quantity of 10,000 minimum lethal doses toxin heated in the same way. The horse was then bled and its blood used in immunity tests [see Section E (*b*), page 1148].

Conclusion.—Heating the toxin to 70° C. for twelve hours seems to be a safe method as far as horses are concerned.

(iv) *Tests on Cattle.*

The majority of the animals mentioned below were injected with heated toxin for the purpose of immunization. Those animals which remained alive were afterwards tested for immunity with unheated toxin.

All experiments dealing with the question of immunity will be discussed in a later section [E. (*a*) (1) (i), page 1129]. Here we are only concerned with the tests in so far as they affect the question of attenuation by means of heat.

Experiment No. 1.—In this experiment five cattle were injected with toxin heated to 75° C. for varying periods. Heifer 4843 received 150 minimum lethal doses of toxin heated to 75° C. for ten minutes. It showed the first symptoms of lamsiekte after seven and a half days, and had to be destroyed ten days later. Cow 2404 received 1,000 minimum lethal doses heated to 78° C. for half an hour, and tollie 218 received the same amount heated to 75° C. for one hour; neither showed any reaction. (See Appendix, page 1181.) Heifer 4752 received 1,000 minimum lethal doses heated to 75° C. for three hours. The first symptoms appeared four and a half days later, and the animal died thirteen days after the injection as a result of an intravenous injection of sodium arsenite (Appendix, page 1182). Cow 2388 received 1,000 minimum lethal doses heated to 75° C. for six hours and showed no ill-effects.

Conclusion.—The method of attenuation adopted in this experiment was not reliable. Perhaps a longer heating at 75° C. would have given satisfactory results, but it was felt that it would probably be better to extend the heating at a lower temperature (say, 70° C.) if a safe vaccine could be obtained in this way.

The experiment also demonstrated the variable behaviour of individual animals towards the toxin. Whereas cow 2404, which received toxin that had been heated to 75° C. for only half an hour, showed no reaction at all, heifer 4752 developed lamsiekte, although it received the same amount of toxin, which had been heated to 75° C. for three hours, and should therefore have been weaker.

Experiment No. 2.—In this experiment the standard method of attenuation (heating to 70° C. for twelve hours) was adopted.

Six animals received as a first injection 1,000 minimum lethal doses heated to 70° C. for twelve hours, and not one of them showed any reaction.

Some of these animals thereupon received larger quantities of heated toxin for the purpose of strengthening their immunity. Tollie 266 was injected with 5,000 minimum lethal doses about three weeks after the first injection of 1,000 minimum lethal doses. Nine days after the second injection the animal showed the first signs of paralysis. Three days later it was completely paralysed, and it died fourteen days after the injection (see Appendix, page 1182). Cow 229 first received 1,000 minimum lethal doses toxin heated to 70° C. for twelve hours. Eighteen days later it was injected 5,000 minimum lethal doses, treated in the same way, and again seventeen days later with

10,000 minimum lethal doses. No ill-effects were noted after any of these injections. Ox 203 received 1,000, 5,000, and 10,000 minimum lethal doses of heated toxin at about three to four weeks' intervals. The first and second injection produced no reaction, but eight days after the third the animal was partly paralysed (see Appendix, page 1183). It died two and a half days later.

Experiment No. 3.—Six more animals were injected with large quantities of toxin heated to 70° C. for twelve hours. None of them showed any symptoms of lamsiekte.

The following table gives the necessary details in connexion with the cattle used for these experiments.

TABLE No. 17.

Experiment No.	Cattle No.	Weight in kg.	Amount of Toxin Expressed in M.L.D.	Heated		Result.	
				To.	For.	Incubation.	Died after.
1	4843	165	150 × M.L.D.	75°	10 minutes	7½ days	Killed after 18 days.
	2404	295	1,000 × M.L.D.	75°	½ hour	No reaction.	
	218	231	1,000 × M.L.D.	75°	1 hour	,,	
	4752	156	1,000 × M.L.D.	75°	3 hours	4½ days	13 days.
	2388	268	1,000 × M.L.D.	75°	6 hours	No reaction.	
2	249	156	1,000 × M.L.D.	70°	24 hours	No reaction.	
	4376	163	1,000 × M.L.D.	70°	12 hours	,,	
	267	252	1,000 × M.L.D.	70°	12 hours	,,	
	266	147	1,000 × M.L.D.	70°	12 hours	,,	
			5,000 × M.L.D.	70°	12 hours	9 days	14 days
	229	359	1,000 × M.L.D.	70°	12 hours	No reaction.	
			5,000 × M.L.D.	70°	12 hours	,,	
			10,000 × M.L.D.	70°	12 hours	,,	
	203	239	1,000 × M.L.D.	70°	12 hours	,,	
			5,000 × M.L.D.	70°	12 hours	,,	
			10,000 × M.L.D.	70°	12 hours	8 days	10½ days.
3	4664	236	1,000 × M.L.D. (intravenously)	70°	12 hours	Shock.	
			5,000 × M.L.D. (intravenously)	70°	12 hours	,,	
	4164	440	5,000 × M.L.D.	70°	12 hours	No reaction.	
			10,000 × M.L.D.	70°	12 hours	,,	
	2994	413	10,000 × M.L.D.	70°	12 hours	,,	
	4681	281	5,000 × M.L.D.	70°	12 hours	,,	
	4346	220	10,000 × M.L.D.	70°	12 hours	,,	
	4683	227	10,000 × M.L.D.	70°	12 hours	,,	

General Conclusions.—From these experiments it would appear to be perfectly safe to inject toxin that had been heated to 70° C. for twelve hours subcutaneously into cattle in quantities up to 1,000 minimum lethal doses (i.e. 0.1 c.c. per kg. body-weight).

If much larger quantities are injected, lamsiekte may result. In Experiment No. 2, two animals (266 and 203), which received 5,000 and 10,000 minimum lethal doses respectively, contracted lamsiekte and died. In both of these cases the long incubation period (nine and eight days) is worthy of note. Another animal in Experiment No. 2 (229) and six animals in Experiment No. 3 also received very large doses without showing any ill-effects.

Should an injection of 1,000 minimum lethal doses heated toxin have given an adequate degree of immunity, the method would have been eminently satisfactory. In how far this proved to be the case will be seen in Section E (*a*) (1), page 1129.

(2) *Repeated Heatings.*

Only one test was carried out with toxin heated to 65° C. for ten minutes on each of three successive days. Forty minimum lethal doses (0.04 c.c. per kg. body-weight) of this toxin were injected into a guinea-pig (44) (see Appendix, page 1183). The first symptoms of lamsiekte were noticed three days after injection, and the guinea-pig died about twelve hours later.

Conclusion.—Three heatings for ten minutes each at 65° C. were not sufficient to attenuate the toxin.

In view of the satisfactory results obtained with the method recorded in the previous sub-section (viz., a single heating to 70° C. for twelve hours) it was considered unnecessary to attempt further ways of attenuation by repeated heatings.

(*b*) By Cold.

Again only a single test was conducted. A quantity of filtered toxin was collected in a test tube which was then covered with solid CO_2. The toxin froze rapidly and was kept in this state for three hours. The tube was then left at room-temperature and the toxin slowly returned to the liquid state.

Ten minimum lethal doses (0.01 c.c. per kg.) of this liquid were thereupon injected into a guinea-pig (27), which was found dead two and a half days after the injection (see Appendix, page 1183).

Conclusion.—The toxin was not attenuated by freezing and being kept in the frozen state for three hours.

(*c*) By Exposure to Sunlight.

Filtered toxin was collected in a clear test tube, which was exposed to the direct rays of the sun for five and a half hours.

Ten minimum lethal doses (0.01 c.c. per kg.) were then injected into rabbit No. 9. The result was that the rabbit contracted lamsiekte about sixty-eight hours, and died about nine and a half days, after the injection (see Appendix, page 1183).

