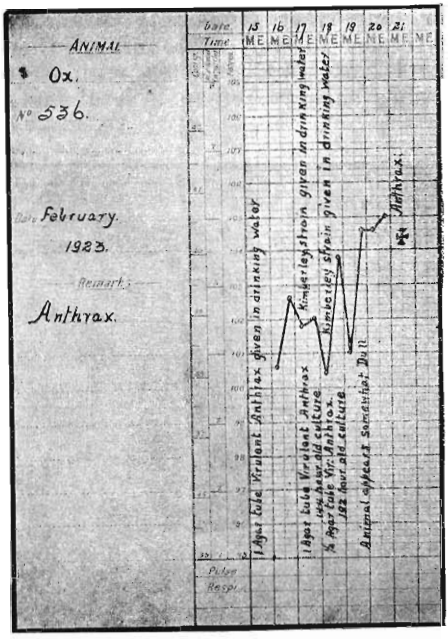
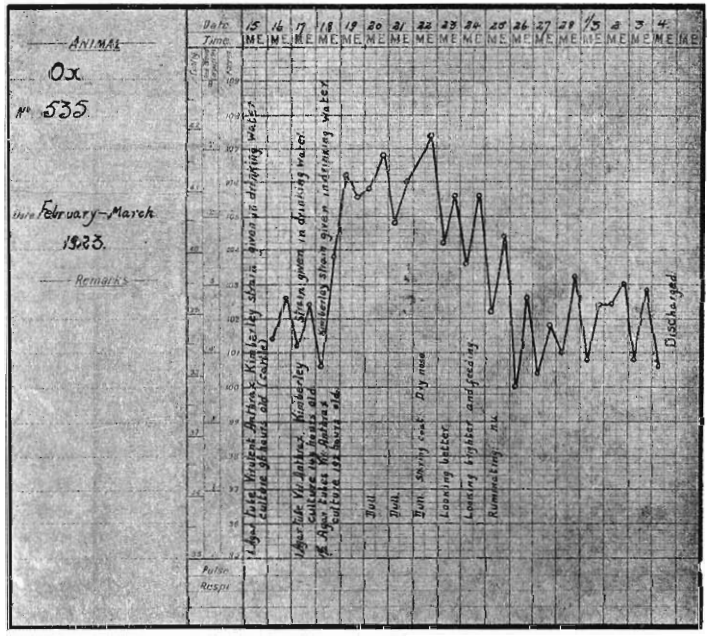
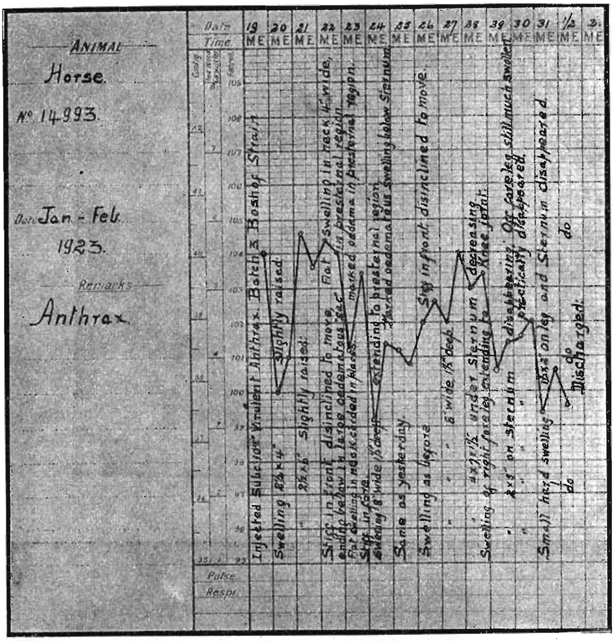
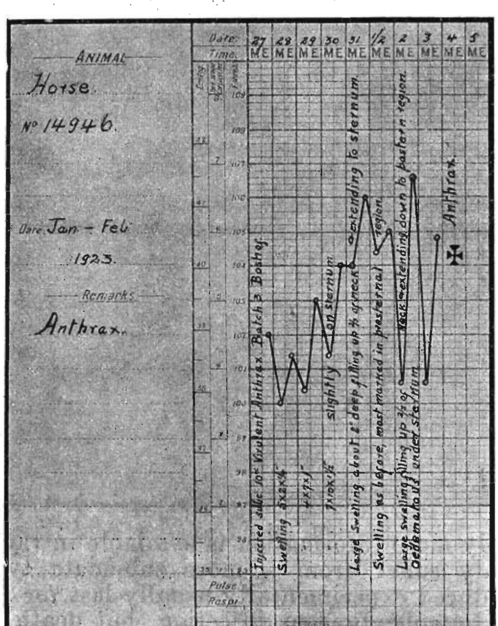




Considerably more information is available in the case of equines which commonly suffer from a more sub-acute type of anthrax. Fatal cases produced experimentally usually last for four to five days after infection by subcutaneous injection, but death may take place as early as three days or as late as ten days. Where the disease lasts

for four days or longer, it is usually accompanied by extensive swelling of the tissues in and surrounding the site of injection. Temperature charts Nos. 14946, 14993 will serve to illustrate the temperature reactions encountered in a fatal and a non-fatal attack of the disease.





