16 c.c. toxin and ostrich 4 received 8.5 c.c. In neither case did any reaction take place.

Conclusion.—The minimum lethal dose in the case of subcutaneous injection of lamsiekte toxin into ostriches is more than 16 c.c. for averaged-sized birds, and the ostrich is therefore relatively resistant.

Summary.

Table No. 11.

Experiment No.	Ostrich No.	Material Used.	Amount.	Mode of Administration.	Result.		Remarks.
					Incubation.	Death after.	
1	2	Rotten bones	1 lb.	Per os.	4 days	5½ days	Probably not much above minimum lethal dose.
2	3	Rotten meat and maggots	1 lb.	Per os.	19½ hours	2½ days	Considerably above minimum lethal dose.
3	1	Culture	20 c.c.	Per os.	6½ days	Recovered after 11 days	Minimum lethal dose probably 20–50 c.c.
	5	,,	100 c.c.	,,	40 hours	4½ days	
	6	,,	200 c.c.	,,	40 hours	20½ days	
	7	,,	200 c.c.	,,	45 hours	5 days	
	8	,,	250 c.c.	,,	45 hours	20 days	
4	1	Toxin	16 c.c.	Subcutaneous	No reaction		Minimum lethal dose more than 16 c.c.
	4	,,	8·5 c.c.	,,	,,		

(*m*) In Turkeys.

The experiments with turkeys were inconclusive and disappointing. The very first bird which was dosed with rotten meat and maggots (turkey 1) developed symptoms two days later, which persisted for more than a day and then disappeared. The test was repeated, using different materials of proved toxicity, but never again did any turkey show any signs of lamsiekte (see Appendix, page 1177).

The results of the tests may be summarized as follows:—

TABLE No. 12.

Experiment No.	Turkey No.	Material Used.	Amount.	Mode of Administration.	Result.	
					Incubation.	Death after.
1	1	Rotten meat and maggots	40 g.	Per os.	2 days	Recovered.
	8	,,	100 g.	,,	No reaction.	
2	3	Culture	5 c.c.	Per os.	No reaction.	
	5	,,	10 c.c.	,,	,,	
	7	,,	70 c.c.	,,	,,	
3	4	Toxin	5 c.c.	Subcutaneous	No reaction.	
	6	,,	8 c.c.	,,	,,	

Conclusion.—From these experiments it is clear that turkeys are not very, if at all, susceptible to lamsiekte.

(*n*) IN DUCKS.

Almost the same results were obtained with ducks as with turkeys. One of the ducks which received rotten meat and maggots (duck 1) was almost completely paralysed seventeen hours later. On the third day after the dosing the symptoms had disappeared (see Appendix, page 1178). Another duck (5), which received ½ c.c. toxin per os, also showed paralytic symptoms which persisted for several days.

None of the other ducks which received toxic material in various ways ever showed any ill-effects.

The results of the experiments are summarized in the following table:—

TABLE No. 13.

Experiment No.	Duck. No.	Material Used.	Amount.	Mode of Administration.	Result.	
					Incubation.	Death after.
1	1	Rotten meat and maggots	40 c.c.	Per os	17 hours	Recovered after 2½ days.
	2	,,	40 c.c.	,,	No reaction.	
	3	,,	40 c.c.	,,	,,	
	4	,,	40 c.c.	,,	,,	
	6	,,	50 c.c.	,,	,,	
2	13	Maggots	Several lb.	Given to eat	No reaction.	
	14	,,	,,	,,	,,	
3	2	Culture	30 c.c.	Per os	No reaction.	
	16	,,	5 c.c.	,,	,,	
	18	,,	10 c.c.	,,	,,	
	19	,,	8 c.c.	,,	,,	
4	5	Toxin	0·5 c.c.	Per os	21 hours	Recovered after 10 days.
	6	,,	0·05 c.c.	,	No reaction.	
	7	,,	0·005 c.c.	,,	,,	
	8	,,	1 c.c.	,,	,,	
5	17	Toxin	5 c.c.	Subcutaneously	No reaction.	
6	9	Toxin	0·05 c.c.	Intravenously	No reaction.	
	10	,,	0·005 c.c.	,,	,,	
	11	,,	0·1 c.c.	,,	,,	
	12	,,	1 c.c.	,,	,,	

Conclusion.—Ducks may undoubtedly contract lamsiekte. Two of the ducks used in the above experiments developed typical symptoms of this disease. On the other hand, several other ducks which received much larger quantities of toxic material showed no symptoms.