Conclusion.—Lamsiekte toxin is not attenuated by exposure to sunlight for five and a half hours.

(*d*) By Drying.

2 c.c. filtered toxin was poured on a piece of blotting-paper, which was then dried in the sun for two and a half hours. The dry-paper was then soaked in 20 c.c. physiological saline solution and the liquid squeezed out. In this way 5.8 c.c. liquid was obtained which was injected into a rabbit (8); two and a half days after the injection the rabbit was found dead (see Appendix, page 1184).

Conclusion.—Drying toxin in the sun did not attenuate it in any way.

(*e*) By Keeping for a Long Time.

A sterile solution of lamsiekte toxin was kept at room-temperature in a dark cupboard.

About two months later each of two cattle was injected with 50 minimum lethal doses (0.005 c.c. per kg.) of this toxin. Heifer 309 was found dead less than three days after the injection without having shown symptoms. Ox 3904 showed definite symptoms of lamsiekte two days after injection, and was found dead at the same time as 309.

About six months after the preparation of the toxin ox 4623 was injected with 50 minimum lethal doses. Five days later the animal showed the first signs of lamsiekte (dullness, salivation). The next day the animal was paralysed (see Appendix, page 1184) and died almost eight days after the injection.

These results may be summarized as follows:—

TABLE No. 18.

Cattle No.	Injected with.	Kept for.	Result.	
			Incubation.	Died after.
309	50 × M.L.D. toxin	2 months	—	Less than 3 days.
3904	50 × M.L.D. toxin	2 months	2 days	Less than 3 days.
4623	50 × M.L.D. toxin	6 months	5 days	8 days.

Conclusion.—The toxin did not seem to have deteriorated at all after two months' keeping. After six months, however, the incubation period was markedly lengthened, and the conclusion seems justified that the toxin had been attenuated to some extent after this period.

(*f*) BY MEANS OF CHEMICALS.

(1) *Iodine.*

The first chemical that suggested itself as a means of attenuating the toxin was iodine. In all the tests it was used in the form of Lugol's solution.

The solution was made up fresh for each test. It was then mixed with the toxin in varying proportions and the mixture injected into susceptible animals to determine what proportion of Lugol's solution was necessary to achieve the desired degree of attenuation.

(i) *Tests on Guinea-pigs.*

Experiment No. 1.—The quantities of Lugol solution used in this preliminary experiment proved to be much too big.

Guinea-pig 38, weighing 465 grams, received 10 minimum lethal doses of toxin (i.e. 0.01 c.c. per kg. = 0.00465 c.c.) in a dilution of 1 to 1,000 (i.e 4.65 c.c. of the dilution) mixed with the same quantity (4.65 c.c.) Lugol solution. This mixture, therefore, contained 1,000 times as much Lugol solution as toxin (4.65 : 0.00465). It was made ten minutes prior to injection.

In this and the following experiments the proportion between Lugol solution and toxin will be calculated in the same way.

Guinea-pig 38 was found dead next morning. There was, however, reason to believe that this guinea-pig had died from some extraneous cause, and another guinea-pig (42) was therefore substituted. This animal showed no reaction.

Guinea-pig 39 received 10 minimum lethal doses mixed with 250 times the amount of Lugol solution. Guinea-pig 40 received 10 minimum lethal doses mixed with 100 times the amount of Lugol solution. Neither of these guinea-pigs showed any reaction. The control guinea-pig (41) received 2 minimum lethal doses of toxin without the admixture of any Lugol solution and died seventeen hours later. Another control guinea-pig (43) was added, which received no toxin, but merely an amount of physiological saline solution corresponding

to the quantity of toxin solution (1 : 1,000), which would have been injected had the guinea-pig received 10 minimum lethal doses mixed with the same quantity Lugol solution.* The object of this injection was to show the effect, if any, of the Lugol solution. Nothing resulted.

Conclusion.—Toxin mixed with 100 to 1,000 times the amount of Lugol solution is harmless when injected into guinea-pigs.

Experiment No. 2.—In this experiment much smaller quantities of Lugol solution were used than in the first experiment.

Guinea-pig 51 received 100 minimum lethal doses of toxin mixed with seven and a half times the amount of Lugol solution. Guinea-pig 52 received 100 minimum lethal doses, plus five times the amount of Lugol soiution, and guinea-pig 53 received 100 minimum lethal doses, plus two and a half times the amount of Lugol solution. None of these guinea-pigs showed any reaction. The control guinea-pig (50)† received 100 minimum lethal doses toxin without any Lugol solution, and died seventeen hours after the injection.

Conclusion.—According to the results obtained in this experiment it appeared to be safe to inject toxin mixed ten minutes previously with two and a half to seven and a half times as much Lugol solution.

These results were thereupon applied to goats.

Table No. 19 gives a summary of the experiments just discussed.

TABLE No. 19.

Experiment No.	Guinea-pig No.	Weight in grm.	Amount of Toxin in M.L.D.	Amount of Toxin in c.c.	Quantity of Diluted Toxin.	Amount of Lugol Solution.	Proportion of Toxin to Lugol Solution.	Result.	Remarks.
1	42	430	10 × M.L.D.	0·0043	4·3 c.c.	4·3 c.c.	1 : 1,000	No reaction	
	39	440	10 × M.L.D.	0·0044	4·4 c.c.	1·1 c.c.	1 : 250	,,	
	40	430	10 × M.L.D.	0·0043	4·3 c.c.	0·43 c.c.	1 : 100	,,	
	41	340	2 × M.L.D.	0·00068	0·68 c.c.	—	—	Died after 17 hours	Toxin control.
	43	380	—	—	3·8 c.c. saline solution	3·8 c.c.	—	No reaction	Iodine control.
2	51	430	100 × M.L.D.	0·043	4·3 c.c.	0·32 c.c.	1 : 7½	No reaction	
	52	430	100 × M.L.D.	0·043	4·3 c.c.	0·21 c.c.	1 : 5	,,	
	53	430	100 × M.L.D.	0·043	4·3 c.c.	0·11 c.c.	1 : 2½	,,	
	50	380	100 × M.L.D.	0·038	3·8 c.c.	—	—	Died after 17 hours	Toxin control.

(ii) *Tests on Goats.*

Experiment No. 1.—Goats 4, 5, and 6 each received 200 minimum lethal doses of filtered and diluted toxin mixed with 50, 10, and 1 times the amount of Lugol solution respectively. Goats 4 and 5 showed no reaction, but goat 6, which received equal quantities of toxin and Lugol solution, developed symptoms of lamsiekte within twenty-four hours and died after thirty to thirty-six hours.

Conclusion.—Whereas in the experiments conducted with guinea-pigs the quantity of iodine was always large enough to attenuate the toxin to such an extent that no reaction followed the injection, we see from the experiment recorded here that if the amount of Lugol solution be reduced to the same quantity as the toxin the latter will prove fatal.

* Guinea-pig 43 weighed 380 grm. 1 minimum lethal dose = 0·001 × 0·380 = 0·00038. 10 minimum lethal doses = 0·0038.

In a dilution of 1 : 1,000 the dose would have been 3·8 c.c. The guinea-pig, therefore, received 3·8 c.c. saline solution plus 3·8 c.c. Lugol solution.

† This guinea-pig also served as control in Experiment No. 3 of Section D (a) (1) (*i*), which was carried out at the same time. (See page 1116.)

We concluded therefore that the smallest quantity of Lugol solution necessary to render the toxin harmless is more than one and less than five times the amount of toxin.

To determine this quantity the following experiments were carried out.

Experiment No. 2.—Goats 5, 4 (both of which had previously been used in Experiment No. 1), 14, and 15 each received 1,000 minimum lethal doses of toxin mixed with one and a half, two, three, and five times the amount of Lugol solution respectively.

