Black-quarter in South Africa: With Special Reference to Improved Methods of Inoculation.

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A.—INTRODUCTION.

Black-quarter in South Africa is an enzootic disease, usually making its appearance in sporadic form, and is of such wide distribution in all parts of the country that State control is not attempted. All the State can do, and does, is to advise stockowners regarding the best methods of combating it. The principal weapon must of course be preventive inoculation of susceptible stock with an efficient vaccine, and in this direction the State can render further assistance by placing a reliable vaccine at the disposal of farmers.

The main object of this report is to place on record the experimental work that has been done during the last three years in connexion with the discovery and preparation of an efficient black-quarter vaccine. Since no publication concerning this disease has appeared in South Africa for a number of years, the authors consider it advisable to submit a few remarks on the more general aspects of the black-quarter problem.

(1) *Historical.*

The first reference to the occurrence of black-quarter in South Africa was in the final report of the Stock Disease Commission in the Cape, published in 1877 (64). In the annual report (1883) of the Colonial Veterinary Surgeon for the Cape Colony, Hutcheon (32) mentioned a large number of districts in which the disease was prevalent. He also referred to certain infected districts in the Transkeian Territory. In Natal the disease was reported on as early as 1884 (3), and, as mentioned elsewhere in this report, black-quarter vaccine had been made use of in the Orange Free State in 1897. In 1894, Theiler (66) reported on the occurrence of black-quarter in the Transvaal and on his early attempts at preventive inoculation in 1893. A further report by Theiler appeared in 1897 (67), in which the results obtained from inoculation are discussed, and in which reference is made to the fact that the disease was very prevalent on some farms during the summer months.

(2) *Occurrence of Black-quarter.*

At the present time the disease is known to occur in all parts of South Africa, but, as in other countries, it has a regional distribution. Generally speaking, black-quarter is more prevalent in the low-lying parts of the country, such as the bushveld or coastal areas, but it must be understood clearly that it is by no means of rare occurrence even in the high veld of South Africa. There is no reason to believe that the disease in South Africa is connected with any special kind of soil, since it is of common occurrence on farms where wet, marshy places do not exist.

Owing to the fact that black-quarter is not a scheduled disease under the Stock Diseases Act, no accurate statistics concerning its distribution are available, but it is generally recognized to be of seasonal occurrence. According to the experience of farmers, the disease is at its worst during the spring or early summer months, and especially after heavy rains. As a matter of fact, this general experience of farmers has led many to believe that the disease is connected with the rapid growth of the young animals during the early summer months. From our records, it would appear that inoculation against the disease is carried out throughout the year, very little difference in the amounts of vaccine issued during the different months of the year being shown. This does not, however, indicate anything, because the time chosen for inoculation will depend on the age of calves; for instance, calves born during September to December may be inoculated when they are about six months old or older, i.e. during April to July. In this connexion it should be stated that some farmers do not undertake preventive inoculation until the calves are 9 to 12 months old. It is interesting to note that, according to Edmunds (14), black-quarter was first recognized in Southern Rhodesia in 1911, and that during 1916 to 1919 the disease spread rapidly through the country.

Further north black-quarter does not appear to be a serious stock disease. At the fifth Pan-African Conference, held at Nairobi in April, 1923, representatives from the Sudan, Nyassaland, and Tanganyika mentioned that the disease had not been recognized in those territories.

(3) *Animals Affected.*

Natural cases occur only in cattle and sheep, and in the latter only in special circumstances.

The most susceptible age of calves appears to lie between 9 and 18 months, but cases of the disease are common in cattle between the ages of six months and three years. The disease also makes its appearance now and again in younger calves (down to one month) and in older cattle (up to six and seven years). Experimentally, calves show a great variation in susceptibility, so much so that we have found them most unsatisfactory to use in our experiments. It is practically impossible to determine the minimum lethal dose of the virus that would in most cases prove fatal to calves by intramuscular injection. Usually about 3–4 c.c. of a 24-hour-old Hibler culture was required to kill the average-size calf of 9 to 12 months old, but very often much bigger doses were necessary.