The minimum lethal dose for ducks cannot be given. One of the ducks which gave a positive reaction received only ½ c.c. toxin per os.

(*o*) In Pigeons.

Pigeons seem to be less susceptible to lamsiekte than the other species of birds used in these experiments. The following table gives a summary of the result (see Appendix, page 1179).

Table No. 14.

Pigeon No.	Material Used.	Amount.	Mode of Administration.	Result.
1	Rotten meat and maggots	10 g.	Dosed per os	No reaction.
5	Toxin	1 c.c.	Dosed per os	No reaction.
2	Toxin	0·1 c.c.	Injected subcutaneously	No reaction.
3	Toxin	0·01 c.c.	Injected subcutaneously	No reaction.
4	Toxin	0·001 c.c.	Injected subcutaneously	No reaction.
6	Toxin	0·5 c.c.	Injected subcutaneously	No reaction.
7	Toxin	1 c.c.	Injected subcutaneously	No reaction.
8	Toxin	2 c.c.	Injected subcutaneously	Died after 24 hours.
9	Toxin	1 c.c.	Injected subcutaneously	No reaction.
10	Toxin	3 c.c.	Injected subcutaneously	No reaction.
11	Toxin	4 c.c.	Injected subcutaneously	No reaction.
12	Toxin	5 c.c.	Injected subcutaneously	No reaction.

Conclusion.—Only one pigeon (8) showed any reaction, and died twenty-four hours after the injection of the toxin; other pigeons which received bigger doses showed no ill-effects. Similar contradictory results were obtained with the other species of birds experimented upon.

It is impossible to draw any definite conclusions in regard to the minimum lethal dose for pigeons, beyond stating that in general they show a high natural immunity.

D.—ATTENUATION OF THE TOXIN.

With a very potent toxin like the one under investigation it seemed reasonable to expect that it would be possible to attenuate it, and that a certain degree of immunity would follow on the injection of the attenuated toxin.

The problem was attacked from two angles, and, as will be seen, both lines of attack proved fairly successful, although of limited practical application. On the one hand, the effect of physical influences on the toxin were tried, and, on the other, various chemicals were added to the toxin and the effect noted. Among the physical factors, *heat* was employed in the first instance; the records of these experiments are given below.

(*a*) Attenuation by Heat.

(1) *By Means of One Heating.*

In most of the experiments the toxin was subjected to one heating only. The heated toxin was then tested on animals for which the toxicity of the unheated material was accurately known. In the first

place, guinea-pigs were used and subsequently goats. The experiments conducted with these two classes of animals will be recorded separately.

(i) *Tests on Guinea-pigs.*

To facilitate a discussion of these experiments, a summary of the results will be given first. The amount of toxin used in these and later experiments will be indicated in multiples of the minimum lethal dose, which for guinea-pigs is taken as 0.001 c.c. per kg. body-weight. The heating of the toxin was carried out on a water-bath, and the temperature of the toxin itself was measured. Where the toxin is stated to have been heated to a certain temperature for a certain length of time, the period was measured from the moment the thermometer inside the toxin first registered the temperature and until the vessel containing the toxin was removed from the water-bath. All injections were made subcutaneously.

TABLE No. 15.