Goat 5 showed the first symptoms of lamsiekte thirty-six hours after the injection, and was dead twenty-four hours later. Goat 4 was found dead thirty-six hours after the injection. Goat 14 was partly paralysed after forty-four hours and died forty-eight hours after injection, and goat 15 was paralysed after thirty-eight hours and died after forty-six hours. The control goat (16), which received no toxin, but only a quantity of Lugol solution corresponding to the amount given to goat 15, showed no reaction.

It should be noted that the toxin used in this experiment had not been passed through a Berkefeld candle, but merely through a muslin cloth. The liquid therefore undoubtedly contained a larger quantity of extraneous organic matter than usually associated with the toxin, and would therefore be expected to react with more iodine (see Appendix, page 1184).

Conclusion.—Apparently too little iodine had been added in this experiment. However, since a Berkefeld filtrate had not been used, the results were regarded as inconclusive and the experiment was repeated.

Experiment No. 3.—In order to retest the results obtained in the previous experiment, goats 17 and 18 were injected with 1,000 minimum lethal doses each, mixed with three and five times the amount of Lugol solution respectively. Neither goat showed any reaction.

Conclusion.—Lamsiekte toxin mixed with three or more times its amount of Lugol solution seemed to be rendered innocuous for goats.

This result appeared to justify a further reduction in the amount of iodine in accordance with the results obtained in guinea-pigs (see page 1123).

Experiment No. 4.—Goats 22 and 23 each received 1,000 minimum lethal doses toxin mixed with two and two and a half times the amount of Lugol solution. Neither animal showed any reaction.

Conclusion.—The amounts of iodine used in this experiment seemed to have attenuated the toxin sufficiently.

It was thereupon decided to inject a number of goats with large quantities of toxin mixed with two and a half times the amount of Lugol solution. The intention was to immunize the goats in this way and then to test their immunity. The next two experiments therefore really belong in Section E (*a*) (3) (ii) (see page 1137). In the present section reference will only be made to the result of injecting the mixture of toxin and iodine.

Experiment No. 5.—Ten goats (24, 25, 26, 27, 28, 29, 30, 31, 32, and 33) were each injected with 1,000 minimum lethal doses (0.1 c.c. per kg.) toxin mixed with two and a half times the amount of Lugol solution. The results were somewhat disastrous.

Goat 24 showed the first symptoms of lamsiekte about four and a half days after the injection (see Appendix, page 1194), but recovered within two days. Goat 25 showed symptoms three days

after injection; these persisted for about three weeks and then disappeared. Goat 26 got ill about two and a half days after injection. Four weeks later it was still paralysed and was then treated with ferrous sulphate, but died three days later. Goat 27 died five and a half days, goat 28 five days, and goat 29 four and three quarter days after injection. Goat 30 was ill four and a half days after injection, and had recovered fifteen days later. Goat 31 died six and a half days after injection, goat 32 four and a half days, and goat 33 (which had received 4,000 minimum lethal doses) two and three-quarter days after injection (see Appendix, page 1186).

After recovery, goat 24 was injected with 5,000 minimum lethal doses toxin mixed with three times the amount of Lugol solution. It showed no reaction, and thereupon received a third injection of 10,000 minimum lethal doses mixed with three times the amount of Lugol solution, again without ill-effects.

TABLE No. 20.

Experiment No.	Goat No.	Weight in kg.	Amount of Toxin in M.L.D.	Proportion of Toxin to Lugol Solution.	Result.		Remarks.
					Incubation.	Died after.	
1	4	40·8	200 × M.L.D.	1 : 50	No reaction		
	5	38·5	200 × M.L.D.	1 : 10	,,		
	6	40	200 × M.L.D.	1 : 1	24 hours	30–36 hours	
2	5	38·5	1,000 × M.L.D.	1 : 1½	36 hours	2 days	
	4	40·8	1,000 × M.L.D.	1 : 2	—	36 hours	
	14	42·2	1,000 × M.L.D.	1 : 3	44 hours	48 hours	
	15	37·5	1,000 × M.L.D.	1 : 5	38 hours	46 hours	
	16	31·3	—	15·6 c.c. Lugol solution	No reaction		Iodine control.
3	17	36·3	1,000 × M.L.D.	1 : 3	No reaction		
	18	31·7	1,000 × M.L.D.	1 : 5	,,		
4	22	22	1,000 × M.L.D.	1 : 2	No reaction		
	23	36	1,000 × M.L.D.	1 : 2½	,,		
5	24	34	1,000 × M.L.D.	1 : 2½	4½ days	Recovered after 6½ days	
			5,000 × M.L.D.	1 : 3	No reaction		
			10,000 × M.L.D.	1 : 3	,,		
	25	43	1,000 × M.L.D.	1 : 2½	3 days	Recovered after 25 days	
	26	47	1,000 × M.L.D.	1 : 2½	2½ days	34 days	
	27	47	1,000 × M.L.D.	1 : 2½	3 days	5¾ days	
	28	47	1,000 × M.L.D.	1 : 2½	3½ days	5 days	
	29	49	1,000 × M.L.D.	1 : 2½	3½ days	4¾ days	
	30	36	1,000 × M.L.D.	1 : 2½	4½ days	Recovered after 20 days	
			5,000 × M.L.D.	1 : 3	—	4½ days	
	31	47	1,000 × M.L.D.	1 : 2½	3½ days	6½ days	
	32	42	1,000 × M.L.D.	1 : 2½	3 days	4½ days	
	33	36	1,000 × M.L.D.	1 : 2½	64 hours	67 hours	
6	44	40	1,000 × M.L.D.	1 : 4	No reaction		
	45	34	1,000 × M.L.D.	1 : 4	,,		
			5,000 × M.L.D.	1 : 3	,,		
	46	59	1,000 × M.L.D.	1 : 4	,,		
			5,000 × M.L.D.	1 : 3	,,		
	47	30	1,000 × M.L.D.	1 : 4	,,		
			5,000 × M.L.D.	1 : 3	,,		
			10.000 × M.L.D.	1 : 3	,,		
	48	39	1,000 × M.L.D.	1 : 4	,,		
	49	57	1,000 × M.L.D.	1 : 4	,,		
	50	57	1,000 × M.L.D.	1 : 4	,,		
			5,000 × M.L.D.	1 : 3	,,		
	51	45	1,000 × M.L.D.	1 : 4	,,		
	52	38	1,000 × M.L.D.	1 : 4	,,		
			5,000 × M.L.D.	1 : 3	,,		
	53	31	1,000 × M.L.D.	1 : 4	,,		
			5,000 × M.L.D.	1 : 3	,,		

Goat 30 also received a second injection of 5,000 minimum lethal doses mixed with three times the amount of toxin. Four and a half days after the injection the goat was found dead.

Conclusions.—The disastrous results of this experiment showed very clearly that the addition of two and a half times the amount of Lugol solution to the toxin is not always sufficient for attenuation. In the case of the second injection into goat 30, even three times the amount of Lugol solution was insufficient to counteract the fatal effect of the toxin.

In the next experiment the proportion between Lugol solution and toxin was therefore increased to four.

Experiment No. 6.—Ten goats (44, 45, 46, 47, 48, 49, 50, 51, 52, and 53) each received 1,000 minimum lethal doses toxin mixed with four times the amount of Lugol solution. None of these goats showed the slightest reaction.

In the case of several of these goats, the injection was repeated with larger quantities of toxin mixed with three times the amount of Lugol solution. In no case did the goats show any reaction.

Conclusion.—Lamsiekte toxin mixed with four times the amount of Lugol solution was found to be perfectly safe for goats. The results of the foregoing experiment have been summarized in Table No. 20.

