(2) *The Time it takes for Immunity to Develop.*—Field experience has taught us that it requires about ten days after vaccination before immunity becomes well established, and this observation has been supported by experiments at the Laboratory. One of these may be given in detail as follows:—

Experiment No. 8.

Sheep No.	Date Vaccinated Subcutaneously with.	Date, Immunity Test.	M.L.D. Given.	Result.
9599	14.11.24 ½ c.c. B. 44, 1 : 20	14.11.24	50	+ 20.11.24
9327	,, ,,	,,	100	+ 19.11.24
9479	,, ,,	,,	500	
9322	14.11.24 ½ c.c. B. 44, 1 : 20	15.11.24	50	+ 18.11.24
9158	,, ,,	,,	100	
9623	,, ,,	,,	500	+ 21.11.24
9502	14.11.24 ½ c.c. B. 44, 1 : 20	17.11.24	50	+ 20.11.24
8677	,, ,,	,,	100	+ 23.11.24
9477	,, ,,	,,	500	+ 20.11.24
9307	14.11.24 ½ c.c. B. 44, 1 : 20	20.11.24	50	
9266	,, ,,	,,	100	+ 24.11.24
9490	,, ,,	,,	500	+ 25.11.24
9577	14.11.24 ½ c.c. B. 44, 1 : 20	24.11.24	50	
8725	,, ,,	,,	100	
9142	,, ,,	,,	500	

+ Equals died of anthrax.

The results show that a well marked immunity is not present until the tenth day after vaccination, all three sheep surviving an injection of virulent material on that day. It is true that one sheep survived the test carried out on the same day as vaccine was injected, and another behaved similarly when tested 24 hours later. These are, however, undoubtedly cases where individual insusceptibility has to be accepted as an explanation; the vaccine could not be held responsible for conferring immunity, for, if this were so, one would have every reason to expect a higher degree of immunity to be present on the third day, and this was certainly not the case. We have previously referred to the fact that the sheep did not show a great variation in its susceptibility to anthrax, but there are of course exceptions; some of them may possibly have acquired some degree of immunity as a result of previous inoculation. Although sheep that are used in our anthrax experiments are as far as possible obtained from certain districts in the Karroo, where no anthrax is present, it is possible that now and again an animal that had previously been inoculated may slip into an experimental lot.

Sheep No. 9490 received by mistake an additional dose of virulent material on the 24th November, and died the following day. It may have died even if no second dose had been given, but it lingered sufficiently long to make one suspect that it had received some degree of protection derived from vaccination. Another sheep in the same

test survived, so that possibly a small degree of immunity may have been present six days after vaccination.

That this conclusion is probably correct is shown by the results of the following experiment:—

Experiment No. 9.—June, 1924.

Animals.	Date of Vaccination.	Method of Vaccination.	Date of Immunity Test.	Results.
Sheep 7005	17.6.24	Subcutaneous	25.6.24	Negative.
„ 7094	„	„	„	„
„ 7083	„	„	4.7.24	„
„ 6979	„	„	„	+ Anthrax.
Goat 8252	„	„	25.6.24	Negative.
„ 8418	„	„	4.7.24	„
Sheep 7740	„	Intradermal	25.6.24	+ Anthrax.
„ 7017	„	„	„	Negative.
„ 7335	„	„	4.7.24	„
„ 6262	„	„	„	+ Anthrax.
Goat 8323	„	„	25.6.24	„
„ 8399	„	„	4.7.24	Negative.
Sheep 6974	„	Scarification	25.6.24	+ Anthrax.
„ 6971	„	„	„	Negative.
„ 7078	„	„	4.7.24	„
„ 7027	„	„	„	+ Anthrax.
Goat 8277	„	„	25.6.24	Negative.
„ 8251	„	„	4.7.24	„

+ Equals died of anthrax.

It will be seen that half the number of animals received an injection of virulent material on the 8th, and the other half on the 17th day, after vaccination. Six out of the eighteen animals succumbed to anthrax, three of the lot tested on the 8th and the same number of those done on the 17th day. It would appear, therefore, that, relatively speaking, quite a good immunity was present on the 8th day after inoculation.

The immunity test in connection with our routine vaccine batches is usually carried out three weeks after the animals received the vaccine. This period has been selected to make quite certain that the immunity had developed to its fullest extent by the time the virulent material was injected. This again conforms to experience gained in the field, several cases having come to our notice where mortality in an outbreak of anthrax did not stop completely until about three weeks after vaccination was applied.

(3) *Duration of Immunity.*—Owing to the great expense involved, it is not an easy matter to have this point settled by experimental evidence. In order to obtain conclusive experimental data it would be necessary to keep and feed a large number of animals for a year or longer, and it is undoubtedly difficult to justify the expenditure that would necessarily be incurred. Such animals have to be kept in a stable or enclosure where anthrax infection is known definitely not to exist, since otherwise the results might be interfered with considerably through the animals developing a further degree of immunity as a result of natural infection.

In this case field experience is of some value, especially when one has to deal with as many as over two million vaccinated animals per annum, some of these running on badly infected farms where non-vaccinated animals are not likely to survive for any length of time. Extensive experience has shown the immunity to last for at least ten to twelve months, breakdowns in immunity during this period being extremely rare. This point will be dealt with more fully at a later stage. As further evidence, may be stated the fact that reinoculation of animals vaccinated twelve months previously is hardly ever followed by well marked reactions, even when the second inoculation is made with a particularly strong vaccine. In the course of our experimental work we have made some observations on the duration of immunity in anthrax and these may be stated briefly as follows:—

Experiment No. 10.