Experiment No.	Guinea-pig No.	Weight in grm.	Amount of Toxin.	Heated		Result.	
				To.	For.	Incubation.	Died After.
1	23	520	10 × M.L.D.	90°	10 minutes	No reaction.	
	24	560	10 × M.L.D.	75°	10 minutes	6½ days	10½ days.
	25	580	10 × M.L.D.	60°	10 minutes	—	22½ hours.
	26	500	10 × M.L.D.	56°	10 minutes	—	2½ days.
	28	480	1 × M.L.D.	Unheated (control)		6½ days	8½ days.
2	29	750	5 × M.L.D.	70°	10 minutes	5½ days	Recovered.
	31	600	5 × M.L.D.	66°	10 minutes	2 days	6½ days.
	33	340	5 × M.L.D.	62°	10 minutes	2½ days	5 days.
	34	500	5 × M.L.D.	Unheated (control)		About 36 hours	41½ hours.
3	47	510	100 × M.L.D.	70°	1 hour.	3½ days	5 days.
	48	550	100 × M.L.D.	70°	2 hours	No reaction.	
	49	450	100 × M.L.D.	70°	3 hours	,,	
	50	380	100 × M.L.D.	Unheated (control)		—	,, 17 hours.
4	54	550	100 × M.L.D.	80°	½ hour	No reaction.	
	55	630	100 × M.L.D.	80°	10 minutes	,,	
	56	460	100 × M.L.D.	78°	1 hour	,,	
	57	430	100 × M.L.D.	78°	½ hour	,,	
	58	480	100 × M.L.D.	78°	¼ hour	,,	
	59	540	100 × M.L.D.	75°	1 hour	,,	
	60	600	100 × M.L.D.	75°	½ hour	3¾ days	4½ days.
	61	540	100 × M.L.D.	Unheated (control)		14 hours	18 hours.

Discussion of Results.

Experiment No. 1.—Four guinea-pigs each received 10 minimum lethal doses (i.e. 0.01 c.c. toxin per kg. body-weight), which had been heated to 90°, 75°, 60°, and 56° C. respectively for ten minutes. The guinea-pigs which received the toxin heated to 56° and 60° C. died after two and a half days and one day respectively; the guinea-pig which received the toxin heated to 90° C. showed no reaction; and the fourth one died after a lapse of ten and a half days. The control guinea-pig, which received one minimum lethal dose (0.001 c.c. per kg.) of unheated toxin, died after eight and a half days.

Conclusion.—From this experiment it appeared that a temperature of 56° or 60° C. did not affect the toxin at all; 90° C. either destroyed the toxin or attenuated it to such an extent that it produced no symptoms, even when injected in a quantity ten times the size of

the minimum lethal dose. At 75° C. the toxin was undoubtedly attenuated, the guinea-pig showing the first symptoms only six and a half days after the injection and dying four days later.

The critical temperature at which the toxin was attenuated was concluded to be between 60° and 90° C.

The attenuation obtained by heating the toxin to 75° C., and keeping it at this temperature for ten minutes, was obviously inadequate. Two methods for rectifying this suggested themselves. The one was to increase the temperature and the other to continue the heating process for a longer space of time. It even seemed likely that the desired object would be achieved if the toxin were to be kept at a lower temperature for a considerably longer period.

The following experiments, which were all of a preliminary nature, were undertaken with the object of establishing the optimum relation between temperature and duration of heating. The experiments were continued with goats and were concluded with cattle. In the next section it will be seen that the optimum relation finally decided upon was: *heating the toxin to 70° C. and maintaining it at this temperature for twelve hours.* How this procedure was arrived at will be seen in the following experiments:—

Experiment No. 2.—This experiment was commenced before guinea-pig 24 in Experiment No. 1 died. It was then thought that the toxin heated to 90° and 75° C. had been destroyed, and that heated to 56° or 60° C. had not been attenuated at all. The object of Experiment No. 2 was therefore to try and determine whether the toxin could be attenuated at an intermediate temperature.

Toxin was heated to 70°, 66°, and 62° C. for ten minutes, and five minimum lethal doses (0.005 c.c. per kg.) of each lot injected into a guinea-pig. The first guinea-pig (29) got ill and recovered. The other two died after six and a half and five days respectively. The control guinea-pig, which received five minimum lethal doses of the unheated toxin, died within two days.

Conclusion.—There appeared to be some attenuation in all three lots of toxin. In the case of the toxin heated to 70° C. for ten minutes the attenuation had proceeded so far that the guinea-pig which received five minimum lethal doses recovered.

In the next experiment the same temperature was adhered to, but the heating prolonged.

Experiment No. 3.—Three guinea-pigs each received 100 minimum lethal doses (0.1 c.c. per kg.) of toxin heated to 70° C. for one hour, two hours, and three hours respectively. The first guinea-pig died after five days, the other two showed no ill-effects. The control, which received 100 minimum lethal doses unheated toxin, died after seventeen hours.