Sheep contract the disease naturally by infection through wounds in the skin, such wounds occurring commonly during shearing, castration, docking, etc. In this country hides removed from cattle are commonly hung out to dry in covered sheds, in which it is the usual practice to carry out shearing operations. Not only that, but the dry hides are very often made use of to keep the sheep clean, and in this way the source of infection can be traced very easily. There being a common source of infection, it is usual in these cases for the disease to break out on a relatively large scale, many sheep showing symptoms within a few days after the operation was performed.

Only one case has so far been brought to our notice where an outbreak of black-quarter in sheep was not preceded by operative interference; this happened on a farm where the disease was extremely prevalent in young cattle, and where it could be stopped only after the use of our improved black-quarter vaccine (filtrate). After the mortality had ceased in cattle, sporadic cases made their appearance in the sheep running on the farm; the owner was obliged to carry out preventive inoculation, and soon after this was done no further losses were experienced.

Experimentally, the sheep is the most susceptible animal we have, much more so than the guinea-pig. Whereas it requires a dose of at least 1 c.c. of virulent culture (our strain 1) to kill the average-sized guinea-pig, sheep will succumb to ½ c.c. of the same virus. Moreover, generally speaking, the difference in susceptibility in sheep of the same breed has been found to be very small, although, as is the case with other animals and other infections, animals possessing well-marked natural resistance have now and again been encountered.

As will be seen later, in our experimental work sheep have been used almost exclusively.

Goats are much more resistant, at least 3 c.c. of virulent culture (strain 1) being necessary to set up a fatal attack of the disease.

Horses, rabbits, and white mice have been injected with virulent cultures, but these animals appear to be in possession of a strong natural immunity.

(4) *Method of Infection.*

This important matter still remains obscure, no definite evidence being forthcoming to prove the manner in which infection occurs under natural conditions.

We have carried out a few experiments to find out whether infection could be producd by the administration of virulent cultures per os, but obtained negative results. No attempt was made at the time to secure wounds or abrasions of the mucous membranes of the gastro-intestinal tract, but experiments along these lines will be tried.

It is common knowledge that in calves suffering from the disease it is rarely possible to demonstrate wounds or abrasions of the skin. Moreover, in our experiments it has been shown that comparatively large quantities (3 c.c. and more) of virulent material is required to produce the disease in calves by intramuscular injection. It is difficult to conceive how such a big dose of virus could enter through the skin under natural conditions, especially when it is remembered that the skin wounds are considered to be so small that they are not easily detected.

It seems to us that some unknown factors are involved concerning the nature of which one can only speculate.

As already indicated, sheep undoubtedly contract the disease through wounds in the skin, but in this case the wounds are relatively large and, moreover, the black-quarter lesion can usually be connected with the skin wounds.

(5) *The Causal Organism.*

It is not intended to give a detailed description of *B. chauvaei*, but merely to mention points of special interest, at the same time showing that we have actually to do with the classical organism.

(a) *Morphology.*—In smears taken shortly after death from the local lesion, the following forms are encountered: bacillary, end-points, rarely granular, orgonts, and spores; the latter are numerous if the smears are made after the carcass is a few hours old. In fresh cases smears made from the liver surface and from a cut surface of the spleen show bacillary forms, occurring singly or in pairs, but neither orgonts or spores are met with. Spores are found in the organs after the carcass has undergone a certain amount of decomposition. In the blood of perfectly fresh cases black-quarter bacilli occur only in small numbers, sometimes so small that one fails to detect them by microscopic examination of smears; they can, however, invariably be demonstrated by cultural methods.

In culture media probably all the forms described in the literature have been observed, including the short chains referred to by v. Hibler (31); in the few cases which have come under observation the chains were made up of up to four or five links.