Natural cases in equines commonly last from two to four days, during which oedematous swellings in certain regions are one of the most characteristic symptoms. The most common sites for swellings to develop are in the region of the udder of the female or sheath of the male, and the ventral aspect of the abdominal floor, but they may be found in other regions, such as the shoulder, precrural region, etc. The initial swelling is of an inflammatory character, being hard and painful to the touch. In cases where the disease is undoubtedly fly-transmitted, the swelling will be seen to develop as a small hard raised area covered by flies, and growing very rapidly by extension to the surrounding tissues. From the udder, for instance, it would extend backwards between the thighs, along the perineum and forward along the ventral aspect of the abdomen to the sternal region. Owing to its rapid development and, frequently, great size, the movements of the affected animal are often interfered with very markedly. For illustrations see end of this article.

In the case of artificial infection with virulent or attenuated cultures the swelling starts to develop at the site of injection and from there it gravitates to the dependent parts of the body; it passes down the side of the neck, involves the region of the pectoral muscles, extends down the forelimbs or passes backwards along the ventral aspect of the sternum or abdomen. These swellings rapidly disappear on recovery of the animal, but occasionally they persist for a week or longer. In one exceptional case, illustrated by a photograph, the swelling lasted for as long as six weeks. There is no doubt that, quite apart from the method of infection, equines are disposed to develop swellings in anthrax infection, but the results obtained from experiments in connection with artificial infection seem to indicate that the site of development of the initial swelling marks the route of infection. This is confirmed by observation in areas where the disease is believed to be fly transmitted that swellings are almost invariably present and that they commence in those regions of the body which are specially favoured by the fly. In these cases the swelling develops in connection with the skin or subcutaneous tissues, whereas in cases where infection was not through the skin, the initial swelling appears in a lymphatic gland and from there extends to the surrounding tissues.

Apart from equines, swellings are commonly met with in pigs, in which the lymphatic glands and surrounding tissues are involved. Here again the site of the initial swelling indicates the route of infection.

It is difficult to offer a satisfactory explanation for the fact that swellings are of such frequent occurrence in equines, even when infection had apparently not taken place through the skin. As far as fly transmission is concerned, the horse appears to be a favourite host of Hippobosca; even when cattle, sheep and goats run on the same pasture with horses, the fly will be found much more abundantly on the horses than on any of the other species mentioned. The skin of sheep and goats is better protected by their thick woolly or hairy coats, the ox has a very thick hide, whereas the horse is thin-skinned and some regions of its body (perineum, udder, sheath, etc.) are almost devoid of hair. These and other factors appear to make it relatively more attractive to the fly.

F.—Diagnosis.

This matter is described so fully in all modern text books that the writers do not feel called upon to refer to it in any detail. Reference is made to it only because of the expressed intention to make this report as complete as possible and because local experience may bring to light interesting information in some directions.

(a) *Clinical.*—In herbivora the disease usually runs a peracute course, as in other parts of the world, and consequently it is uncommon for the veterinarian to be called upon to make a diagnosis on the clinical aspect alone. In these animals anthrax does not, however, always run a peracute course, cases having been brought to the notice of the writers where affected cattle lived several days, some actually recovering. In one such outbreak the disease was first of all diagnosed post-mortem; further cases continued to occur and among the more subacute the following clinical symptoms were predominant: High temperature, dullness and swelling of the prescapular or precrural glands. It was possible to make a diagnosis on the clinical signs, this being confirmed by bacterioligical investigation of the cases that terminated fatally. In equines it is usually not difficult to make a diagnosis of anthrax by the clinical manifestations, and this is especially so when more than one case occurs in the same lot of horses or on the same farm. The disease conmmonly occurs in the subacute form, accompanied by the appearance of characteristic oedematous swellings, and a well-marked fever reaction. When these symptoms are present in characteristic form there is no great difficulty in arriving at a diagnosis. At any rate, in this country one must always suspect anthrax in equines showing fever accompanied by oedematous swellings.