Animal.	Date of Inoculation.	Date of Test.	Period after which Immunity Tested.	Results.
Sheep 2317...	25.10.20	19. 4.21	6 months	Died after two days.
,, 2267...	,,	9. 5.21	6½ ,,	Alive.
,, 2256...	,,	,,	6½ ,,	Died after two days.
,, 2217...	,,	,,	6½ ,,	,, ,,
,, 2215...	,,	9. 8.21	9½ ,,	Alive.
,, 2147...	,,	9.10.21	11½ ,,	Died after six days.
,, 2115...	,,	25.12.21	14 ,,	Alive.
,, 2145...	,,	25. 1.22	15 ,,	,,
,, 2183...	,,	25. 8.21	10 ,,	Died after eight days.
Cattle 4465...	15.11.20	1. 2.22	14½ ,,	Alive.
Horses 15046..	28. 2.22	19. 1.23	11 ,,	Died; poverty and weakness.
,, 14279..	10. 5.21	,,	20½ ,,	Alive.
,, 13460..	30. 8.21	,,	16½ ,,	,,
,, 14275..	10. 5.21	27. 1.23	20½ ,,	,,
,, 14109..	30. 8.21	3. 1.23	17 ,,	,,
,, 14195..	30.12.21	19. 1.23	2 years	Died.
,, 14940..	26. 1.22	3. 2.23	12 months	,,
,, 14936*.	26. 1.22	27. 1.23	12 ,,	,,
,, 14478..	30. 8.21	15. 2.23	17½ ,,	Alive.
,, 15002..	,,	,,	17½ ,,	,,

(b) *Immunity following other Methods of Administering the Vaccine.*—Although subcutaneous inoculation has been, and still is, the most commonly practised method of administering anthrax vaccine, other routes of introducing the vaccine into animals merit discussion, particularly in view of the statements made recently by Besredka and others concerning cuti-vaccination and local immunity. The recent literature on this subject may be reviewed briefly as follows:—

Besredka (1921) described several experiments carried out on guinea-pigs and rabbits in connection with immunity. He pointed out that these animals do not lend themselves to the production of immunity against anthrax as do the large domesticated animals, i.e.

* In low condition and suffering from strangles.

by a method of vaccination such as Pasteur's. This author, however, succeeded in protecting guinea-pigs and rabbits by the local application of vaccine material to the skin, or as he states:—" Surtout après une irritation de la peau," such as would be produced by shaving the skin or better still by scarification. In the case of guinea-pigs, Besredka found it necessary to apply both first and second vaccines, but he showed that the interval between the application of these vaccines could be considerably reduced, a most important point in combatting anthrax in the field. It was also demonstrated that intradermal administration answered as satisfactorily as superficial application. Further, another important point of great practical importance was the fact that doses of virulent material which were fatal when given intradermally did not cause death when administered intratracheally, intraperitoneally or intravenously, provided that the skin was not injured during the operation. Noetzel, however (Gay, 1924), " had noted as early as 1898 that rabbits will withstand 200 times the fatal subcutaneous dose of anthrax bacilli when the injection is practiced intraperitoneally without skin injury."

Arising from Besredka's work it may be stated that his evidence showed that the skin is the susceptible organ in anthrax, but that if locally immunized, as by scarification, a general immunity against infection by any path could be produced. It was also found that the serum of cuti-vaccinated guinea-pigs did not protect against anthrax infection.

Dozens of contributions have appeared supporting Besredka's observations and the more important are referred to briefly in the following paragraph. Besredka's (1922) later paper on " Immunite générale par immunisation locale " should, however, also be perused.

Brocq-Rousseu and Urbain [1923 (*a*) (*b*) (*c*) (*d*)] (1924) have contributed several articles confirming Besredka's work, and in their experiments on horses have come to the following conclusions:—

(i) the possibility of vaccinating horses by the cutaneous route;
(ii) that the resulting immunity is unaccompanied by the production of antibodies of which there is an insignificant amount in the serum;
(iii) that the serum of such an animal does not protect guinea-pigs against anthrax;
(iv) the possibility of immunising more rapidly than is the case with the usual Pasteur method;
(v) the cuti-immunity in the horse is a strong immunity in addition to its being more rapidly brought about.

Vallée (1923) carried out experiments on heifers in connection with the same subject and came to the same conclusion as did Besredka, namely that the skin is exceedingly susceptible to anthrax. Velu, who carried out his experiments in Morocco has gone a step further and submits evidence which shows—

(i) that in sheep it is possible to produce a strong immunity by a single intradermal injection (1924*a*);
(ii) that this immunity is produced so rapidly that it is possible to vaccinate animals with success *during the incubation period* (1924*b*).

Besredka's observations have found support from workers such as Balteano (1922), Mazucchi (1923), and Plotz (1924). Mazucchi, who specialises in the serotherapeutic aspect of the problem, agrees that cuti-immunisation is possible and that it is unaccompanied by a

marked thermal or local reaction, and adds that the serum of a protected animal is of no value for conferring passive immunity on a susceptible subject. He further states that in order to secure anti-anthrax serum of any value, immunisation must be repeated, and the material must be of such a nature (e.g. massive doses) that marked thermal reactions follow. It is obvious that such conditions are impossible with cuti-vaccination.

Combiesco (1923), (1924), while in accord with Besredka in as far as the question of cuti-infection is concerned, believes that for intravenous, intraperitoneal, and subcutaneous infection to succeed, it is necessary for the dose of virus to exceed a certain amount. Further, he believes that other organs besides the skin are very susceptible to anthrax. With regard to the mechanism of infection he considers that the following points merit special attention:—

(i) The rapidity with which the *B. anthracis* adapts itself to an albuminuous medium, the result of which is the formation of a capsule in a few minutes.
(ii) The absence of phagocytic action on capsulated organisms.
(iii) The fact that to produce anthrax infection the contamination of the skin is not essential.

With Popesco (1924) the same author, in experiments on guinea-pigs concluded that in cuti-vaccination the question of local immunity is not the only factor, but that other phenomena such as opsonins and their action on phagocytes should also be taken into consideration. They suggest that in cuti-infection bacteria become adapted better to the defensive mechanism of the body and thus resist phagocytosis more effectively. In another paper, Combiesco and Dumitresco (1924) describe observations in the rabbit which support this view. In a few words then, Combiesco (with co-workers) concludes that the skin is not specially sensitive as believed by Besredka and his school; but that by cuti-infection phagocytosis is inhibited and multiplication of anthrax organisms is facilitated.