(b) *Cultural Characters.*—Our black-quarter strains do not grow in broth, glucose broth, agar, or glucose-agar; they thrive best in media consisting of liver broth to which pieces of liver and brain are added. Growth was obtained on serum, but for a solid medium, glucose-blood-agar has been used in all routine work. Zeissler first recommended this medium, and we can support him whole-heartedly, especially when a medium is required for isolation and identification of the organism. A certain amount of variability in the appearance of the colonies was encountered, but these atypical forms we thought might be due to a difference in the quantity of moisture present on the surface or to a difference in the age or condition of the seed material. Furthermore, some strains were encountered that did not form typical colonies until they had been accustomed to the medium by repeated subculturing, alternating the solid with a liquid medium.

(c) *Biochemical Features.*—Owing to stress of work in other directions it has not been possible to pay a great deal of attention to this matter, but fermentation tests have been carried out in connexion with some of our strains. This was done by Mr. A. D. McEwan, who worked in the black-quarter laboratories for a few months. He obtained satisfactory results with a medium composed of fermented liver broth to which a small quantity of sterile tissue, such as liver, kidney, or brain, had been added after sterilization. On the whole, the sugar reactions corresponded with those described by previous workers, but since the tests were undertaken on only a small scale, it is not considered advisable to publish detailed results. Further work in this connexion is now in progress and it is hoped to have the results available for publication within the next few months.

(d) *Pathogenicity.*—This question is discussed fully elsewhere in this report, so that at this stage brief reference may be made only to the fact that there is great variation in the virulence of different strains of the organism. The virulence of any particular strain is highest soon after isolation from a natural case of black-quarter. Most strains are inclined to become weaker after repeated subcultivation on artificial media, but it is usually possible to restore the virulence completely by passage through animals. As a matter of fact, we make it a rule to pass our strains through guinea-pigs before they are used in any crucial tests; by doing this it is possible to obtain fairly uniform results in such experiments as immunity tests. Elsewhere in this report reference is also made to the part played by aggressins in determining the pathogenicity of black-quarter organisms.

(e) *Mode of Life.*—*B. chauvaei* is generally believed to be an ordinary facultative parasite, capable of existing and multiplying in the soil of certain localities. No experimental evidence to prove this contention or to show how long the organisms can exist outside the animal body has ever been advanced. It is admittedly a difficult question, but with the advance of our scientific knowledge attempts at its solution should not be beyond the bounds of practicability. Beyond saying that, as is the case with anthrax, the infected carcass must play a considerable rôle in the maintenance and spread of infection, we are not in a position to bring forward any information that would throw light on the matter.

B.—CONTROL OF BLACK-QUARTER.

It has already been mentioned that in this country State control in connexion with black-quarter is not carried out. The State veterinarians, by means of lectures, popular articles, etc., advise the farmers concerning the nature of the disease, the methods of spread and infection, and the best means of combating it. Special stress is laid on the necessity of disposing of the infected carcasses in a proper manner, and finally the stockowners are given full advice in regard to the methods of preventive inoculation. Owing to the fact that black-quarter is a disease which, for all practical purposes, only attacks the young animal, it can be controlled easily by means of vaccination without any great expense or hardship to the owner. The farmer has learnt from experience that all he has to do is to get his young cattle safely over the susceptible age, and this can be done by one or two or, at most, three annual inoculations, the number of inoculations depending on the age at which the first injection is made, on the degree of infection that exists on the farm, and of course on the reliability of the vaccine employed.

When one compares the disease with such a dangerous condition as anthrax, which attacks all ages and all species of farm animals, and against which, in consequence, annual inoculation has to be carried out for years on end, one begins to appreciate the simple way in which black-quarter can be controlled.

In the following pages will be found a detailed discussion of the various methods of preventive inoculation that have been, and are still being, employed against this disease.

I.—Bacterial Vaccines.

Under this heading will be discussed vaccines containing live or "attenuated" organisms. The term "attenuated" is employed here in the sense in which it is being applied by present workers, namely, that the material containing black-quarter organisms has been *reduced in virulence*. This reduction in virulence, at least as far as powder vaccines are concerned, is now accepted to mean that the numbers of organisms have been reduced, and not that the organisms have become attenuated in the correct sense. As a matter of fact, there is no reason to believe that black-quarter spores, which are employed in the bacterial vaccines, ever become attenuated.