(b) *Blood Examination.*—The writers have often been able to demonstrate the presence of anthrax bacilli by the microscopic examination of smears made from the blood several hours before the death of the animal. Of course, there is no difficulty in making a diagnosis by these means after the death of the animal; in ruminants the blood usually swarms with anthrax bacilli, while in equines one meets with cases now and again where the bacilli are rare and likely to escape observation unless the examination is made carefully. For staining blood smears Giemsa solution has been used with great success in this Laboratory for many years. With experience in the diagnosis of anthrax one has no difficulty in recognising the organisms, even when large numbers of putrefactive bacteria are present. The anthrax bacilli can be distinguished by the presence of typical capsules, their morphology and by their tendency to disintegrate. We have frequently encountered smears in which capsulated organisms of the putrefactive type were present, but anthrax bacilli could be distinguished from these by their morphology and tendency to disintegrate, etc. By adopting Giemsa solution as a routine method of staining, it is possible to arrive at a diagnosis of the large majority of cases; in cases where we failed to arrive at a decision, the adoption of other methods of staining (including McFadyean's Methylene blue reaction) did not afford any assistance.

One other interesting point which is worth recording is the occurrence of anthrax spores in smears sent in for examination from the field. This usually applies to smears that had been spread too

thickly, and not allowed to dry before being wrapped up for despatch to the Laboratory; very often two smears were placed together while still wet, and in this way drying was hindered or completely prevented. In such smears putrefactive bacteria multiply and especially round the edges sporulation of anthrax bacilli takes place. In a few instances smears have been encountered where the anthrax bacilli multiplied, appearing in long chains (as in culture) and presenting spore formation in some places.

Where spore formation is present in a smear we have had no difficulty in isolating the anthrax strain in pure culture, either by animal inoculation or cultural examination.

(c) *Animal Inoculation.*—White mice and guinea-pigs are commonly employed for this purpose. It is a very useful method for establishing the existence of anthrax spores in suspected material of all kinds, and by its application we have often succeeded in making a positive diagnosis in the case of dry hides, bones, bonemeal, etc. The suspected material is cut or chopped into small pieces which are placed in a small quantity of saline contained in a large test tube and pasteurized in a water bath at 60° C. for one hour. By these means non-sporulating organisms are killed off while anthrax and other spores are left intact. The liquid is filtered through paper and 1 or 2 c.c. injected into white mice and guinea-pigs. If any anthrax spores are present at all the white mice will be killed off rapidly. Small animal inoculation is, of course, employed in many other directions, for instance, in confirming a diagnosis made by cultural examination.

(d) *Cultural Examination.*—The different methods of cultivation that may be employed for the isolation of anthrax bacilli are so well known that there is no need to repeat them here. Cultural methods are very useful in making a diagnosis of the disease in either live or recently dead animals in which the organisms are not present in the blood in sufficiently large numbers to permit of their detection by the ordinary smear examination. Such cases are fairly frequently met with in animals that have succumbed to attenuated (vaccine) strains of anthrax; cultures are made from material taken from the spleen and usually there is no difficulty in obtaining the organisms in pure culture.

In anthrax swellings the causal organisms also occur in only small numbers and here cultural method often give the desired results.

(e) *Precipitin Test.*—With the methods of diagnosis already enumerated it was usually possible to arrive at a correct decision, so that we rarely had occasion to fall back on Ascoli's precipitin reaction.

G.—Control and Eradication.

Under this chapter it is proposed to deal with (*a*) legislative measures taken to cope with the anthrax menace, and (*b*) protective vaccination giving details with regard to all aspects of this important problem. The chapter will necessarily be long, but for convenience the subject matter is discussed under the following headings:—

I. Introduction.

II. Legislation.

III. Protective Inoculation.
(1) Introduction.
(2) Historical Survey.
(3) Spore Vaccine.
(4) Selection of Strains.
(5) Preparation.
(6) Immunity.
(7) Vaccination in Practice.

I.—*Introduction.*

Anthrax can be eradicated from any locality only if all sources of infection can be removed and the introduction of any further infection prevented. In practice it is rarely possible to remove all sources of infection, for the simple reason that they are not often known with any degree of certainty. Where this can be done, for instance when the infection has been introduced through the medium of foodstuffs, no further difficulty is experienced. In the large majority of cases, however, the problem is a much more difficult one, e.g., it is not known how the infection was introduced, to what extent it exists on the farm and—what is still more important—how long it is likely to presist. This brings us to the greatest lack of our knowledge concerning this disease, namely, in connection with the biology of the organism outside the body, under natural field conditions. In spite of this lack of knowledge, the disease can be kept under control by paying attention to the following points:—

(1) By rendering all susceptible animals on the farm insusceptible to the disease and keeping them in this insusceptible condition so long as they are exposed to infection. This can be done by annual inoculation with a reliable anthrax vaccine.