Gratia (1924) too, follows Combiesco in that he does not agree that the skin is the only sensitive organ to anthrax. Shigeru Tada (1924) carried out a number of experiments in guinea-pigs which he vaccinated with the same material, and at the same times, in three different ways, namely: (*a*) subcutaneously, (*b*) shaving of the skin, and (*c*) intramuscularly; in the last mentioned method every precaution was taken to exclude infection of the skin. His results went to show that there were no appreciable differences in the degree of immunity conferred by the different methods of vaccination and that the skin was not concerned specially or exclusively in the development of immunity against anthrax.

From these few references to the literature it becomes clear that there is a lack of unanimity among the different workers in regard to the role played by the skin in anthrax infection or immunization. To settle the different points that have been raised, some experiments were carried out at this Laboratory. These may be discussed under the following headings:—

(1) *The Sensitiveness of the various Paths of Infection.*—In so far as infection with virulent anthrax spores is concerned, experiments were carried out in connection with the following paths: subcutaneous, intradermal, scarified areas of the skin, intravenous, submucous and intramucous. In the case of the last three mentioned routes, every care was taken to prevent contamination of the skin.

The results were shown in experiments Nos. 2, 3, and 4 (see pages 464 and 465).

From these it becomes quite clear that the skin is not the only sensitive organ and that a susceptible animal can be infected successfully by almost any path, always provided of course that a sufficiently large dose of virulent material is employed. Of all the routes tried, infection through the scarified skin was perhaps the least certain, while injection of the virus into the deeper layers of the skin (intradermal) was followed regularly by positive results.

(2) *The Comparative Value of Vaccination when applied in the following ways: Subcutaneously, Intradermally, and to Scarified Areas of the Skin.*—In connection with this, quite a number of experiments were carried out by us. Before supplying details of these, the following points in connection with the technique employed should be made clear:—

Scarification was done on the inside of the thigh; the skin was first thoroughly cleaned and shaved, then with the ordinary scarifier used in small-pox vaccination, scarifications were made on the shaved area in exactly the same manner as it is done in small-pox vaccination in human beings; needless to say, either lymph or blood was brought to the surface during the operation.

When carrying out vaccination by the different routes, every endeavour was made to introduce about the same number of attenuated spores into the animal; this was effected by using a more concentrated spore emulsion in the case of the intradermal and scarified routes, with, of course, a correspondingly smaller dose of vaccine.

The following are the particulars of the experiments in question:—

Experiment No. 11.—*June*, 1924.

To ascertain the value of subcutaneous, intradermal and scarification methods of vaccination against anthrax.

This experiment has been given in detail on page 497, to show that some degree of immunity can become established as early as eight days after vaccination. It is now being considered from a different point of view, and for the sake of completeness is again shown in detail.

Experiment No. 11.

Animals.	Date of Vaccination.	Method of Vaccination.	Immunity Test.	Result.
			M.L.D.	
Sheep 7005	17.6.24	Subcutaneous	1,000	Negative.
,, 7094	,,	,,	500	,,
,, 7083	,,	,,	1,000	,,
,, 6979	,,	,,	500	+Anthrax.
Goat 8252	,,	,,	250	Negative.
,, 8418	,,	,,	250	,,
Sheep 7440	,,	Intradermal	1,000	+Anthrax.
,, 7017	,,	,,	500	Negative.
,, 7335	,,	,,	1,000	,,
,, 6262	,,	,,	500	+Anthrax.
Goat 8323	,,	,,	250	,,
,, 8399	,,	,,	250	Negative.
Sheep 6974	,,	Scarification	1,000	+Anthrax.
,, 6971	,,	,,	500	Negative.
,, 7078	,,	,,	1,000	,,
,, 7027	,,	,,	500	+Anthrax.
Goat 8277	,,	,,	250	Negative.
,, 8251	,,	,,	250	,,

+ Equals died of anthrax.

The results show that immunity against anthrax can be established by introducing the vaccine through any of the three routes, but that in this case the subcutaneous method proved to be the most reliable.

Experiment No. 12.—*July*, 1924.

To bring out minor differences a weaker vaccine was employed in this case.

Experiment No. 12.

Animals.	Date of Vaccination.	Method of Vaccination.	Immunity Test.	Results.
			M.L.D.	
Sheep 8595*	10.7.24	Subcutaneous	500	Negative.
,, 6523*	,,	,,	500	,,
,, 8591	,,	,,	500	+ Anthrax.
,, 8590	,,	,,	500	,,
,, 7025*	,,	Intradermal	500	Negative.
,, 8592*	,,	,,	500	+ Anthrax.
,, 8596	,,	,,	500	,,
,, 8611	,,	,,	500	,,
,, 8593*	,,	Scarification	500	Negative.
,, 6758*	,,	,,	500	+ Anthrax.
,, 8597	,,	,,	500	,,
,, 7797	,,	,,	500	,,

+ Equals died of anthrax.
* Tested 17.7.24, remainder 31.7.24.

In half the number of animals the immunity test was applied on the 7th, and in the other half, on the 21st day after vaccination. Of the first lot, only two died, showing a well marked immunity to be present seven days after vaccination. In this connection it should be noted that the most favourable results were again obtained from the subcutaneous method of vaccination.

It is remarkable that all the sheep tested on the 21st day after vaccination died in the virulence test; it is of course inconceivable that the immunity could have diminished in that time, but, since comparative results are under discussion, it seems unnecessary to offer an explanation for this occurrence at this stage.

Experiment No. 13.—*July*, 1924.

In this case again a fairly weak vaccine was employed.

Experiment No. 13.