It would serve no useful purpose to discuss in any detail the historical development of this form of vaccination against black-quarter; it might suffice to remind the reader that the first workers to practise a method of protective inoculation against the disease were Arloing, Cornevin, and Thomas (5). They tried to immunize animals by repeated injection of small quantities of virulent material, and in this they were fairly successful. At any rate they were able to work out a double method of inoculation by employing diseased muscle or muscle juice after proper "attenuation" by heat.

In 1893 Kitt (37) tried to simplify the method by introducing "single" vaccination. He also attempted attenuation by heat, but later on realized that there could be no question of true attenuation, the material employed becoming less virulent simply because a reduction in the number of virulent spores had taken place.

According to statistics, these early methods of vaccination were responsible for reducing the mortality from black-quarter to some considerable extent. That they were never really satisfactory is proved by the fact that attempts at improvement have always been in progress.

These older methods of vaccination were employed in South Africa from the very early times and have been in use until a few years ago. As long ago as 1885 black-quarter powder vaccine was imported into Natal from France by Wiltshire (75), and from 1887 attempts at the preparation of a reliable vaccine had been made at Grahamstown. From the veterinary laboratory at the latter place a vaccine was issued in 1897 (56), and according to reports by Hutcheon (32) proved to be effective and reliable. During the same year the Orange Free State obtained vaccine from Grahamstown (56), while Natal commenced the preparation of its own black-quarter vaccine during the following year (59). From 1905 Theiler prepared black-quarter vaccine in the Transvaal (58), and soon after Union of the four Colonies mentioned all activities in connexion with the preparation of this and other vaccines were concentrated at the main laboratories at Onderstepoort (57).

That the vaccine, in spite of its shortcomings, was extensively employed in the Union is proved by the fact that during the fiscal year 1919-20 the issues reached the high figure of over half a million doses.

Methods of Preparation and Testing of the Powder Vaccine.

The methods employed at the Onderstepoort Laboratories were briefly as follows:—

(a) *Collection of Virulent Material.*—Strips of diseased muscles taken from natural cases were dried, then powdered, and mixed with normal saline or sterile water for injection into susceptible animals. Originally calves were used for this purpose, but later on sheep were substituted as being equally suitable, and of course much cheaper. After the death of the animal, affected muscle was collected, cut into thin strips, and then dried in a hot-air oven, or preferably in the open air. As soon as the muscle strips were perfectly dry—and sporulation complete—they were rendered into a fine powder, which was intended for storing as virulent strains or for injection into further animals for preparation of the vaccine.

(b) *Preparation of the Vaccine.*—In the earlier days heating at 85–90° C. for twelve hours was the common practice. The vaccine was then injected into animals to test its safety; if it proved to be too virulent, reheating and retesting followed until attenuation was considered sufficient to render the vaccine safe for use in practice. According to Walker (72), the total period of heating required varied from 19 to 141 hours, the average being about 44 hours.

At a later stage a shorter period of heating at a higher temperature was tried; for instance, batch 62 vaccine was found to be suitable for issue as a single vaccine after heating in a Koch's pot for only six hours. In fact, about this time the system of reheating was completely discontinued and a standard method of attenuation adopted. To obtain a suitably attenuated vaccine for the first injection in the double method of inoculation steaming in a Koch's pot was carried out for nine hours, while for the single vaccine and second injection in the double method the period of heating was fixed at six hours.

(c) *Methods Employed for Testing the Vaccine.*—At first tests on animals were carried out only to determine the safety of the vaccine, while immunity tests were completely disregarded. Sheep were the test animals commonly employed, from one to ten doses of the newly prepared vaccine being injected. This method of testing for safety was the best one available, but could not be considered to be reliable at all times. The reason for this is that, in spite of all efforts to get the powder thoroughly mixed, there was no certainty that the organisms present would be distributed evenly throughout the mixture. To illustrate the uncertainty of the method the following examples may be given:—

(1) In April, 1918, batch 21 was issued as first of double after passing the following test:—

EXPERIMENT No. 1.