(2) By avoiding in every possible way further infection of the farm. The greatest and most dangerous source of infection is the infected carcase, and if its destruction is carefully attended to, a good deal of progress towards the eradication of the disease has been made, because it is reasonable to assume that the original infection will not persist indefinitely. Infection from outside sources may be brought on to the farm by means of infected animals or animal products and this has, of course, to be guarded against. All the steps enumerated in the next section are governed by regulations under the Stock Diseases Act, but it is clear that they cannot be carried out properly unless there is the closest co-operation between the stock-owners and government officials concerned with the control of the disease.

II.—*Legislation.*

It is considered advisable at this stage to give a short summary of the Union Government regulations under which the control of anthrax is carried out.

It may be mentioned that prior to Union in 1910, no very active steps were taken against the disease. Anthrax was placed on the list of scheduled diseases in the Orange Free State as long ago as 1891, but in other parts of the Union it does not appear to have been

definitely scheduled until the time of Union. In the Transvaal, government regulations for the suppression of anthrax were drawn up as long ago as 1903. There is no need to go into this question any further, excepting perhaps to point out that no active measures were taken by the different states simply because the disease did not appear to be sufficiently serious and because they had other, more serious, problems to deal with.

The present regulations governing the control of anthrax are all based on the provisions of Stock Diseases Act No. 14 of 1911, and may be explained briefly, as follows:—

There are virtually three sets of regulations, differing in their stringency and framed in such a way as to allow the proper control of anthrax under different conditions; they are:—

(*a*) Regulations applicable all over the Union.
(*b*) Regulations applicable in proclaimed anthrax areas.
(*c*) Regulations applicable on the Witwatersrand.

(*a*) The Union regulations contain the following provisions:—

(1) Compulsory notification to the nearest police officer, magistrate, Government veterinary officer, etc. Owing to the difficulty encountered by the farmer in diagnosing the disease, this measure is not generally enforced, excepting in areas that have been proclaimed infected areas.

(2) No person other than a Government veterinary officer is allowed to dissect an anthrax carcase. For the purpose of obtaining material for diagnosis, the owner is allowed to take a blood smear from the ear, while an officer of the Department or a police officer may cut off a piece of skin. Usually this refers to dried hides or skins that had been removed from suspected cases.

(3) The owner must dispose of the carcase by burning or proper burial, the burial ground being fenced off.

(4) Infected animals have to be isolated and quarantine of in-contacts is usually enforced for fourteen days after the last death or proper vaccination.

(5) Exhuming of buried carcases or removal of portions of such carcase is strictly prohibited, excepting by the authority of the Government veterinary officer.

(6) Disinfection of persons who have been in contact with infected animals, or of stables, bedding, excreta, etc., is demanded.

(7) The owner has to allow in-contact animals to be vaccinated at the discretion of the Principal Veterinary Officer. Vaccine for this purpose is issued free of charge.

(8) Ox or equine transport is not allowed along infected routes, unless these animals had been inoculated against anthrax within the preceding twelve months. The person in charge of such animals has to carry, and produce on demand, a certificate showing that such vaccination had been performed.

(9) Any case of sickness or death occurring in stock travelling on a public road has to be reported to the authorities as well as to the owner of the land. In the event of death or destruction of the animal, the owner or person in charge has to attend to the proper disposal of the carcase.

(*b*) In proclaimed anthrax infected areas the following additional regulations apply:—

(1) All sudden deaths of stock, excepting as a result of an accident, have to be reported immediately to the proper authorities.

(2) A blood smear is to be taken from the ear and immediately after that the carcase must be disposed of in a proper manner, either by burning or burial.

(3) Compulsory vaccination within fourteen days after notification by the Government veterinary officer has to be carried out. Vaccination must be repeated thereafter annually until the owner is notified that this is no longer necessary.

(4) No transport animals unless vaccinated within the previous twelve months shall be allowed to be outspanned or to graze in such an area.

(5) Similarly, no stock unless vaccinated during the preceding twelve months are allowed to graze there.

(*c*) On the Witwatersrand special regulations exist as follows:—

(1) Compulsory notification of all deaths to the nearest police officer.

(2) The carcase shall not be incised in any way, blood smears in this case being taken by an officer of the Department or a police officer. Only the Government veterinary officer has the right to carry out further dissection.

(3) In the event of an authorized officer not attending to take a blood smear within twelve hours after death the owner shall have the right to do so and immediately after that the carcase may be disposed of, subject to bye-laws of the local authority.

Analysing these regulations we find that all the necessary powers for the suppression of the disease are provided, and that the provisions are particularly stringent as far as proclaimed infected areas and the Witwatersrand are concerned.