(2) *Other Chemicals.*

(i) *Carbolic Acid.*

Rabbit 21 was injected with 10 minimum lethal doses toxin (0.01 c.c. per kg.) in a dilution of 1 in 200 parts of physiological saline solution containing 0.5 per cent. carbolic acid. Forty-three hours after injection the rabbit was found dead.

(ii) *Alcohol.*

Rabbit 25 received 10 minimum lethal doses toxin mixed with five times the amount of absolute alcohol. This rabbit was also found dead forty-three hours after the injection.

(iii) *Ether.*

Rabbit 24 received 10 minimum lethal doses toxin mixed with five times the amount of ether. Forty-three hours later the rabbit was very ill, and it died fifty hours after the injection.

(iv) *Chloroform.*

Rabbit 23 was injected with 10 minimum lethal doses toxin mixed with five times the amount of chloroform. The incubation period lasted about forty-three hours and the rabbit died forty-eight hours after the injection.

(v) *Boracic Acid.*

Rabbit 22 received 10 minimum lethal doses toxin in a dilution of 1 in 100 parts of saline containing 3 per cent. boracic acid. This rabbit also was seen to be ill after forty-three hours, and died forty-five hours after the injection (see Appendix, page 1187).

Conclusion.—None of the chemicals mentioned above, when mixed with lamsiekte toxin in the concentration indicated, had any effect upon its virulency.

The results of these experiments have been summarized in the following table:—

TABLE No. 21.

Rabbit	Weight in grm.	Amount of Toxin in M.L.D.	Mixed with.	Concentration of Chemical or Proportion of Toxin to Chemical	Result.	
					Incubation.	Died after.
21	1,230	10 × M.L.D.	Carbolic acid	0·5 %	—	43 hours.
25	1,490	10 × M.L.D.	Alcohol	1 : 5	—	43 hours.
24	1,190	10 × M.L.D.	Ether	1 : 5	43 hours	52 hours.
23	1,890	10 × M.L.D.	Chloroform	1 : 5	43 hours	48 hours.
22	1,190	10 × M.L.D.	Boracic acid	3 %	43 hours	45 hours.

(*g*) By Occlusion with Agar.

It was thought that it might be possible to retard the absorption of the toxin by mixing it with a substance such as agar, which, when injected under the skin, would perhaps liberate the toxin slowly.

Experiment No. 1.—Rabbit 301 was accordingly injected with 2 c.c. toxin (1,000 × minimum lethal doses) mixed with 5 c.c. bouillon and 5 c.c. melted agar. The mixture was allowed to cool to body-temperature and then, before setting, was injected under the skin of the rabbit. Twenty-two hours later the rabbit was found dead.

Experiment No. 2.—Smaller quantities of toxin were mixed with varying amounts and concentrations of agar and injected into rabbits.

Rabbit 303 received 2 minimum lethal doses toxin mixed with 5 c.c. of a 1 per cent. agar solution. It died after three days.

Rabbit 304 received 100 minimum lethal doses toxin mixed with 5 c.c. of a 2 per cent. agar solution. It died forty-two hours later.

Rabbit 305 received 2 minimum lethal doses toxin mixed with 10 c.c. of a 2 per cent. agar solution. After two and three-quarter days it looked ill and died twenty-four hours later.

The control rabbit (302) received 2 minimum lethal doses toxin and died three days after the injection (see Appendix, page 1187).

Conclusion.—From these experiments it was concluded that the admixture of an agar solution had no effect upon the virulency of the lamsiekte toxin.

Table No. 22 summarizes the results.

TABLE No. 22.

Rabbit No.	Weight in grm.	Amount of Toxin in M.L.D.	Quantity of Agar Solution.	Concentration of Agar.	Result.	
					Incubation.	Died after.
301	2,000	1,000 × M.L.D.	5 c.c.	2 %	—	22 hours.
303	1,700	2 × M.L.D.	5 c.c.	1 %	—	3 days
304	1,870	100 × M.L.D.	5 c.c.	2 %	—	42 hours.
305	2,000	2 × M.L.D.	10 c.c.	2 %	2¾ days	3¾ days.
302	1,600	2 × M.L.D.	—	—	—	3 days.

E.—ATTEMPTS AT IMMUNIZATION.

Before proceeding to describe the numerous experiments which were undertaken in the hope of finding a practical method of immunizing cattle against lamsiekte, it is necessary to point out that *a priori* there seemed to be very little likelihood of ever discovering a method whereby a strong artificial immunity could be conferred on cattle. In nature there seemed to be no such thing as immunity against lamsiekte. Cattle which recover from an attack of lamsiekte have been known to contract the disease a second, third, or even a fourth time, the last time usually with fatal results. It was clear therefore that a simple method whereby a beast was made to contract the disease in a non-fatal form would have very little chance of success. Most of the efforts were accordingly directed towards giving the animal a large amount of attenuated toxin in the hope of inducing the production of large quantities of immune bodies in this way. Other avenues in which there appeared a faint hope of success were also explored—with what results will be seen in the following pages.

(*a*) Active Immunization.

Various methods of active immunization were attempted. Animals were injected with (1) heated toxin, (2) spores, i.e. cultures freed from toxin, (3) toxin treated with iodine (Lugol solution), (4) heated or unheated toxin injected into the tail, (5) increasing doses of toxin, commencing with very small sublethal doses.

These methods will be dealt with in succession.

(1) *By means of Heated Toxin.*

In Section D (*a*)—see page 1115—experiments are recorded which led to the conclusion that lamsiekte toxin could be injected in large quantities into cattle and goats with comparative safety if it was previously heated to 70° C. for twelve hours. This method was accordingly tested on a fairly large number of cattle and goats. Some of the animals injected for the purpose of producing immunity died as a result of the injection; these have already been dealt with in Section D (*a*). In the present section only those animals are enumerated which withstood the injection of the heated toxin and were subsequently tested for immunity, and those which died from other causes.

(*i*) *In Cattle.*

In four animals only, which had withstood the injection of 1,000 minimum lethal doses of heated toxin, was the immunity test carried out.

Tollie 218, which had received 1,000 minimum lethal doses toxin heated to 75° for one hour, and had shown no reaction, was injected twenty days later with 10 minimum lethal doses ordinary toxin (0.001 c.c. per kg. body-weight). Thirteen days later it showed the first definite symptoms of lamsiekte, which gradually lessened until the sixteenth day after the injection, when the animal had recovered completely.

This result was distinctly promising. The very long incubation period and the ultimate recovery showed that the animal had a very marked resistance against a dose of toxin which would, in an untreated animal, probably have proved fatal in about two to four days.

The question now was whether this injection of 10 minimum lethal doses, from the effects of which tollie 218 had recovered, had increased its immunity.

It was accordingly injected, about six days after its final recovery, with 50 minimum lethal doses of unheated toxin. After thirty-nine hours it showed well-marked symptoms of lamsiekte, and was found dead the next morning (sixty-two hours after injection).

Tollie 249 received 1,000 minimum lethal doses toxin heated to 70° C. for twenty-four hours and showed no reaction. Twenty-three days later its immunity was tested with 10 minimum lethal doses unheated toxin. After five and a half days it showed the first symptoms of lamsiekte. Three days later there was a temporary improvement, but twelve days after the injection the animal was *in extremis* and had to be killed.

Tollie 267 was immunized with 1,000 minimum lethal doses toxin heated to 70° C. for twelve hours without showing any reaction. Twenty-five days later its immunity was tested with 10 minimum lethal doses unheated toxin. Lamsiekte developed after four days, and the animal died about seven and a half days after the injection.

Tollie 4376 was also immunized with 1,000 minimum lethal doses toxin heated to 70° C. for twelve hours without any ill effects. Twelve days later it was injected with 10 minimum lethal doses unheated toxin. About seven days later it showed the first symptoms of lamsiekte, and died eleven and a half days after the injection (see Appendix, page 1189).