Conclusion.—In all three cases the toxin was attenuated. In the case of the toxin heated for one hour the attenuation was not sufficient to prevent the appearance of the disease and death.

The result of this and other experiments led to the formula finally adopted, namely, heating the toxin to 70° C. for twelve hours.

Experiment No. 4.—The effect of higher temperatures was tried in this experiment. Toxin was heated to 75° C. for half an hour and one hour; to 78° C. for quarter of an hour, half an hour, and one hour; and to 80° C. for ten minutes and half an hour. 100 minimum lethal doses of each lot were injected into a guinea-pig. Only the guinea-pig which received the toxin heated to 78° C.

for half an hour developed the disease and died after four and a half days. None of the others showed any symptoms. The control, which received 100 minimum lethal doses unheated toxin, died after eighteen hours.

Conclusion.—Any temperature above 75° C. seemed to be sufficient to attenuate the toxin to such an extent that it produced no symptoms. At 75° C. the heating had to be continued for more than half an hour to render the inoculation of a comparatively large quantity, such as 0.1 c.c. per kg., safe.

(ii) *Tests on Goats.*

The same procedure was followed in goats as in guinea-pigs, with only this difference, that larger doses of heated toxin were used.

Experiment No. 1.—Three goats (7, 8, and 9) received 1,000 minimum lethal doses (i.e. 0.1 c.c. per kg.) toxin heated to 65° C. for one hour, two hours, and three hours respectively. Goat 7 died forty-four hours, goat 8 forty-two hours, and goat 9 about fifty hours after injection. (See Appendix, page 1180.)

Conclusion.—The attenuation adopted in this experiment was totally inadequate. The very large dose which was injected was partly responsible for the rapid course the disease took in these three goats.

Experiment No. 2.—Goat 10 received 1,000 minimum lethal doses heated to 70° C. for two hours; goat 11, 1,000 minimum lethal doses heated to 70° C. for three hours; goat 12, the same amount heated to 75° C. for one hour; and goat 13, the same quantity heated to 75° C. for two hours.

Goat 10 died after two and a half days, goat 11 after four days. Goat 12 showed the first symptoms seven days after the injection; a fortnight later the goat was still alive, but completely paralysed (see Appendix, page 1180). It died after an intravenous injection of ferrous sulphate. Goat 13 also showed the first symptoms seven days after the injection and died twenty-four days later.

Conclusion.—The toxin used in this experiment was not sufficiently attenuated.

On the assumption that a longer period of heating would attenuate the toxin further, the following experiment was carried out:—

Experiment No. 3.—Goats 19, 20, and 21 each received 1,000 minimum lethal doses of toxin heated to 70° C. for twelve hours and showed no ill-effects at all.

These three goats, together with others, were later on used for immunity experiments [see Section E (*a*) (1) (ii), page 1131, and Appendix, page 1189].

Conclusion.—The method of attenuation used in this experiment, namely, heating to 70° C. for twelve hours, seemed to be adequate for the amount of toxin used (1,000 minimum lethal doses, i.e. 0.1 c.c. per kg. body-weight).

This method seemed, therefore, to lend itself to the routine immunization of animals against lamsiekte by means of heated toxin [see Section E (*a*) (1)].

Experiment No. 4.—This experiment was undertaken to determine whether toxin heated to 70° C. for twelve hours could with safety be injected into goats in quantities larger than 1,000 minimum lethal doses. It was considered advisable to start with the smaller amount (1,000 minimum lethal doses) and then to give 5,000 or 10,000 minimum lethal doses.

Ten goats (54, 55, 58, 59, 60, 61, 62, 63, 64, and 67) each received 1,000 minimum lethal doses heated toxin with no ill-effects whatever. Thereupon each of these goats was injected with 5,000 minimum lethal doses toxin heated to 70° C. for twelve hours. In one (60), symptoms of lamsiekte developed after an incubation period of six days; the disease lasted about three weeks and the animal recovered completely. The other nine goats showed no reaction.

Eight of the goats received a further injection of 10,000 minimum lethal doses heated toxin (i.e. 1 c.c. per kg. body-weight) without showing any ill-effects.