Certain areas are declared anthrax "infected" when the prevalence of the disease and local conditions are such that active steps have to be taken by the State. In such cases extra veterinary supervision is provided in connection with the disposal of carcases, quarantine regulations and, above all, compulsory vaccination. By adopting these methods it is possible to get the disease under proper control in any particular area. Regarding the special regulations in force in the Witwatersrand area reference has already been made to the peculiar conditions existing there.

Apart from the regulations already referred to, one or two others deserve special mention. Under the Fertilizers, Farm Foods, Seeds and Pest Remedies Act (1917), it is laid down that artificial fertilizer and bonemeal must be submitted to efficient sterilization, the method of sterilization being clearly defined. Moreover, under the provisions of this Act buyers of bonemeal can demand from the vendors a certificate of sterilization.

Regulations prohibiting the importation of vaccines, excepting under official permit, are now also in force. These are of the utmost importance, because unsafe and inefficient vaccines can be excluded from the country.

Earlier in this report we showed no hesitation in supplying the fullest details concerning the prevalence of anthrax in this country; we did this knowing full well that in spite of the marked prevalence of the disease, it is at the present time probably under better State control than in most other parts of the world.

That the Union Government has been fully alive to the seriousness of the position and that it means to suppress anthrax was explained in the introductory remarks to this article. When it is remembered that the average annual issue of free anthrax vaccine exceeds two million doses and that a great proportion of this vaccine is used under the personal supervision of Government officials it will be understood to what extent the Union Government assists and controls the campaign which is being carried out against the disease. Vaccination is, after all, the finest weapon we have against anthrax and if this weapon is used wisely we can safely hope to see a great improvement in the position in a few years' time.

Although anthrax is a disease largely affecting livestock and thus comes under control chiefly of the veterinary branch of the Department of Agriculture, its appearance now and again in human beings has brought about the necessity of legislation being passed and applied by departments other than that of Agriculture. Thus, under the Public Health Act anthrax in human beings is a notifiable disease and every case has to be reported to the local authority who, again, is required to send a weekly return to the central authority, the Chief Health Officer. Provision is made for mutual notification and consultation between the Public Health and Agricultural Departments. The Department of Public Health exercises control over the importation of certain articles made from animal products and intended for use by persons, e.g., shaving brushes. It may be mentioned that in 1920 anthrax was detected in a consignment of shaving brushes imported from Japan and that further importation was then prohibited under proclamation by the Public Health Department.

Under the Department of Labour the protection of workers in factories was sought for by the promulgation of regulations in 1920 under the Factories Act. Persons suffering from abrasions on exposed parts of the body are prohibited from working with hides and skins. Provision is also made for washing facilities and for storage of food and clothing.

The transport of animals or animal products by rail, places in the hands of the Railway Department a certain amount of control. Where infection is discovered to be present in animals or animal products conveyed in railway trucks, the latter have to undergo thorough disinfection and this is done according to methods laid down by the Veterinary Division. Of course, suspected cases that have died in railway trucks or on railway premises have to be reported immediately to the veterinary authorities.

III.—*Protective Inoculation.*

(1) *Introduction.*—We have already referred to the fact that vaccination with a reliable vaccine is the most effective weapon we can employ in our fight against anthrax. The importance of this weapon cannot be over emphasized. Provided vaccination can be applied regularly at least once annually, and provided care is taken to prevent further infection on the farm, it is reasonable to assume that the disease can be eradicated from any locality after a certain

number of years. Owing to our lack of knowledge of the biology of the causal organisms under natural conditions, it is not yet possible to give even an approximate estimate of this period. In any case it is safe to say that losses from the disease can be kept down to a minimum—or even avoided altogether—by the regular and systematic employment of a reliable vaccine. What we understand by a reliable vaccine is one that possesses all the following properties:—

(*a*) It must be perfectly safe for use in animals since otherwise the "cure might be worse than the disease." There is nothing that discourages preventive inoculation so much as a vaccine which is likely to be followed by undesirable sequelae.

(*b*) It must possess good immunizing properties and the immunity conferred must last for a reasonably long period, nine to twelve months. When a farmer goes to the trouble of inoculating his stock, he has the right to expect the vaccinated animals to be protected against the disease in practically all cases.

Failure to protect is not only a great disappointment, but definitely discourages the farmer to repeat the inoculation.

(*c*) It must have good keeping properties, especially under the conditions prevailing in this country, where very often the vaccine has to be sent long distances and where farmers are inclined to keep supplies on hand for some time. With a vaccine of doubtful keeping quality, the producer is never certain of the results he is going to obtain from its use in practice. Failure to confer the desired immunity is a common complaint and this in spite of the fact that the original tests showed the immunizing properties to be good. This was a common experience with the vaccines prepared according to Pasteur's method and containing few or no spore-bearing organisms.