Animals.	Date of Vaccination.	Method of Vaccination.	Immunity Test.	Results.
			M.L.D.	
Sheep 7484.....	29.7.24	Subcutaneous	500	+ Anthrax.
,, 7004.....	,,	,,	500	,,
,, 6573.....	,,	,,	200	Negative.
,, 6468.....	,,	,,	200	,,
,, 7301.....	,,	Intradermal	500	,,
,, 7040.....	,,	,,	500	+ Anthrax.
,, 8601.....	,,	,,	200	,,
,, 8328.....	,,	,,	200	Negative.
,, 8608.....	,,	Scarification	500	+ Anthrax.
,, 7034.....	,,	,,	500	,,
,, 6938.....	,,	,,	200	Negative.
,, 7293.....	,,	,,	200	,,

+ Equals died of anthrax.

Animals receiving the larger dose of virulent anthrax were tested on the 10th day and the others on the 20th day after vaccination.

For all practical purposes, the results obtained from the three methods of vaccination were identical, two sheep of each lot succumbing to the virulence test.

Experiment No. 14.—*July*, 1924.

In this case a different vaccine of greater immunizing value was employed.

Animals.	Date of Vaccination.	Method of Vaccination.	Immunity Test.	Results.
			M.L.D.	
Sheep 8605.....	29.7.24	Subcutaneous	500	+ Anthrax, 24/8/24.
„ 5861.....	„	„	500	Negative.
„ 7790.....	„	„	1,000	„
„ 6358.....	„	„	500	„
„ 7670.....	„	Intradermal	500	„
„ 7030.....	„	„	500	„
„ 7788.....	„	„	1,000	„
„ 7051.....	„	„	500	„
„ 7098.....	„	Scarification	500	„
„ 8594.....	„	„	500	„
„ 7773.....	„	„	1,000	„
„ 6967.....	„	„	500	„

+ Equals died of anthrax.

In this case the first two sheep in each lot were tested on the 10th day, the remaining being done on the 20th day after vaccination. Only one death took place, sheep No. 8605 dying on the 16th day after application of the virulence test. This animal was in weak condition and was found lying in water contained in the drinking trough. For all practical purposes, it may be left out of consideration, in which case the results can be accepted to show that with a good vaccine a strong protection can be conferred on sheep, no matter what route for introducing the vaccine is adopted.

Experiment No. 15.—*July*, 1924.

This was carried out as a parallel experiment to the preceding two, a different vaccine of good immunizing value being employed.

Animals.	Date of Vaccination.	Method of Vaccination.	Immunity Tests.	Results.
			M.L.D.	
Sheep 7310.....	29.7.24	Subcutaneous	500	Negative.
„ 8603.....	„	„	500	„
„ 6533.....	„	„	1,000	„
„ 6260.....	„	„	500	„
„ 7033.....	„	Intradermal	500	„
„ 7540.....	„	„	500	„
„ 7022.....	„	„	1,000	+ Anthrax.
„ 6644.....	„	„	500	Negative.
„ 7100.....	„	Scarification	500	„
„ 7728.....	„	„	500	„
„ 8610.....	„	„	1,000	„
„ 8607.....	„	„	500	+ Anthrax.

+ Equals died of anthrax.

In this case the virulence test was applied in the same way as was done in the previous experiment, the first two sheep of each lot on the 10th and the remaining on the 20th day after vaccination.

The results show clearly that with an efficient vaccine sheep can be protected against anthrax, no matter what method of vaccination is adopted. If anything, the results are more favourable when vaccination is applied by the subcutaneous route.

According to observations made by Velu, immunity is developed much more rapidly when vaccination is applied to scarified areas of the skin. To test this point the following experiment was carried out: —

Experiment No. 16.—*November*, 1924.

To compare the relative value of vaccination when applied by scarification and subcutaneously and to ascertain whether the immunity is produced sufficiently rapidly to enable a sheep to withstand infection introduced subcutaneously 1, 2, 3, 6 and 10 days after vaccination.

Sheep No.	Date of Vaccination by *Scarification* with.	Date, Virulent Material Given.	M.L.D. Given.	Result.
9223	14.11.24 ⅛ c.c. B. 44, 1 : 50	14.11.24	50	+ 17.11.24
9403	,, ,,	,,	100	
9399	,, ,,	,,	500	+ 20.11.24
8944	14.11.24 ⅛ c.c. B. 44, 1 : 50	15.11.24	50	
9221	,, ,,	,,	100	
9244	,, ,,	,,	500	+ 18.11.24
9594	14.11.24 ⅛ c.c. B. 44, 1 : 50	17.11.24	50	+ 17.11.24
9170	,, ,,	,,	100	+ 23.11.24
9318	,, ,,	,,	500	+ 21.11.24
9308	14.11.24 ⅛ c.c. B. 44, 1 : 50	20.11.24	50	
9374	,, ,,	,,	100	
8984	,, ,,	,,	500	+ 24.11.24
8739	14.11.24 ⅛ c.c. B. 44, 1 : 50	24.11.24	50	
9400	,, ,,	25.11.24	100	
9447	,, ,,	24.11.24	500	+ 27 11.24

+ Equals died of anthrax.

Sheep No.	Date of *Subcutaneous Vaccination* with.	Date, Virulent Material Given.	M.L.D. Given.	Result.
9599	14.11.24 ½ c.c. B. 44, 1 : 200	14.11.24	50	+ 20.11.24
9327	,, ,,	,,	100	+ 19.11.24
9479	,, ,,	,,	500	
9322	14.11.24 ½ c.c. B. 44, 1 : 200	15.11.24	50	+ 18.11.24
9158	,, ,,	,,	100	
9623	,, ,,	,,	500	+ 21.11.24
9502	14.11.24 ½ c.c. B. 44, 1 : 200	17.11.24	50	+ 20.11.24
8677	,, ,,	,,	100	+ 23.11.24
9477	,, ,,	,,	500	+ 20.11.24
9307	14.11.24 ½ c.c. B. 44, 1 : 200	20.11.24	50	
9266	,, ,,	,,	100	+ 24.11.24
9490	,, ,,	,,	500	+ 25.11.24
9577	14.11.24 ½ c.c. B. 44, 1 : 200	24.11.24	50	
8726	,, ,,	,,	100	
9142	,, ,,	,,	500	

+ Equals died of anthrax.