Conclusions.—The result of these tests was exceedingly disappointing. In only one case (218) was the immunity, produced by the injection of 1,000 minimum lethal doses attenuated toxin, sufficient to protect the animal against 10 minimum lethal doses of unheated toxin, although even in this case the animal contracted lamsiekte in a mild form. The other three animals died after the injection of 10 minimum lethal doses.

The injection of 1,000 minimum lethal doses heated toxin undoubtedly did confer some immunity on the animals—218 proved this, and also in the other three animals the incubation period and the duration of the disease was much longer than would have been the case had they not been previously treated. Whether these animals would have been able to withstand an immunity test with a smaller dose of toxin (say, 1 or 2 minimum lethal doses) cannot be stated with certainty, but is very likely. However, it was felt that the method would have no practical value unless it could protect animals against at least 10 minimum lethal doses.

The result of the second immunity test in tollie 218 was also very interesting. Fifty minimum lethal doses of unheated toxin killed the animal in two and a half days. This seems to indicate that whatever immunity was conferred on the animal by the injection of the 1,000 minimum lethal doses of heated toxin, this immunity was not increased by the slight attack of lamsiekte following on the first test. This result is in complete agreement with the view expressed at the beginning of this section (page 1129), namely, that practically no immunity is present in an animal which has recovered from an attack of lamsiekte.

The above experiment is summarized in the following table:—

TABLE No. 23.

Cattle No.	Weight in kg.	Immunized with.			Result.	Immunity Test.		Result.	
		Amount of Toxin.	Heated.			Carried Out after Previous Injection.	With Toxin.	Incubation.	Died after.
			To.	For.					
218	231	1,000 × M.L.D.	75°	1 hour	No reaction	20 days	10 × M.L.D.	13 days	Recovered after 16 days.
						22 days	50 × M.L.D.	39 hours	62 hours.
249	156	1,000 × M.L.D.	70°	24 hours	No reaction	23 days	10 × M.L.D.	5½ days	12 days.
267	252	1,000 × M.L.D.	70°	12 hours	No reaction	25 days	10 × M.L.D.	4 days	7½ days.
4376	163	1,000 × M.L.D.	70°	12 hours	No reaction	12 days	10 × M.L.D.	7 days	11½ days.

(ii) In Goats.

Experiment No. 1.—Three goats (19, 20, and 21) were injected with 1,000 minimum lethal doses toxin heated to 70° C. for twelve, six, and twenty-four hours respectively. None of the animals showed any reaction. About three weeks later each of these goats was tested with 5 minimum lethal doses of unheated toxin. Goats 19 and 21 showed no reaction, whereas goat 20 showed distinct signs of lamsiekte five days after the injection, but had recovered four days later.

About three weeks after the first immunity test all three goats were again subjected to a test, but this time with 100 minimum lethal doses unheated toxin. It was thought at the time that the first test with virulent material would have increased the immunity of the animals to such an extent that they would resist 100 minimum lethal doses. However, two days after the injection all three goats showed the first signs of paralysis and were dead two days later (see Appendix, page 1189).

Conclusion.—The result of this experiment (which was carried out a few days before the experiment with cattle recorded above) seemed to be fairly hopeful at first. All three goats, which were immunized with 1,000 minimum lethal doses heated toxin withstood an injection of 5 minimum lethal doses virulent material, although one showed a distinct reaction.

All hopes which had been centred on this result were, however, destroyed when the second immunity test was undertaken. As stated, all three goats died after receiving 100 minimum lethal doses toxic material. We must conclude, therefore, that practically no active immunity is produced by the injection of unheated toxin.

Experiment No. 2.—It was deducted from the results of Experiment No. 1 that the immunity against lamsiekte could not be appreciably increased by the injection of non-fatal doses of toxin. Accordingly an attempt was made to increase the immunity by injecting larger quantities of heated toxin.

Ten goats (34, 35, 36, 37, 38, 39, 40, 41, 42, and 43) received as a first injection 1,000 minimum lethal doses toxin heated to 70° C. for twelve hours without showing any reaction. Goats 36 and 38 were

tested a fortnight later with 10 minimum lethal doses unheated toxin and both developed typical lamsiekte. Goat 42 was tested with 50 minimum lethal doses toxin and died two and a half days later.

The remaining seven goats received a second injection of 5,000 minimum lethal doses toxin heated in the same way as at the first injection. Two of the goats (35 and 40) developed symptoms of lamsiekte, but both recovered. The other five goats (34, 37, 39, 41, and 43) showed no reaction after this large dose of heated toxin. Goat 34 was tested with 5 minimum lethal doses toxin and showed very slight symptoms of lamsiekte, from which it recovered. Goat 43 received 10 minimum lethal doses toxin and died six and a half days later of lamsiekte. Goat 39 also received 10 minimum lethal doses, and goat 41 15 minimum lethal doses unheated toxin; both developed typical lamsiekte and had to be destroyed about three weeks later. Goat 37 was tested with 20 minimum lethal doses toxin and died three and a half days later (see Appendix, page 1190).

TABLE No. 24.

Experiment No.	Goat No.	Weight in kg.	Immunized with.			Result.	Immunity Test.		Result.	
			Amount of Toxin.	Heated.			Carried Out after Previous Injection.	With Toxin.	Incubation.	Died after.
				To.	For.					
1	19	43	1,000 × M.L.D.	70° C.	12 hours	No reaction	19 days	5 × M.L.D.	No reaction	—
							19 days	100 × M.L.D.	2 days	4 days.
	20	42	1,000 × M.L.D.	70° C.	6 hours	No reaction	19 days	5 × M.L.D.	5 days	Recovered.
							19 days	100 × M.L.D.	2 days	4 days.
	21	36·5	1,000 × M.L.D.	70° C.	24 hours	No reaction	19 days	5 × M.L.D.	No reaction	—
							19 days	100 × M.L.D.	2 days	4 days.
2	34	43	1,000 × M.L.D.	70° C.	12 hours	No reaction	—	—	—	—
			5,000 × M.L.D.	70° C.	12 hours	,,	17 days	5 × M.L.D.	4½ days	Recovered.
	35	43	1,000 × M.L.D.	70° C.	12 hours	,,	—	—	—	—
			5,000 × M.L.D.	70° C.	12 hours	Symptoms of lamsiekte, but recovered	—	—	—	—
	36	51	1,000 × M.L.D.	70° C.	12 hours	No reaction	13 days	10 × M.L.D.	5 days	16 days.
	37	43	1,000 × M.L.D.	70° C.	12 hours	,,	—	—	—	—
			5,000 × M.L.D.	70° C.	12 hours	,,	11 days	20 × M.L.D.	2½ days	3½ days.
	38	37	1,000 × M.L.D.	70° C.	12 hours	,,	13 days	10 × M.L.D.	5 days	12 days.
	39	51	1,000 × M.L.D.	70° C.	12 hours	,,	—	—	—	—
			5,000 × M.L.D.	70° C.	12 hours	,,	17 days	10 × M.L.D.	3 days	18 days (killed).
	40	43	1,000 × M.L.D.	70° C.	12 hours	,,	—	—	—	—
			5,000 × M.L.D.	70° C.	12 hours	Symptoms of lamsiekte, but recovered	—	—	—	—
	41	41	1,000 × M.L.D.	70° C.	12 hours	No reaction	—	—	—	—
			5,000 × M.L.D.	70° C.	12 hours	,,	17 days	15 × M.L.D.	4 days	18 days (killed).
	42	41	1,000 × M.L.D.	70° C.	12 hours	,,	13 days	50 × M.L.D.	2 days	2½ days.
	43	43	1,000 × M.L.D.	70° C.	12 hours	,,	—	—	—	—
			5,000 × M.L.D.	70° C.	12 hours	,,	11 days	10 × M.L.D.	3½ days	6½ days.
3	65	29	1,000 × M.L.D.	70° C.	12 hours	,,	—	—	—	—
			5,000 × M.L.D.	70° C.	12 hours	Symptoms of lamsiekte, but recovered	—	—	—	—
			10,000 × M.L.D.	70° C.	12 hours	No reaction	7 weeks	40 × M.L.D.	42 hours	2½ days.