Apart from these points, there is the question of cost to the stock owner, which determines to quite an appreciable extent the regular and systematic manner in which vaccination should be carried out. This factor is of special importance in a country like ours where large numbers of livestock are kept by individual owners and where farmers very often are loth to spend any appreciable amount of money on the purchase of vaccines. The only way to overcome this difficulty, which may provide a real obstacle to successful vaccination, is for the State to consider the advisability of supplying anthrax vaccine free of charge to all bona fide stock owners. To avoid wastage, etc., vaccination should, of course, be carried out under veterinary supervision as far as possible. This is actually what the Union Government has decided upon and proof is already forthcoming that it was a wise step, vaccination being practised more extensively and reports of large outbreaks of the disease showing a marked decrease.

(2) *Historical Survey.*—Under this heading it is proposed to give a short historical review of vaccination against anthrax up to 1920, when the so-called "Spore-vaccine" was first introduced. At a later stage the development of spore vaccine—i.e. vaccination during the last few years —will be discussed in detail. Pasteur vaccine was used by Hutcheon in the Cape Province as long ago as 1882. At that time

he was investigating the cause and nature of Heartwater and believing it to be a form of anthrax he accordingly attempted protective inoculation. The experiment was repeated the following year and the conclusions arrived at are best given in Hutcheon's own words: "But although heartwater is thus satisfactorily shown to be a distinct disease from anthrax or charbon, I am still strongly of opinion that its cause is an agent of a character similar to the one which produces anthrax." He also tried anthrax vaccine as a prophylactic against horsesickness, inoculating nine horses of which three died from the effects of the vaccine. As far back as 1883 Wiltshire in Natal appeared to have been very anxious to obtain the necessary authority to import anthrax vaccine for use in that colony, but he apparently received no encouragement from the local administration. By 1890 he appeared to have had some anthrax vaccine in his possession, but no records are available to show the extent to which it was used.

In 1894 Henning advised the farmers of Griqualand West, where anthrax was very prevalent, to adopt preventive inoculation and two years later a supply of vaccine for use in this area was ordered from Europe.

From 1897 Pasteur anthrax vaccine was prepared at Grahamstown and supplies from there were despatched during the same year to neighbouring territories, Transkei, Rhodesia, and Orange Free State. Two years later a supply was also sent to the Transvaal. The preparation of anthrax vaccine at Grahamstown continued until 1905 after which the necessary supplies were obtained from Europe.

During this period protective inoculation against anthrax gained somewhat in popularity, but it was never really practised on a large scale until some time after Union in 1910. That even in the earlier days the results obtained from the use of Pasteur vaccine were not always satisfactory, can be gleaned from the following:—In 1904 Dixon noted that anthrax vaccine to be efficacious should be "fresh" and "full doses" used. In 1905 the same authority expressed the opinion that the usual dose of vaccine does not confer immunity where the infection is "strong." In such cases he used double the dose generally employed.

As a result of reorganisation following on Union, Onderstepoort Laboratories, Pretoria, became the headquarters of Veterinary Research in this country, and consequently assumed control of the preparation and issue of vaccines employed against stock diseases. Anthrax vaccine continued to be imported from the Pasteur Institute until 1914 when its local preparation was recommenced. More attention was paid to the control of anthrax which seemed to be becoming increasingly prevalent, and in consequence there was a corresponding increase in the demand for vaccine. From a few thousand doses the annual issue increased to 190,605 doses for the financial year ended 31st March, 1915.

With the greater experience gained in the use of Pasteur vaccine, unsatisfactory results became more and more apparent. Thus, in the report of the Director of Veterinary Research for the year ended 31st March, 1914, reference was made to failures on the part of Pasteur vaccine to confer the necessary immunity. This information was conveyed to the Pasteur Institute, which provided material which was stated to be more efficient, but field experience in this country failed to support this contention.

It was then believed that the lack of immunizing value might probably be due to bad keeping properties, that, in other words, the vaccine contained very few or no living organisms after its long journey from France. The underlying principle on which the preparation of Pasteur vaccine is based, is the complete avoidance of spore formation in cultures, the vaccine containing only vegetative forms. In this form it is generally accepted that anthrax organisms do not remain alive for more than a few weeks, and it stands to reason, therefore, that to be of any value such a vaccine must be used fresh. As a matter of fact, it can be accepted that spores are not entirely absent from Pasteur vaccine, and that the small immunizing value which is still present in "old" vaccine is due to these spores.