Controls.

Sheep No.	Date, Virulent Material Given.	M.L.D.	Result.
9569	14.11.24	50	
9326	,,	100	+20.11.24
9363	,,	500	+19.11.24

+ Equals died of anthrax.

Since experience with the subcutaneous method of vaccination has taught us not to expect much immunity to develop before the 8th or 10th day after vaccination, we purposely employed a comparatively small dose of virulent spores in the immunity test. Analyzing the results obtained, one fails to detect any well marked difference in the extent to which immunity had been conferred by the two respective methods of vaccination. It is true that a few sheep survived the virulence test applied during the first two days after vaccination, but these cases can be explained by small variations in individual insusceptibility and by the fact that the standard spore emulsion (which was then a few years old) did no longer kill all sheep even when 50 M.L.D. were employed.

All the sheep tested on the third day after vaccination succumbed to anthrax, thus showing the absence of immunity after both methods of vaccination. Of those tested on the sixth day two sheep vaccinated by the subcutaneous method died, compared to only one vaccinated

by scarification, but one of the two sheep had received by mistake a second dose of virulent material and this may have helped to cause its death. At any rate, of those tested on the tenth day after vaccination, one of the three vaccinated by scarification died of anthrax, while of those done by the subcutaneous method all survived.

It becomes quite clear therefore, that vaccination by scarification of the skin does not produce a higher degree of immunity than that following on the subcutaneous method, nor does immunity develop more rapidly in animals done by the former method.

Summary.—Summarizing the results obtained from experiments carried out in connection with vaccination and infection through the skin, we are entitled to draw the following conclusions:—

(1) The outer layers of the skin of sheep are not particularly sensitive to anthrax infection.

(2) The disease can be set up by introducing anthrax spores into the body along practically every recognised path of of infection, provided a sufficiently large dose of material is used.

(3) Infection can be brought about by introducing the spores into mucous membranes and straight into the blood stream, without coming into contact with the skin.

(4) Introduction of virulent spores into the deeper layers of the skin (intradermal injection) appears to be one of the most certain methods of infection.

(5) There is no well marked difference in the degree of immunity conferred on sheep by vaccine applied in the following ways: Subcutaneously, intradermally, and scarification of the skin. If any difference at all, it will be found to favour the subcutaneous route.

(6) In sheep the development of a well marked immunity takes place in seven to eight days after vaccination and is strong by the tenth day; this applies to all three methods of vaccination mentioned above. Immunity does not develop more rapidly in sheep vaccinated by the intradermal method or after scarification of the skin.

(c) *Immunity following Combined Vaccination against both Anthrax and Black Quarter.*—Where stock farming is carried out under ranching conditions, young cattle are usually untrained and extremely difficult to handle. On many areas both anthrax and black quarter are prevalent, and consequently young stock must be protected against both. Owing to the difficulty and trouble involved in handling such animals, farmers have often enquired as to whether vaccination against these two diseases cannot be carried out at the same time, thus avoiding handling them twice. Up to recently we have not been able to give a definite reply to this question. Since black quarter filtrate (artificial aggressin) is absolutely germ-free and produces practically no reaction in animals, it was clear that no harm could follow its injection at the same time as anthrax vaccine, but it was not certain whether the animal tissues would respond to

both injections and produce antibodies against both diseases. To clear up this point the following experiments were carried out:—

Experiment No. 17.

Animal.	Method of Vaccination.	Result.	Method of Testing Immunity.	Result.
Sheep 9237	Both anthrax and black quarter	Lived.	Only black quarter tested	Lived.
,, 9209	,, ,,	,,	,, ,,	,,
,, 9421	,, ,,	,,	Both anthrax and black quarter tested	+Not anthrax or black quarter.
,, 9542	,, ,,	,,	,, ,,	Lived.
,, 9415	,, ,,	,,	,, ,,	,,
,, 9355	,, ,,	,,	,, ,,	,,
,, 9333	,, ,,	,,	,, ,,	,,
,, 8785	,, ,,	,,	,, ,,	,,
,, 8670	,, ,,	,,	,, ,,	,,
,, 9248	,, ,,	,,	,, ,,	,,

+ Equals died.

Conclusion.—The results show that simultaneous inoculation with both vaccines can be carried out successfully.

(7) *Vaccination of the different species of Animals and the results obtained in practice.*—Under this heading will be discussed the practical application of protective inoculation in the various species of animals running under different field conditions, the results obtained and the modifications or improvements that we were able to bring about as the result of field observations or laboratory tests.

In a discussion of this kind one should always bear in mind the essential characters of a good vaccine, previously given, namely:—

(*a*) Absolute safety for use in the particular species of animals for which the vaccine is intended, with no undesirable sequelae.

(*b*) Good immunizing value, the immunity lasting for a reasonably long period (ten to twelve months).

(*c*) Sound keeping properties (already discussed).

A vaccine producing no immunity is worse than useless, while one which is unsafe greatly hinders the successful application of any method of inoculation.

The undesirable sequelae that may follow on vaccination are:—

(1) Deaths from anthrax caused by the vaccine, even in only a small percentage of animals.

(2) Unduly severe reactions, resulting in loss of condition, decrease in milk yield, and dangerous swellings.

We have already referred to the earlier history of vaccination with spore vaccine in this country, and have indicated the shortcomings of some of the first batches issued from this Institute. These may be recapitulated briefly as follows:—

(1) Most batches were far too strong, causing death or dangerous reactions in all domestic animals. Goats, in

particular, suffered severely, a mortality of between 20 and 30 per cent. being produced by vaccination. Not many reports of mortality in sheep resulting from vaccination in the field have been received, but laboratory tests proved the vaccine to be dangerous, producing a fatal disease in a few instances. In equines, a large percentage of vaccinated animals showed alarming swellings, in some cases leading to death. Cattle suffered least, but cases are on record where loss in condition, decrease in milk yield and marked swellings were observed.