Sheep No.	Vaccine Injected.	Reaction.
10994	1 dose subcut.	Little or no swelling.
11129	1 ,, ,,	Very slight swelling, not lame.
12450	5 doses ,,	Small swelling, painful, lame.
12458	5 ,, ,,	Fairly large swelling, carries leg.
13328	10 ,, ,,	Small swelling, carries leg.
13338	10 ,, ,,	Large hot swelling, lame.

All sheep recovered and the vaccine was passed for issue.

Some time later reports were received from a few farmers complaining that the vaccine was responsible for causing the death of calves.

(2) As a result of these complaints a further animal test was carried out with a sample of vaccine actually returned by one of the farmers. These are the results:—

EXPERIMENT No. 2.

	Vaccine Injected.	Result.
Sheep No.		
14094	5 doses from tube I subcut.	Died from black-quarter.
14095	2 ,, ,, I ,,	Survived.
14096	5 ,, ,, II ,,	,,
14097	5 ,, ,, II ,,	Died from black-quarter.
Calves No.		
4081	1 dose from tube I subcut.	Survived.
4007	2 doses ,, I ,,	Died from black-quarter.
4061	1 dose ,, II ,,	,, ,,
4080	2 doses ,, II ,,	Survived (no symptoms).

They show quite clearly that some tubes of vaccine were far too virulent, a calf being killed in one case from a single dose only.

(3) A further test with another sample of the same batch of vaccine was carried out, the results being as follows:—

EXPERIMENT No. 3.

Sheep No.	Vaccine Injected.	Result.
14098	1 dose subcut.	Survived.
14099	1 " "	"
14101	5 doses "	"
14102	5 " "	"
14103	10 " "	"
14104	10 " "	"

Here it was clearly shown that the particular sample submitted to the test was not too strong. One must conclude therefore that different samples taken from the same batch of vaccine varied greatly in their virulence.

Numerous examples from experiments carried out at Onderstepoort may be given to show the unreliability of this method of preparing and testing black-quarter vaccine, but the following further ones should be sufficient:—

(1) Vaccine batch 25 was prepared in March, 1919, and tested out in sheep, with the following results:—

EXPERIMENT No. 4.

Sheep No.	Vaccine Injected.	Result.
16114	10 doses subcut.	Died.
16074	10 " "	Survived.
16154	5 " "	"
16193	5 " "	"
16092	1 dose "	"
16186	1 " "	"

One sheep receiving ten doses died from black-quarter, and hence the vaccine was considered too strong for use as a first of double for which it was intended.

(2) After reheating, a further test on sheep gave the following results:—

EXPERIMENT No. 5.

Sheep No.	Vaccine Injected.	Result.
15497	10 doses subcut.	Died.
15423	10 " "	"
15479	5 " "	"
15389	5 " "	"
15376	1 dose "	Survived.
15602	1 " "	"

These were certainly remarkable, in that, in spite of reheating, the sample tested appeared to be more virulent than the one tested before reheating was carried out. One can only conclude that further heating did not have very much effect on the organisms, and that there was again a marked difference in the virulence of the two samples that had been submitted to the test.

(3) The same vaccine was attenuated further by heating for twenty hours, and a sample tested on sheep gave satisfactory results, no mortality or severe reactions being produced. Following are the details of this test:—

EXPERIMENT No. 6.

Sheep No.	Vaccine Injected.	Result.
15421	10 doses subcut.	Slightly lame, survived.
15371	5 ,, ,,	,, ,,
15431	5 ,, ,,	No reaction, ,,
15462	2 ,, ,,	,, ,,
15491	1 dose ,,	,, ,,
15473	1 ,, ,,	,, ,,

While in the safety tests for the first of double vaccine the death of any sheep was taken as an indication that the vaccine was too strong, in the case of the second of double the vaccine was considered safe for issue when one or two 10-dose, or one 10-dose and one 5-dose, or only one 5-dose sheep died from black-quarter. In the following experiment will be seen the results, which were considered to be satisfactory for a second of double vaccine:—

EXPERIMENT No. 7.