It was now decided to prepare Pasteur vaccine locally, with the object of having fresh material for issue to the stock owners. Moreover, the difficulty of obtaining regular supplies from Europe during the war period (1914-1918) was another deciding factor.

Up to March, 1917, both locally produced and imported Pasteur vaccines were used, but apparently there was very little to choose between these two products. In both cases the results continued to be rather unsatisfactory, in some cases excessive swellings being produced while in others the resulting immunity appeared to be nil. In this connection it should be pointed out that, although the vaccine was produced locally, in some cases several weeks or even a few months might elapse before it was actually injected by the farmer, so that even here some of the unsatisfactory results might be put down to the bad keeping properties. On the other hand, the particular strains used for vaccine production—they originated from Pasteur Institute—may not have had sufficient immunizing properties to protect against local virulent strains, thus accounting for its failure in so many cases.

In 1918 Kehoe described in great detail the results obtained from the use of Pasteur vaccine, so that it seems to be unnecessary to pay much more attention to this side of the question. We might add, however, that since Kehoe's article was published, much additional information in connection with this matter has been collected and that, briefly stated, the results obtained from the use of Pasteur vaccine were in many cases extremely disappointing. To quote only a few examples:—

(1) During November-December, 1922, a severe outbreak of anthrax occurred in the Kimberley district, involving a number of farms in a wide area. Horses were the principal sufferers, but cattle, goats, sheep and wild herbivora were also attacked. In some cases the affected animals had been inoculated with Pasteur vaccine a few months previously, while in others the vaccine was used as soon as cases of the disease appeared. In no instance did vaccination seem to have the slightest influence on the course of the disease, and it only abated after the use of spore vaccine. One farmer used double Pasteur vaccine 5-6 months before the outbreak on 500 horses, and the same animals were again vaccinated as soon as the first cases of anthrax made their appearance. In spite of this he lost 320 out of 500 horses during this single outbreak. Another farmer inoculated 400 horses with Pasteur vaccine soon after the

disease made its appearance and in this case 105 died from anthrax within a few weeks.

(2) In the Griqualand West area where Pasteur vaccine has been in use for a number of years, very similar experiences can be related. To quote only a few recent instances:—

(*a*) One farmer inoculated about 100 equines with Pasteur vaccine, soon after the disease had broken out. During the next month his losses amounted to 44 per cent. and the disease showed no signs of abating until after the use of spore vaccine.

(*b*) Another farmer inoculated 32 donkeys with Pasteur vaccine and two months later moved them to an infected farm. A week after arrival the disease broke out amongst them and was only stopped after reinoculation with spore vaccine.

As a result of similar experiences in many parts of the country the demand for Pasteur vaccine has become so small that it no longer pays agents to import it.

This vaccine has certainly been of service in the past, when there was no better available, but under our South African conditions a much more stable and reliable product is required.

(3) *Spore Vaccine.*—This is an old established method of preparing anthrax vaccine, it having been used for many years, particularly in Russia. During recent years it has been employed extensively in Australia, Japan and North America. A certain amount of prejudice against the method has existed for some time, because it was believed that the introduction of resistant spores under the skin of an animal would be attended by a considerable risk of setting up infection. This suspicion appears to be groundless, since there is no reason to believe that infection is more likely to result from spores than from vegetative forms, provided they are from the same anthrax strain. On the contrary, our experience with virulent anthrax strains has taught us that young cultures, containing mostly bacillary forms, are more dangerous than old cultures, containing only spores. When spores are injected into the tissues of an animal, they very soon vegetate and thereafter behave in the same way as bacillary forms, being subject to attacks on the part of the body tissues to the same extent as the latter. Both forms must multiply to some extent at the site of inoculation, since otherwise it is not conceivable that much reaction on the part of the body, with the production of immune bodies, would result.

There appears to be no essential difference in the reaction produced in, and the immunity conferred on, animals by freshly prepared Pasteur and spore vaccines. In practice the only real difference is that in the case of spore vaccine the number of living organisms contained in the vaccine remains fairly constant for many months and that consequently the results obtained from its use in animals are much more reliable. From the practical point of view, however, this factor makes all the difference between success and failure, because with a spore vaccine, containing a fairly constant number of live organisms, failure to confer immunity is rarely met with, provided, of course, the initial tests of the vaccine showed it to possess a good immunizing value. A further advantage is provided by the fact that infection of the finished product can be avoided

altogether by the addition of a fairly strong preservative agent, e.g., glycerine, which would prevent multiplication of any organisms. Such a preservative agent would not for some time, affect the highly resistant anthrax spores. Work in connection with the production of a spore vaccine was first commenced in South Africa in 1920 by Kind (1922). Kind aimed at the production of a very high degree of immunity in animals; in this he succeeded, but only by sacrificing one of the most essential characters of a good vaccine, namely its safety. The batches of vaccine prepared by Kind, with the exception of one, undoubtedly protected animals against anthrax, but in many cases they were also responsible for mortality which was sufficiently serious to demand immediate investigation. The worst sufferers in these cases were animals of the more susceptible species, goats and horses, but fatal results were also produced in sheep and cattle.