(2) Owing to faulty technique, resulting in defective spore formation some batches produced no immunity and had to be destroyed. One batch actually passed the immunity test, but on re-testing a few months later, was found to be useless as it contained no living organisms. Apparently vegetative forms must have been responsible for the presence of immunity when the vaccine was first tested. On keeping in glycerine-saline solution for a few months these organisms probably died and the vaccine thus became useless.

Generally speaking, the earlier batches of vaccine conferred a very strong immunity, stronger, apparently than was actually required in practice; they were tested against 5,000 M.L.D. or more and, as will be seen later, this standard was unnecessarily high.

The next step in the development of spore vaccine was to lower its strength considerably; instead of immunizing against 5,000 M.L.D. it was considered sufficient if protection was afforded against 2,000 or 3,000 M.L.D. This was found to be a great improvement in so far as the inoculation of cattle and sheep was concerned, but the vaccine was still too strong for goats and still produced swellings in horses. At this time also several batches of vaccine were found at the time of testing to confer practically no immunity. We, therefore, had to face the position that some batches produced no immunity and that on the other hand, others were too strong.

It was then found that the technique previously employed, namely, to grow the cultures intended for vaccine production for several weeks was wrong, in that it might lead to the death of the majority of organisms in the cultures. This was remedied, as discussed elsewhere in this report, by constant observation of sporulation in the cultures and by preparing the vaccine only when the optimum sporulation was present.

With regard to the safety of the vaccine, it was realised that each species of animal had to be dealt with separately and that, if necessary, separate vaccines had to be issued. It would have been quite a simple matter to reduce the strength of our vaccine to such an extent that it would have been safe for all species, but this could have been done only by sacrificing one of the other essential properties of a reliable vaccine, namely, its immunizing value.

We have already discussed the susceptibility of the different species of domestic animals, and have drawn attention to the variations that occur, and especially to the remarkable susceptibility of the goat. Knowing this, it seems to be no more than reasonable to take into consideration this important factor and to arrange the strength of all vaccines accordingly

Having arrived at this important point, we may now discuss the protective inoculation of each species separately, as follows:—

(1) *Sheep.*—Merino sheep with which we have to deal very largely in this country, do not show any great variation in their susceptibility to anthrax. Although they are undoubtedly very susceptible to the virulent disease, sheep show a marked resistance to infection by attenuated anthrax organisms. This factor is of outstanding importance, and renders the inoculation of sheep against anthrax a comparatively simple matter. As long as one employs for the manufacture of the vaccine, a strain that has been well attenuated and one that no longer kills rabbits, no difficulty will be experienced.

The routine method of preparing a vaccine for sheep and cattle has already been given in detail, so that further discussion is unnecessary. The only point that may be emphasized is that for the sake of safety under all possible conditions, the strength of the vaccine now being issued has been reduced so as to protect against at least 1,000 M.L.D. of virus instead of 2,000 or 3,000 M.L.D. as previously employed. Such a vaccine has been found to answer well in practice, breakdowns in immunity being practically unheard of, even on the worst infected farms. There is no doubt that sheep can stand a stronger vaccine quite easily, and such a vaccine could be supplied in special cases whenever the necessity should arise.

(2) *Cattle.*—As previously indicated, cattle are not easy to kill with anthrax by artificial means. Owing to the expense involved, experimental infection has been tried in comparatively few animals, and then it was possible to set up a fatal attack of the disease in some cases only after the administration of relatively large quantities of virulent material, two agar slants subcutaneously. This might lead one to believe that this species is not very susceptible to the disease, were it not for the fact that under natural conditions cattle are known to be the worst sufferers from anthrax.

Moreover, it has to be pointed out that the injection of such a small dose as 1 c.c. of attenuated virus (vaccine) may result in dangerous swellings and even death. Such an occurrence was not infrequent at the time when strong spore vaccines were employed. For these reasons a considerable amount of caution has to be exercised in the preparation and issue of anthrax vaccine for cattle. This is effected by the use of only properly attenuated strains which under no circumstances will prove fatal to rabbits in the ordinary test doses. During the last two years cattle have been vaccinated very successfully with a vaccine having sufficient immunizing value to protect against 1,000 M.L.D. of virus and being safe for sheep even when 20 c.c. are injected subcutaneously. In the case of one batch the sheep receiving 20 c.c. of undiluted vaccine died from the effects, but it was considered reasonably safe to issue the vaccine for use in cattle. The results were not so satisfactory as with other batches, at least one report of undesirable sequelae having reached us subsequently. On investigation about 30 per cent. of vaccinated animals showed signs of stiffness in the limbs, some having enormous swellings extending down the front limbs, involving the pectoral region and passing back to the ventral aspect of the abdomen. All these animals made a good recovery, but suffered severe discomfort leading to loss in condition, decrease in milk yield, etc. This is rather an exceptional case, but serves to illustrate the point.

By following the routine technique, previously described, and exercising reasonable care, one can obtain an anthrax vaccine that will prove safe and efficient for use in cattle under all circumstances. For the sake of convenience the same vaccine is being issued for use in both sheep and cattle, and that the results have been remarkably good during the last few years is shown by the fact that in spite of the enormous scale on which vaccination is carried out in these two species, complaints concerning the safety and efficacy of the vaccine are practically unknown. Apart from the one case quoted, where swellings were produced by one particular batch, one other complaint was made to us; this was in connection with the immunity produced by the vaccine. The farmer complained that in spite of vaccination, one heifer died from anthrax about a month later. We were seriously perturbed by this occurrence, and advised reinoculation with a stronger vaccine; two days later, however, the owner sent a message containing his apologies for the unfounded complaint made by him. He had, in the meantime, discovered that the heifer in question escaped vaccination and was the only one to contract and die from anthrax subsequent to vaccination of his herd. Similar experiences are not by any means rare and numerous instances can be quoted. In this connection Spreull,* writing of the results obtained from vaccination in the field, makes the following statement: "Where certain animals were known to have missed being inoculated, it was remarkable how the infection picked them out afterwards on more than one infected farm."