Sheep No.	Vaccine Injected.	Result.
209	1 dose subcut.	Lived.
220	1 ,, ,,	,,
270	5 doses ,,	Died.
905	5 ,, ,,	Lived.
920	10 ,, ,,	Died.
922	10 ,, ,,	Lived.

Complaints in connexion with the immunizing properties of the vaccine had also been of rather frequent occurrence, and as long ago as 1918 it was considered necessary to make certain that *Vibrion septique* was not mistaken for *B. chauvaei* and actually used in the preparation of the vaccine. A simple method of settling this point was to include in each safety test a few guinea-pigs and a few rabbits. This meant, of course, that the rabbits which are not susceptible to black-quarter should not die in the test.

At the same time every effort was made to improve the method, particularly in regard to obtaining good sporulation in the muscle material and an even distribution of the organisms in the powder.

At a later stage it was believed that some strains of black-quarter may not immunize against others, and to overcome this difficulty strains from various parts of the country were collected and mixed for use in the preparation of our powder vaccine.

In some cases also the usual dose of 10 mg. was increased to 20 mg. In spite of these efforts to improve the vaccine, reports received from the practice at different times went to show that its immunizing effect was not always satisfactory.

It was then fully realized that every batch of vaccine should be submitted to an immunity test carried out in susceptible animals and passed out for issue only when the results of such a test proved to be satisfactory. As soon as this method of testing was put into practice it was found that black-quarter vaccine in powder form conferred very little immunity on the experimental animals. Numerous experiments to prove this point could be given, but a few of the later ones should be sufficient.

(1) EXPERIMENT No. 8. *August*, 1921.
Second of Double and Single Vaccine.

	Vaccine Subcut.	Virus Intramusc.	Result.
Sheep No.			
1950	10 doses	—	Died from vaccine.
2116	10 ,,	15 mg. (Fuls.)	,, (black-quarter).
2228	5 ,,	—	,, from vaccine.
2461	5 ,,	10 mg. (Fuls.)	,, (black-quarter).
2608	1 dose	5 ,,	,, ,,
2692	1 ,,	5 ,,	,, ,,
Calves No.			
231	5 doses	No virus given	No reaction on vaccine.
233	1 dose	,,	,, ,,
1 rabbit	3 doses	,,	Lived.
1 ,,	1 dose	,,	,,
1 guinea-pig	3 doses	,,	Died (black-quarter).
1 ,,	1 dose	,,	Lived.

The minimum lethal dose of virulent powder (Fuls. strain) for a sheep was determined as 5 mg. The results show absence of immunity, and this in spite of the fact that the vaccine was sufficiently virulent to kill a guinea-pig and a sheep injected with five doses.

(2) EXPERIMENT No. 9. *October*, 1921.
Second of Double and Single Vaccine.

Sheep No.	Vaccine, 13.10.21.	Virus Intramusc.		Result.
3854	1 dose subcut.	15 mg. (Kitchener),	27.11.21	Died from black-quarter.
3893	1 ,, ,,	10 ,, ,,	23.11.21	,, ,,
3898	5 doses ,,	15 ,, ,,	27.11.21	Survived.
3900	5 ,, ,,	12 ,, ,,	14.11.21	Died from black-quarter.
3902	10 ,, ,,	15 ,, ,,	27.11.21	,, ,,
3905	10 ,, ,,	10 ,, ,,	14.11.21	Survived.

Here the minimum lethal dose of the virulent powder for sheep was found to be between 10 and 15 mg. Practically no immunity was present in sheep that had received one dose of vaccine, while some degree of immunity could be demonstrated in those that had received five and ten doses of vaccine.

(3) Experiment No. 10. *November*, 1921.

Second of Double and Single Vaccine.