In spite of these unsatisfactory results, it was felt that the solution of our difficulties still lay in the discovery of a safe and efficient spore vaccine, and with this object in view anthrax experiments were continued by us. The strength of the vaccine was gradually reduced, either by using weaker attenuated strains or by reducing the number of spores contained in the dose of glycerine-saline emulsion. At the same time, efforts were directed to the improvement of the technique employed in its preparation.

(4) *Selection of Strains.*—To be able to prepare an efficient spore vaccine, one must be in possession of an anthrax strain that has been attenuated properly and which has a high immunizing value. Such strains can be obtained from other bacteriological institutes or one may undertake one's own attenuations with local virulent strains of anthrax.

It is not considered necessary to describe the method of attenuation in any detail, but the more important steps may be referred to briefly, as follows:—

(*a*) From the virulent culture available a single colony is obtained by plating out several times. By starting with a single organism one is less likely to encounter great variations in subsequent attenuations.

(*b*) An absolutely reliable incubator which can be regulated in such a way that a temperature of not less than 42 and not more than 43° C. is constantly maintained, is essential.

(*c*) For the actual attenuation broth cultures are made use of and these are incubated at the above temperature either (*a*) continuously, or (*b*) intermittently for periods varying up to eighty days or longer. In the former case (*continuous*) the organisms are kept in the same medium during the whole period. At daily intervals or longer, if desired, subcultures on agar are made, grown at an ordinary temperature of 37° C. and put away at room temperature for subsequent testing. In our experience the required attenuation rarely takes place before the 30th day, so that one need not pay particular attention to cultures that have been grown for, say, up to 20 days. When taking out a broth tube for subculturing on agar, it should be replaced in the special incubator as soon as possible, since otherwise sporulation might take place.

The tubes are marked so that later on one would know exactly how long they had been attenuated. In the *intermittent* method, only one or two broth tubes containing virulent anthrax bacilli are placed in the incubator at 42-43° C. and after an interval of four or five days further broth tubes are inoculated from these. Subculturing is done quickly and the new tubes placed back in the special incubator at 42° C. immediately. In this case the underlying idea is to provide fresh nutriment for the growing organism as soon as the old medium is thought to have become exhausted. There is a further possible advantage, namely, that the organisms are not allowed to remain in their own products for too long a period. The further steps are essentially the same as those employed in the continuous method; subcultures are made on agar at intervals of a day or longer and, after these have grown sufficiently at 37° C. they are put away at room temperature for subsequent tests.

(*d*) Having now obtained a big range of agar slopes of varying virulence, it is necessary to determine the extent to which attenuation of the organisms has taken place. It would be too wearisome and costly to submit every tube to a test, so that at this preliminary stage only certain likely tubes, say, those attenuated for 25, 30, 35, 40, 50 and 60 days, respectively, are selected. The test is carried out on small animals, $\frac{1}{2}$ c.c. of a 24-hour old broth culture being injected subcutaneously into one or two rabbits and guinea-pigs. A properly attenuated strain, i.e. for cattle and sheep will still kill guinea-pigs, but not rabbits. If none of the tubes selected show this degree of attenuation, others that have been incubated longer must be tried; similarly, if no guinea-pigs are killed, attenuation must be considered to have been carried too far.

(*e*) Having obtained a number of tubes which seem to show the desired degree of attenuation, the next step is to determine their immunizing value. In our experience neither guinea-pigs nor rabbits lend themselves to immunization, and hence another species has to be employed; we have found the sheep the most reliable and under our conditions the cheapest animal to use for this purpose. A small trial batch of vaccine is prepared from each attenuated strain, according to the method to be described later, and is injected into a few (four to six) sheep in varying doses; about three weeks later the immunity of these sheep is tested against from 500 to 1,000 M.L.D. for sheep of virulent anthrax. If the immunity conferred by any particular strain is considered to be satisfactory, a further batch of vaccine is made from that strain and tested on a larger number of sheep and goats. If the results are still satisfactory, the test is repeated in different species of animals running under natural field conditions, and the vaccine is issued for general use to farmers only if this last test proves the vaccine to be absolutely safe. Such precautions are always necessary when one has to deal with new vaccine strains.