(3) *Equines.*—In South Africa vaccination against anthrax is carried out in horses, mules and donkeys; the relative susceptibility of these animals appears to be in the order given, although no accurate experimental data are available.

Horses are not easily killed with anthrax experimentally; to obtain positive results relatively large doses of virulent cultures have to be injected subcutaneously. As previously stated, the M.L.D. by this route seems to be in the neighbourhood of 10 c.c. of our standard spore emulsion (i.e. 10,000 M.L.D. with sheep as standard) or one full agar slant. Even with such material one is not always certain of producing a fatal attack of the disease. This might convey the impression that horses are not very susceptible to anthrax, but experience has shown that under natural conditions they contract the disease readily and that they may be very sensitive to subcutaneous injections of even small doses of attenuated organisms. One might say that the same remarks apply to cattle, but not nearly to the same extent. Extensive experience with the earlier spore vaccines has shown us that where a vaccine can be used in cattle with comparative safety, injection of the same vaccine may prove disastrous in horses.

Why equines should be so sensitive to attenuated anthrax organisms is impossible to say with the present state of our knowledge concerning the natural method of infection, etc. Not only are equines particularly susceptible as a species, but certain breeds, and animals bred in certain localities, seem to possess more than the average degree of susceptibility. This has been shown over and over again by the results of vaccination in the field carried out during the last four to five years. A batch of vaccine is issued for use in equines in

* Spreull, J., Senior Veterinary Officer, Cape Province. Unpublished report on an outbreak of anthrax investigated in January, 1923.

different parts of the country; in perhaps the majority of cases no untoward results are experienced but for some unaccountable reason in a small minority of cases remarkably severe effects are produced in anything up to 30 or 40 per cent. of animals. These suffer from extensive oedematous swellings, commencing at the site of inoculation, and spreading rapidly to the more dependent parts, as described in Chapter E.

The majority of affected animals make a good recovery but 10 or more per cent. of fatal cases were not uncommon with the earlier and stronger vaccines that were employed. What is remarkable is that in these fatal cases it is not easy to demonstrate the organisms in the blood. These have, however, been recovered from the local swelling when care was taken to obtain material from the initial inflammatory area. In this connection it should be pointed out that it is not possible to isolate the organisms from the purely oedematous swelling formed as a result of gravitation to the more dependent parts.

At first it was thought that some other organism might be responsible for the production of these swellings, but extensive cultural work failed to demonstrate the presence of either aerobes or anaerobes. Moreover, the appearance of these swellings was characteristic for anthrax, namely, complete absence of any pus formation, typical sero-gelatinous infiltration, etc. That the swellings were the direct result of vaccination was clear from the fact that they always commenced at the site of inoculation. We, therefore, have no doubt that the attenuated organisms must be held directly responsible for their production; that they develop so frequently in horses must be attributed to a peculiar local sensitiveness possessed by that species, and particularly by some individuals of the species. What role is played by toxins developed during the growth of the cultures from which the vaccine is prepared we are not in a position to say, especially in view of the general belief that toxins are not produced by anthrax organisms. We have no good reason to differ from this generally accepted view, but experiments to settle the point are being carried out. What we do know is that when swellings were of such frequent occurrence our vaccine cultures were grown for several weeks, during which ample opportunity for the development of toxic substances in the cultures was present. Since our new technique was applied and the vaccine cultures only grown for a few days, swellings in horses are of infrequent occurrence, but it must be stated that important modifications in other directions were also brought about. One of the chief modifications was the selection of another attenuated strain and the reduction in the strength of the vaccine itself, to be discussed later.

To minimise or do away with these undesirable sequelae, experiments were carried out as follows:—

(1) Reduction in the strength of the vaccine.
(2) Introducing the attenuated spores in a less concentrated form.
(3) Introducing the vaccine by different routes.
(4) Introducing the vaccine along with different chemical substances.
(5) Improvement in the technique employed in its preparation.
(6) Selection of a specially mild strain and issue of a relatively weak vaccine.

(1) *Reduction in Strength.*—This was effected at the same time as a similar change made in connection with vaccines intended for the inoculation of cattle and sheep. At first the vaccine was expected to protect against 5,000 and more M.L.D., and at this stage the standard was reduced to 3,000 and then 2,000 M.L.D. A slight improvement could be observed, but dangerous swellings were not by any means infrequent. This was shown by Batches 11, 12 and 13 used in combating a big outbreak of anthrax in the Kimberley district.

(2) *Different Dilutions.*—Approximately the same number of spores was suspended in different quantities of glycerine-saline solution—$\frac{1}{4}$, 1 and 5 c.c.—for injection into horses. The results appeared to show that when the spores were given in less concentrated suspension, the production of swellings was minimized to some extent, but was not prevented. It was clear that this method would not give the desired results.

(3) *Different Routes.*—Comparative results were obtained by injecting the vaccine subcutaneously (*a*) on the neck in front of the shoulder, (*b*) behind the shoulder, and (*c*) on the under surface, near the root of the tail. In all cases swellings developed in about the same percentage of animals, but the tail method proved to be the most dangerous, one animal actually succumbing to peritonitis. In this method there is a great risk of the swelling extending into the pelvis involving the rectum, bladder, etc., and interfering largely with the function of these organs. Worse still, infection of open wounds—which are easily created in this region—may result and extend to the peritoneum, with fatal results, as in the case quoted. This method cannot, therefore, be recommended. Inoculation on the neck, especially when this is done rather high up towards the head, may result in the swelling extending towards the head and involving the throat region. This danger becomes a real one when the animals run under veld conditions and are forced to keep their heads on the ground in search of food for long periods. Under such conditions there is a natural tendency for the oedematous swellings to gravitate to the head. This danger becomes less when the injection is made behind the shoulder, but in this case the swelling may extend along the abdomen to the inguinal region and will almost certainly involve the pectoral region causing great inconvenience in the movements of the animal. Owing to its accessability the region in front of the shoulder is still the common site selected for injection of the vaccine, and so long as it is not done too far up the neck, there can be no real objection to the method.