Conclusion.—Toxin heaed to 70° C. for twelve hours may be injected into goats in amounts of 5,000 minimum lethal doses (½ c.c. per kg.) or 10,000 (1 c.c. per kg.) without ill-effect. In some instances, however, these large quantities will produce symptoms of lamsiekte.

The following table summarizes the results of the experiments recorded above:—

TABLE No. 16.

Experiment No.	Goat No.	Weight in kg.	Amount of Toxin.	Expressed in M.L.D.	Heated		Result.	
					To.	For.	Incubation.	Died after.
1	7	36	3·6 c.c.	1,000 × M.L.D.	65°	1 hour	39 hours	44 hours.
	8	33	3·3 c.c.	1,000 × M.L.D.	65°	2 hours	39 hours	42 hours.
	9	32	3·2 c.c.	1,000 × M.L.D.	65°	3 hours	39 hours	About 50 hours.
2	10	41	4·1 c.c.	1,000 × M.L.D.	70°	2 hours	—	2½ days.
	11	39	3·9 c.c.	1,000 × M.L.D.	70°	3 hours	3½ days	4 days.
	12	36	3·6 c.c.	1,000 × M.L.D.	75°	1 hour	7 days	Killed after 27 days.
	13	33	3·3 c.c.	1,000 × M.L.D.	75°	2 hours	7 days	31 days.
3	19	43	4·3 c.c.	1,000 × M.L.D.	70°	12 hours	No reaction.	
	20	42	4·2 c.c.	1,000 × M.L.D.	70°	12 hours	,,	
	21	36·5	3·65 c.c.	1,000 × M.L.D.	70°	12 hours	,,	
4	54	28	2·8 c.c.	1,000 × M.L.D.	70°	12 hours	No reaction.	
			14 c.c.	5,000 × M.L.D.	70°	12 hours	,,	
			28 c.c.	10,000 × M.L.D.	70°	12 hours	,,	
	55	21	2·1 c.c.	1,000 × M.L.D.	70°	12 hours	,,	
			10·5 c.c.	5,000 × M.L.D.	70°	12 hours	,,	
			21 c.c.	10,000 × M.L.D.	70°	12 hours	,,	
	58	21	2·1 c.c.	1,000 × M.L.D.	70°	12 hours	,,	
			10·5 c.c.	5,000 × M.L.D.	70°	12 hours	,,	
			21 c.c.	10,000 × M.L.D.	70°	12 hours	,,	
	59	29	2·9 c.c.	1,000 × M.L.D.	70°	12 hours	,,	
			14·5 c.c.	5,000 × M.L.D.	70°	12 hours	,,	
			29 c.c.	10,000 × M.L.D.	70°	12 hours	,,	
	60	26	2·6 c.c.	1,000 × M.L.D.	70°	12 hours	,,	
			13 c.c.	5,000 × M.L.D.	70°	12 hours	6¼ days	Recovered.
	61	38	3·8 c.c.	1,000 × M.L.D.	70°	12 hours	No reaction.	
			19 c.c.	5,000 × M.L.D.	70°	12 hours	,,	
			38 c.c.	10,000 × M.L.D.	70°	12 hours	,,	
	62	24	2·4 c.c.	1,000 × M.L.D.	70°	12 hours	,,	
			12 c.c.	5,000 × M.L.D.	70°	12 hours	,,	
			24 c.c.	10,000 × M.L.D.	70°	12 hours	,,	
	63	26	2·6 c.c.	1,000 × M.L.D.	70°	12 hours	,,	
			13 c.c.	5,000 × M.L.D.	70°	12 hours	,,	
			26 c.c.	10,000 × M.L.D.	70°	12 hours	,,	
	64	23	2·3 c.c.	1,000 × M.L.D.	70°	12 hours	,,	
			11·5 c.c.	5,000 × M.L.D.	70°	12 hours	,,	
			23 c.c.	10,000 × M.L.D.	70°	12 hours	,,	
	67	27	2·7 c.c.	1,000 × M.L.D.	70°	12 hours	,,	
			13·5 c.c.	5,000 × M.L.D.	70°	12 hours	,,	

(iii) *Test on Horse.*

Horse 13340 received 1,000 minimum lethal doses toxin heated to 70° C. for twelve hours and showed no reaction. It thereupon received 5,000 minimum lethal doses, and about a month later a further