Sheep No.	Vaccine Subcut.	Virus Intramusc.	Result.
3861	10 doses, 8.11.21	7 mg. (Fuls.), 29.11.21	Survived.
3866	10 ,, ,,	15 ,, (Kitchener), ,,	,,
3896	5 ,, ,,	10 ,, ,, ,,	,,
3974	5 ,, ,,	6 ,, (Fuls.), ,,	Died from black-quarter.
3934	1 dose, ,,	10 ,, (Kitchener), ,,	Survived.
3990	1 ,, ,,	5 ,, (Fuls.), ,,	Died from black-quarter.

In this case quite an appreciable degree of immunity could be demonstrated, at least one animal which had received one dose of vaccine being protected against one minimum lethal dose of virus. As a matter of fact from our extensive experience with powder vaccine we could safely say that this represents about the highest degree of immunity that could be expected after the use of this form of vaccine.

(4) Experiment No. 11. *January*, 1923.

Single and Second of Double Vaccine.

Sheep No.	Vaccine, 30.1.23.	Virus, 27.2.23.	Result.
4995	20 doses subcut.	½ c.c. v. cult. intramusc.	Lived.
5498	20 ,, ,,	10 mg. v .musc. pd. ,,	,,
5301	10 ,, ,,	½ c.c. v. cult. ,,	Died (black-quarter).
5065	10 ,, ,,	10 mg. v. musc. pd. ,,	Lived.
5300	2 ,, ,,	½ c.c. v. cult. ,,	Died (black-quarter).
5377	2 ,, ,,	10 mg. v. musc. pd. ,,	Lived.
5264	Controls	½ c.c. v. cult. intramusc.	Died (black-quarter).
5030		½ c.c. v. cult. ,,	,, ,,
4966		10 mg. v. musc. pd. ,,	,, ,,

In this case animals receiving the bigger dose (20 c.c.) of vaccine were protected against one minimum lethal dose of virus, while those that were injected with smaller doses (10 and 2 c.c.) had sufficient immunity to withstand the virus in only two out of four cases.

Attempts at Improvement by Other Workers.

Numerous attempts at improving this method of vaccination have been made by workers all over the world, but only a few of these need be mentioned here. Zschokke in 1917 (79) referred to the advantages connected with the use of muscle juice instead of the muscle itself, but this would be ruled out on account of expense. To obtain a very fine powder the use of sets of sieves with various meshes was recommended. Straining through muslin was tried and even ready-made emulsions in closed glass tubes were sold, but none of these methods proved to be satisfactory.

To make certain that each animal receives the correct dose, the vaccine has been put up in pellet form, so-called Blacklegoids. We submitted several of these preparations to animal tests, and found them far from satisfactory, as the following experiment will show:—

EXPERIMENT No. 12. *July*, 1924.

Test of Blacklegoids (Imported).

Sheep No.	Vaccine Subcut.	Virus intramusc.	Result.
6276	1 dose (single), 8.7.24	1 c.c. of v. cult. I, 29.7.24	Lived.
6486	"	1 " " "	Died (black-quarter).
6490	1 dose (double), 8.7.24 and 21.7.24	1 " " 7.8.24	" "
6553	"	1·5 " " "	" "
8723	Controls for single vaccine	0·7 c.c. of v. cult. I, 29.7.24	Died (black-quarter).
8733		0·7 " " "	" "
8782	Controls for double vaccine	1 c.c. of v. cult. I, 7.8.24	Died (black-quarter).
8792		1·5 " " "	" "

As will be seen, very little immunity could be demonstrated. At the same time the vaccine was shown to be grossly contaminated with other organisms, such as cocci, *B. welchii* group, etc. It may also be stated that from the single vaccine no growth could be obtained, so that it is doubtful whether the one animal which survived the test was really protected by the vaccine. We are inclined to think that it was one of the rare cases met with in sheep showing a strong natural resistance against black-quarter.

It may be mentioned that we prepared artificial aggressin from the black-quarter organisms isolated from the second of double blacklegoids and that this aggressin conferred a strong immunity on sheep. Unsatisfactory results obtained from tests carried out with pellets are also reported on by Haslam and Franklin (1920) (29), but they worked with so-called pseudo-blackleg pellets.