Conclusions.—Even in so large a quantity as 5,000 minimum lethal doses toxin heated to 70° C. for twelve hours proved to be inadequate to protect goats against doses of virulent toxin varying from 10 to 20 minimum lethal doses.

Experiment No. 3.—Goat 65 was injected with 1,000 minimum lethal doses toxin heated to 70° C. for twelve hours and showed no reaction. Fifteen days later it received 5,000 minimum lethal doses toxin heated in the same way. Nearly three weeks later the goat was noticed to show a slight weakness of the muscles, but the condition soon improved. Thereupon the goat received a further injection of 10,000 minimum lethal doses toxin heated to 70° C. for twelve hours without any ill effect.

About seven weeks after this last injection the immunity of this goat was tested by injecting 40 minimum lethal doses unheated toxin. In less than forty-eight hours the goat was almost completely paralysed, and it died about two and a half days after the injection (see Appendix, page 1192).

Conclusions.—Although only one goat was used in this experiment it seems safe to conclude that the protection afforded by very large quantities of heated toxin is very limited. The method appears to have no practical value.

The results of the above experiments are summarized in Table No. 24.

(1A) *By Drenching with Heated Toxin.*

The unsatisfactory results obtained with the injection of heated toxin scarcely warranted a test by means of drenching. Nevertheless the method was given a trial on one animal. It was felt that, should it be possible to obtain any immunity by drenching with heated toxin, this method would be far preferable to the injection of large quantities of liquid.

Heifer 4550 was drenched with 2,000 minimum lethal doses toxin heated to 70° C. for three hours. No reaction was noticed; eighteen days later the immunity test was carried out by injecting 20 minimum lethal doses unheated toxin. The animal was paralysed three days later and died the same day (see Appendix, page 1192).

Conclusion.—In this one test apparently no immunity was conferred on a heifer by drenching her with 2,000 minimum lethal doses of heated toxin.

(2) *By means of Spores.*

In Section D it was shown that a temperature of 80° C. or more destroyed the toxin of lamsiekte. However, at this temperature the spores of the lamsiekte organisms are not killed. Cultures heated to 80° C. were therefore free from toxin, but contained the organisms in viable form. An attempt was made to produce immunity by injecting such material into cattle.

Heifer 3906 received 100 c.c. culture heated to 82° C. for ten minutes. No reaction followed. A fortnight later an immunity test was carried out with 10 minimum lethal doses unheated toxin. The animal showed the first signs of lamsiekte after five days and died about nine and a half days after the injection.

Tollie 4842 was injected with 5 c.c. culture heated to 85° C. for ten minutes. After eighteen days it received another 30 c.c. culture heated to 85° C. for 15 minutes. No ill effects were noticed.

Seventeen days after the second injection this animal was tested with 2 minimum lethal doses of unheated toxin without showing any reaction. A week later a second immunity test was carried out with 10 minimum lethal doses unheated toxin. After five days symptoms of lamsiekte were noticed. The animal was treated with lithium carbonate, but died eleven and a half days after the second test (see Appendix, page 1192).

Conclusions.—The results of tests carried out with tollie 4842 show that some immunity is actually produced by injecting spores. This animal withstood an injection of 2 minimum lethal doses virulent material, but succumbed when 10 minimum lethal doses were injected. Heifer 3906 also died when tested with 10 minimum lethal doses. The degree of immunity obtained in this way appears therefore to be very limited.

Table No. 25 summarizes the results.

TABLE No. 25.

Cattle No.	Weight in kg.	Immunized with.			Result.	Immunity Test.		Result.	
		Amount of Culture.	Heated.			Carried Out after Previous Injection.	With Toxin.	Incubation.	Died after.
			To.	For.					
3906	229	100 c.c.	82° C.	10 minutes	No reaction	14 days	10 × M.L.D.	5 days	9½ days.
4842	183	5 c.c. 30 c.c.	85° C. 85° C.	10 minutes 10 minutes	No reaction ,,	— 17 days 7 days	— 2 × M.L.D. 10 × M.L.D.	— No reaction 5 days	— 11½ days.

(3) *By mixing Toxin with Iodine.*

This method was considered as an alternative method to that described in Section E 1, namely, the immunization by means of heated toxin. Both methods were regarded as being comparatively safe. In Section D (*f*) 1 it was shown that the addition of three or four times the amount of Lugol solution to even large quantities of filtered toxin rendered the latter innocuous. Attempts were therefore made to immunize cattle and goats by the injection of large doses of toxin so treated.

(*i*) *Tests on Goats.*

Experiment No. 1.—Two goats (17 and 18) were each injected with 1,000 minimum lethal doses filtered toxin mixed with three and five times its volume of Lugol solution respectively and showed no reaction. A fortnight later the immunity of these two goats was tested by the injection of 3 minimum lethal doses virulent toxin. Both animals resisted the injection and showed no reaction at all.

This encouraging test was followed up a week later by the injection of 10 minimum lethal doses toxin into each goat. Again both animals proved to be immune.

This result was considered very satisfactory and it was confidently expected that the goats would now be able to withstand an injection of 100 minimum lethal doses toxin. This test was carried out nineteen days after the previous test. Two days later the one goat showed signs of lamsiekte and on the third day they were both paralysed. The one died about four days and the other about five days after the injection (see Appendix, page 1193).

Conclusions.—Just as hopeful as the first two tests seemed to be, so discouraging was the result of the final test. Some measure of immunity appeared to have been conveyed by the injection of the attenuated toxin, which enabled the goats to survive first the injection of 3 minimum lethal doses and then 10 minimum lethal doses virulent toxin. However, when the goats were subjected to an immunity test with 100 minimum lethal doses toxin, both died.

Experiment No. 2.—Goats 22, 23, and 25 received 1,000 minimum lethal doses toxin mixed with two or two and a half times the amount of Lugol solution. The first two showed no reaction, whereas goat 25 showed signs of paralysis about four days after the injection, but recovered in about three weeks.

Goats 22 and 23 were tested with 10 minimum lethal doses toxin thirteen days after the injection of the attenuated material and showed no reaction. They were then injected with 100 minimum lethal doses as in Experiment No. 1, and died within six days after the injection.

Goat 25 was also tested with 10 minimum lethal doses three and a quarter days later; it showed signs of paralysis and died about ten days after the injection (see Appendix, page 1194).

Conclusions.—The results were approximately the same as in Experiment No. 1. Two of the three goats were able to withstand an injection of 10 minimum lethal doses, but succumbed when 100 minimum lethal doses were given. The third goat did not even survive the injection of 10 minimum lethal doses.

Experiment No. 3.—The results of Experiments Nos. 1 and 2 showed clearly that 1,000 minimum lethal doses attenuated toxin is not sufficient to confer an appreciable degree of immunity on goats. It was therefore attempted to enhance the immunity by increasing the amount of attenuated toxin gradually.

Goat 24 first received an injection of 1,000 minimum lethal doses toxin mixed with two and a half times the amount of Lugol solution. Slight symptoms of lamsiekte appeared after about four and a half days, but the animal recovered within two days. It thereupon received 5,000 minimum lethal doses toxin mixed with three times the amount of Lugol solution, and then a further 10,000 minimum lethal doses toxin treated in the same way. Neither of these two injections produced any ill effects.