(4) *Injection of Vaccine along with chemical substances* was tried chiefly because it was believed that extraneous organisms might be responsible for the swellings. Among the drugs used were 1 per cent. Camphor, 1 per cent. Thymol, 1 and 10 per cent. Alcohol, $\frac{1}{2}$ and 2 per cent. Phenol, 1 per cent. Trypanblue, 10 and 50 per cent. Ether, and 1 per cent. Jeyes fluid. No real differences in the relative frequency in which swellings developed in the various cases could be observed.

(5) *Improved Technique in Preparation.*—This matter has already been referred to, when it was pointed out that this change affected mainly the length of time the cultures intended for vaccine

production were allowed to grow. Further experiments will show whether toxic substances can be demonstrated in anthrax cultures, and if so what factors are responsible for their production.

(6) *Selection of a Mild Strain and issue of a Relatively Weak Vaccine, etc.*—This strain was selected because in our initial tests it proved to be safe for goats, non-fatal to rabbits and only killing guinea-pigs receiving the larger doses of concentrated spore emulsion. As previously pointed out, the goat is particularly susceptible to both virulent and attenuated anthrax organisms, and for this reason is an excellent subject to employ in experiments to test the strength of a vaccine. At the same time, the strength of the vaccine was reduced to such an extent that a certain protection against 1,000 M.L.D. in sheep and 500 M.L.D. in goats was not insisted upon.

Excepting for the introduction of the goat as a test animal and a reduction of the standard virulent test, the technique of the immunity test remains the same as that employed for vaccines intended for use in cattle and sheep.

The position will become clear if a few examples of the actual tests are given:—

Experiment No. 18.—*May*, 1923.

To determine the safety and efficacy of vaccine Batch 21.

Animals.	Dose of Vaccine Emulsion.	Results.	Dose of Virulent Spores.	Results.
Sheep 1	20 c.c.	Negative.	1,000 M.L.D.	Negative.
„ 2	20 „	„	„	„
„ 3	1 „	„	„	„
„ 4	1 „	„	„	„
„ 5	·1 c.c.	„	„	„
„ 6	·1 „	„	„	„
„ 7	·02 „	„	„	+Anthrax.
„ 8	·02 „	„	„	Negative.
„ 9	·01 „	„	„	+Anthrax.
„ 10	·01 „	„	„	Negative.
„ 11	·005 „	„	„	„
„ 12	·005 „	„	„	„
Goat 1	10 „	„	500 M.L.D.	„
„ 2	1 „	„	„	„
„ 3	·1 „	„	„	„
„ 4	·02 „	„	„	„
„ 5	·01 „	„	„	+Anthrax.
„ 6	·005 „	„	„	Negative.

+ Equals died of anthrax.

The results show that the vaccine is perfectly safe for goats and sheep, even when injected in large doses. The virulence test showed the immunity to be fairly strong in animals which received .02 c.c. and larger doses. It is true that one sheep receiving this amount of vaccine died in the virulence test, but the goat receiving the same dose survived.

The vaccine was issued for use in horses in a dose of .02 c.c. and excellent results were obtained from its use in practice.

Guinea-pigs receiving the same vaccine reacted as follows:—

1 receiving .1 c.c. died from anthrax.
1 receiving .02 c.c. survived.
1 receiving .01 c.c. survived.

Experiment No. 19.—*December*, 1923.

To test the safety and efficacy of vaccine Batch 32.

The small animal test gave the following results:—

1 rabbit receiving .1 c.c. survived.
1 guinea-pig receiving 1 c.c. died of anthrax.
1 guinea-pig receiving .01 c.c. survived.

This test on sheep and goats was as follows:—

Animal Nos.	Dose of Vaccine.	Results.	Dose of Virulent Material.	Results.
Sheep 7468	20 c.c.	Negative.	1,000 M.L.D.	Negative.
„ 7424	20 „	„	„	„
„ 7502	1 „	„	„	„
„ 7821	1 „	„	„	„
„ 7369	·1 c.c.	„	„	„
„ 7525	·1 „	„	„	„
„ 7432	·02 „	„	„	+ Anthrax.
„ 7689	·02 „	„	„	„
„ 7477	·01 „	„	„	„
„ 7526	·01 „	„	„	„
„ 7458	·005 „	„	„	„
„ 7352	·005 „	„	„	„
Goat 8139	10 „	„	500 M.L.D.	Negative.
„ 8122	1 „	„	„	„
„ 8130	·1 „	„	„	„
„ 8151	·02 „	„	„	„
„ 8158	·01 „	„	„	+ Anthrax.
„ 8144	·005 „	„	„	„

+ Equals died of anthrax.

The results show the vaccine to be quite safe for sheep and goats, even when large doses are injected. Those obtained from the virulence test, carried out 19 days later, reveal the fact that a good immunity was established by the vaccine in doses of above .02 c.c. and that this dose was still sufficient to protect a goat.

Horses inoculated at the Laboratory showed practically no sign of any swelling.

The vaccine was issued for use in parts of the country where equines had been observed to be particularly sensitive, and the dose selected for this purpose was .01 c.c. The results obtained in practice were excellent, no reports of any undesirable sequelae having reached us. In one case only, a complaint was made that vaccinated animals succumbed to anthrax subsequent to the use of this vaccine. This concerned a farm that was badly infected and where apparently the careless handling of carcases was responsible for spreading the infection in a wholesale manner.

These two examples of safety and immunity tests give a fairly good idea of the standards applied when a decision has to be taken regarding the suitability of a vaccine for use in equines. Some of