About six weeks after the last injection the immunity of this goat was tested with 30 minimum lethal doses toxin. Three days later it was paralysed and died on the fourth day (see Appendix, page 1194).

Conclusions.—Although goat 24 received no less than 16,000 minimum lethal doses of attenuated toxin, its immunity was not strong enough to resist 30 minimum lethal doses virulent material.

Experiment No. 4.—This experiment was really a repetition of Experiment No. 2, with only this difference, that the toxin was mixed with four times the amount of Lugol solution so as to render the injection quite safe.

Four goats (44, 48, 49, and 51) each received 1,000 minimum lethal doses filtered toxin mixed with four times the amount of Lugol solution. None of them showed any reaction.

Their immunity was then tested with virulent material, goats 44, 48, and 49 receiving 10 minimum lethal doses, goat 51, 50 minimum

lethal doses toxin. All four animals reacted to this injection. Goat 44 showed the first symptoms about five days and died about nine days after the injection. Goat 48 began to react five days after the injection and remained half paralysed for over a month, but eventually recovered. Goat 49 also showed symptoms after five days, and then gradually began to improve, but was killed about eighteen days after the injection. Goat 51, which received 50 minimum lethal doses toxin, reacted after two days and was dead within three days (see Appendix, page 1195).

Conclusions.—The immunity present in these four goats was very slight; whereas the majority of goats used in Experiments Nos. 1 and 2 resisted the injection of 10 minimum lethal doses toxin, the three goats tested with this amount in Experiment No. 4 contracted lamsiekte. It would appear as if the addition of the larger amount of Lugol solution reduced the immunizing properties of the toxin.

Experiment No. 5.—In this experiment another attempt was made to improve the immunity by the injection of larger amounts of toxin.

Goats 45, 46, and 53 received an initial injection of 1,000 minimum lethal doses mixed with four times the amount of Lugol

TABLE No. 26.

Experiment No.	Goat No.	Weight in kg.	Immunized with.		Result.	Immunity Test.		Result.	
			Amount of Toxin.	Mixed with Lugol Solution in Proportion.		Carried Out after Previous Injection.	With Toxin.	Incubation.	Died after.
1	17	36·3	1,000 × M.L.D.	1 : 3	No reaction	14 days	3 × M.L.D.	No re	action.
						7 days	10 × M.L.D.		
						19 days	100 × M.L.D.	3 days	" 5 days.
	18	31·7	1,000 × M.L.D.	1 : 5	"	14 days	3 × M.L.D.	No re	action.
						7 days	10 × M.L.D.		
						19 days	100 × M.L.D.	2 days	" 4 days.
2	22	22	1,000 × M.L.D.	1 : 2	No reaction	13 days	10 × M.L.D.	No re	action.
						19 days	100 × M.L.D.	2 days	6 days.
	23	36	1,000 × M.L.D.	1 : 2½	"	13 days	10 × M.L.D.	No re	action.
						19 days	100 × M.L.D.	4 days	6 days.
	25	43	1,000 × M.L.D.	1 : 2½	Slight reaction, but recovered	6 weeks	10 × M.L.D.	3 days	10 days.
3	24	34	1,000 × M.L.D.	1 : 2½	Slight reaction	—	—	—	—
			5,000 × M.L.D.	1 : 3	No reaction	—	—	—	—
			10,000 × M.L.D.	1 : 3	"	17 days	30 × M.L.D.	3 days	4 days.
4	44	10	1,000 × M.L.D.	1 : 4	No reaction	5 weeks	10 × M.L.D.	5 days	9 days.
	48	39	1,000 × M.L.D.	1 : 4	"	11 days	10 × M.L.D.	5 days	Recovered after 7 weeks.
	49	57	1,000 × M.L.D.	1 : 4	"	11 days	10× M.L.D	5 days	Killed.
	51	45	1,000 × M.L.D.	1 : 4	"	11 days	50 × M.L.D.	2 days	3 days.
5	45	34	1,000 × M.L.D.	1 : 4	No reaction	—	—	—	—
			5,000 × M.L.D.	1 : 3	"	12 days	25 × M.L.D.	2 days	3 days.
	53	31	1,000 × M.L.D.	1 : 4	"	—	—	—	—
			5,000 × M.L.D.	1 : 3	"	12 days	15 × M.L.D.	3 days	4 days.
	46	59	1,000 × M.L.D.	1 : 4	"	—	—	—	—
			5,000 × M.L.D.	1 : 3	"	—	—	—	—
			10,000 × M.L.D.	Heated toxin	"	6 weeks	20 × M.L.D.	4 days	6 days.

solution without ill effects. After eight days goats 45 and 53 received 5,000 minimum lethal doses mixed with three times the amount of Lugol solution and showed no reaction. Twelve days later their immunity was tested, goat 45 receiving 25 minimum lethal doses and goat 53 15 minimum lethal doses toxin. Goat 45 died of lamsiekte about two and a half days and goat 53 about three and a half days after the injection.

Goat 46 received, in addition to the initial 1,000 minimum lethal doses attenuated toxin, 5,000 minimum lethal doses toxin mixed with three times the amount of Lugol solution, and eighteen days later another 10,000 minimum lethal doses heated to 70° C. for twelve hours. No reaction was noticed. About six weeks after this latter injection the immunity of this goat was tested with 20 minimum lethal doses virulent material. Four days later the first symptoms of lamsiekte appeared, and the goat died about six days after the test (see Appendix, page 1196).

Conclusions.—These results were just as bad as those obtained in the previous experiments (especially Experiment No. 3). Even the very large quantities of attenuated toxin could not protect these goats against 15, 20, or 25 minimum lethal doses of toxin.

The results of these experiments are summarized in Table No. 26.

(ii) *Tests on Cattle.*

Four cattle were used in this experiment.

Tollie 4847 received 1,000 minimum lethal doses filtered toxin mixed with two and a half times the amount of Lugol solution subcutaneously. No reaction followed. A fortnight later its immunity was tested by an injection of 10 minimum lethal doses virulent toxin. Six days after this injection the animal showed the first symptoms of lamsiekte and died twenty-four hours later.

Heifer 4737 was injected subcutaneously with 1,000 minimum lethal doses toxin mixed with three times the amount of Lugol solution without any ill effects. In the hope of increasing its immunity, the animal was given a second injection of 1,000 minimum lethal doses toxin mixed with twice the amount of Lugol solution thirteen days after the first. Seven days later the immunity test was carried out with 10 minimum lethal doses toxin. The first symptoms of lamsiekte appeared six days later and the heifer died seven and a half days after the test.

Cow 3320 was injected intravenously with 1,000 minimum lethal doses toxin mixed with four times the amount of Lugol solution and showed no reaction. Thirteen days later it received 10 minimum lethal doses toxin to test its immunity. Symptoms of lamsiekte appeared two and a quarter days after the injection and the animal died within twenty-four hours of the onset of symptoms.

Heifer 4663 received 5,000 minimum lethal doses toxin mixed with four times the amount of Lugol solution subcutaneously without ill effects. It was hoped that the large quantity of toxin would give the animal sufficient immunity to resist the injection of 50 minimum lethal doses toxin material. This quantity was accordingly injected thirteen days after the first injection. However, the animal died in less than forty-four hours (see Appendix, page 1196).

Conclusion.—The results obtained in this experiment were very disappointing. 1,000 minimum lethal doses of toxin attenuated by the addition of Lugol solution gave practically no immunity to three

animals. When tested with 10 minimum lethal doses virulent material all three animals died. Heifer 4737, which received two injections of 1,000 minimum lethal doses attenuated toxin, seemed to be no better protected than the others. Even an injection of 5,000 minimum lethal doses attenuated toxin which was given to heifer 4663 proved to be inadequate to afford any protection against 50 minimum lethal doses toxin.

On the whole the results obtained by this method were worse than those obtained with heated toxin and recorded in Section E (*a*) (1) (*i*) on page 1129.

The above experiment is summarized in Table No. 27.

TABLE No. 27.

Cattle No.	Weight in kg.	Intermixed with.		Result.	Immunity Test.		Result.	
		Amount of Toxin.	Mixed with Lugol Solution in Proportion.		Carried Out after Previous Injection.	With Toxin.	Incubation.	Died after.
4847	215	1,000 × M.L.D.	1 : 2½	No reaction	14 days	10 × M.L.D.	6 days	7 days.
4737	250	1,000 × M.L.D. 1,000 × M.L.D.	1 : 3 1 : 2	No reaction ,,	— 7 days	— 10 × M.L.D.	— 6 days	— 7½ days.
3320	372	1,000 × M.L.D.	1 : 4	No reaction	13 days	10 × M.L.D.	2¼ days	3 days.
4663	273	5,000 × M.L.D.	1 : 4	No reaction	13 days	50 × M.L.D.	—	44 hours.

(4) *By means of Inoculation into the Tail.*

The method of inoculation into the tail is well known in South Africa, where it was practised extensively in the campaign against lung-sickness (pleuro-pneumonia in cattle). The idea underlying this method is that by injecting virulent material into a poorly vascularized tissue such as is found at the tip of the tail, growth of the organism or absorption of the toxin takes place so slowly that the animal has time to produce antibodies and so acquire the necessary immunity.

The method which was practised in these experiments consisted in clipping and shaving the hair and disinfecting the skin near the tip of the tail, and then injecting the material about 1 inch from the tip into the hard fibrous tissue on the dorsal side of the tail. Sometimes the tissue was so hard here that the ventral surface had to be selected.

Experiment No. 1.—Heifer 4839 received 1 minimum lethal dose toxin into the tip of the tail without showing any reaction. A fortnight later it again received 5 minimum lethal doses toxin into the tip of the tail without any ill effects.

Eleven days after this second injection an immunity test was carried out with 2 minimum lethal doses toxin given subcutaneously. No reaction followed.

A second immunity test was thereupon conducted, the heifer receiving 10 minimum lethal doses toxin subcutaneously. Again the animal withstood the test.

This result was considered very satisfactory and it was confidently expected that the animal would prove to be immune against a fairly large dose of toxin. In order to improve the immunity still further

25 minimum lethal doses toxin were injected into the tail, the ventral surface being chosen on this occasion. Two days later the animal showed the first signs of lamsiekte, and died twenty-seven hours later.

Conclusions.—The initial results obtained in this experiment were very encouraging. A beast which had received only 6 minimum lethal doses toxin in the tail withstood 2 minimum lethal doses and later 10 minimum lethal doses virulent material. However, all hopes of success which were entertained after this result were considerably reduced when the animal died on receipt of a further quantity of 25 minimum lethal doses into the tail.

The fact that heifer 4839 withstood the first test seemed to indicate that some immunity is actually produced by this method. It was therefore decided to continue the experiments.

Experiment No. 2.— Cow 230 was injected into the tail with 10 minimum lethal doses toxin with no ill effects. Three weeks later it was tested with 5 minimum lethal doses toxin injected subcutaneously and showed no reaction. After another ten days it received 25 minimum lethal doses toxin into the tissue of the ventral side of the tail. Less than two days after this injection the cow was paralysed and it died the same day.

Conclusions.—The results obtained in this experiment were very similar to those of Experiment No. 1. 10 minimum lethal doses toxin injected into the tail seemed to protect against 5 minimum lethal doses given subcutaneously. However, when the animal received a further twenty-five doses it died of lamsiekte.

Experiment No. 3.—Tollie 4354 was injected with 20 minimum lethal doses toxin into the tip of the tail. About three and a half days later it showed the first signs of lamsiekte. The disease progressed steadily and the animal was killed eight days after the injection through an intravenous injection of iron sulphate solution.

Conclusion.—The injection into the tissues at the tip of the tail is not always a safe procedure, which fact in itself would prevent the method from being generally applied.

Experiment No. 4.—Cow 1936 and cow 2658 received 5 minimum lethal doses and 10 minimum lethal doses respectively into the tip of the tail without showing any ill effects. Eleven days later both were tested with 25 minimum lethal doses toxin injected into the ventral side of the tail. Cow 1936 showed symptoms of lamsiekte three days and cow 2658 two days after the injection; both died within twenty-four hours after the onset of the symptoms.

It should be explained here that the injection into the hard fibrous tissue on the dorsal side of the tail is sometimes very difficult, so that, whenever fairly large quantities of liquid had to be injected, the ventral surface was chosen. Here the tissue is looser and more vascularized. The injection, therefore, is easier, but at the same time it appeared that the effect of injecting toxin here was practically the same as in the case of an ordinary subcutaneous injection. The injections mentioned in the above experiment may therefore be regarded as a simple immunity test.

Conclusion.—In this experiment an injection into the tail of 5 or 10 minimum lethal doses toxin could not protect the animals against 25 minimum lethal doses toxin.

Experiment No. 5.—Both animals used in this experiment had previously been used in other tests (see Appendix, page 1198). In the case of heifer 4686 the previous test consisted in an injection

of heated toxin into the tail, and in the case of heifer 4740 in a drenching with bleached bones. In neither case was any reaction noticed.

Both animals then received virulent toxin into the tip of the tail. Heifer 4686 received 5 minimum lethal doses and showed a slight reaction, but recovered. Heifer 4740 received 10 minimum lethal doses and showed symptoms of lamsiekte four days later, the disease proving fatal eight days after the injection.

About a fortnight after the first injection heifer 4648 received a second injection of 10 minimum lethal doses toxin into the tip of the tail. Twenty-two hours later it was partly paralysed. Its condition grew steadily worse, and three weeks after the injection it was killed.

Conclusion.—In this experiment two cases of lamsiekte were produced by the injection of 10 minimum lethal doses toxin into the tail. This method proved to be both unsafe and ineffective, and the experiments were stopped.

General Discussion.—The results of the foregoing experiments seem to justify the conclusions (1) that most cattle will withstand a greater quantity of toxin when injected into the tissues near the tip of the tail than when given subcutaneously, (2) that a certain amount of immunity is produced in this way, but not sufficient to be of practical value, (3) that the method is neither *safe* (several animals having contracted lamsiekte after the injection of 10 or more minimum lethal doses into the tail) nor *effective* (the immunity of all animals having broken down after the injection of toxic material).

Table No. 28 gives a summary of the results.

TABLE No. 28.

Experiment No.	Cattle No.	Weight in kg.	Immunized with.		Result.	Immunity Test.		Result.	
			Amount of Toxin.	Injected into.		Carried Out after Previous Injection.	With Toxin.	Incubation.	Died after.
1	4839	139	1 × M.L.D. 5 × M.L.D.	Tail „	No reaction „	— 11 days 7 days 26 days	— 2 × M.L.D. 10 × M.L.D. 25 × M.L.D.	— No reaction. „ 2 days	— „ 3 days.
2	230	295	10 × M.L.D.	Tail	No reaction	22 days 10 days	5 × M.L.D. 25 × M.L.D.	No reaction. About 1½ days	2¼ days.
3	4354	218	20 × M.L.D.	Tail	Contracted lamsiekte and was killed	—	—	—	—
4	1936 2658	367 401	5 × M.L.D. 10 × M.L.D.	Tail „	No reaction „	11 days 11 days	25 × M.L.D. 25 × M.L.D.	3 days 2 days	4 days. 3 days.
5	4686	241	5 × M.L.D. 10 × M.L.D.	Tail „	Slight reaction Contracted lamsiekte and was killed	— —	— —	— —	— —
	4740	197	10 × M.L.D.	„	Contracted lamsiekte and died	—	—	—	—