Other Bacterial Vaccines.

Apart from the infected muscle used in the preparation of powder and similar vaccines, other material containing black-quarter spores has been tried to obtain a reliable vaccine. A few of these methods may be mentioned:—

Spores and "toxins" have been separated from the muscle and coagulated albumen; a liquid vaccine was obtained by adding hypotone NaCl solution after heating. This form of vaccine proved to be too dangerous.

As long ago as 1889 Kitasato (36) tried artificial cultures grown for fourteen days and then heated for half an hour at 80° C.

In 1913, Leclainche and Vallée (42) tried 5-8 days old cultures after they had been heated for two hours at 70° C. These methods are, however, attended with a great deal of risk.

In 1900, Thomas (68) tried vaccination with silk threads impregnated with spores obtained from frogs.

In 1911, Foth (17) reported on the preparation of a thread vaccine, the threads being impregnated with a white amorphic powder obtained from precipitating a bouillon culture with alcohol.

All these methods are obviously very unsatisfactory and have not been paid any serious attention at our laboratories. A few experiments were made in connexion with the method referred to by Leclainche and Vallée, but the results were disappointing.

Discussion on Bacterial Vaccines.

The idea underlying all methods of protective inoculation against black-quarter with vaccines containing the causal organisms has been that the organisms themselves are responsible for the production of immunity; it was generally believed that a reaction set up by bacterial action in the animal body had to take place and that as a result of this anti-bodies were produced. In other words, the bacilli or spores were regarded as the essential part of the vaccine, thus leading us to conclude that we had to deal with an anti-bacterial immunity.

This idea had taken root so deeply that when the principle of immunizing against black-quarter with germ-free preparations was first mentioned, it met with vigorous protests from different quarters. Even Foth, who in 1911 (17) had suggested that guinea-pigs could be immunized with germ-free material, came forward in 1922 (18) with the contention that with the ordinary methods of filtering it was practically impossible to obtain germ-free filtrates from black-quarter cultures. He had come to this conclusion after having experimented with germ-free filtrates obtained from different sources, in some of which he could demonstrate the presence of black-quarter organisms by the injection of comparatively large quantities into guinea-pigs. As late as 1924, Foth (19) expressed the same views at a meeting of the German Biological and Medical Society held at Innsbruck. He then stated clearly his belief that even with our modern technique it was quite impossible to prepare an absolutely germ-free filtrate from black-quarter cultures. He was of the opinion that some organisms pass even the finest of filters and that, chiefly owing to their small numbers, their presence in the filtrate could be demonstrated only by guinea-pig inoculation.

As far as our filtrates are concerned, we have no hesitation in saying that they are *entirely germ-free* and that Foth's arguments, therefore, fall to the ground.

As far as the anti-bacterial immunity is concerned, it should be pointed out that it has been proved experimentally over and again that the black-quarter organisms by themselves are not capable either of killing animals or of conferring immunity on them. Most of this work has been done in America, but the following experiment carried out by the authors will serve to throw further light on the subject:—

EXPERIMENT No. 13.

Guinea-pig No.	Original Culture.	Washed Culture.	Black-quarter Culture Filtrate.	Result.
1	—	3 c.c. intramusc.	—	No reaction.
2	—	5 c.c. ,,	—	Very slight reaction.
3	—	3 c.c. intramusc.	3 c.c. intramusc.	Fairly marked reaction
4	—	5 c.c.	5 c.c. ,,	Died within 48 hours.
5	2 c.c. int. musc.	—	—	Died within 24 hours.
6	3 c.c. ,,	—	—	,, ,,

The washed cultures were prepared by centrifugalizing the original emulsion three times, the supernatant liquid being pipetted off each time and the original volume of fluid restored by adding normal saline.

The results show clearly that the organisms suspended in normal saline were not pathogenic to guinea-pigs, whereas the addition of filtrate (aggressin) served to restore their virulence to some extent. Filtrate (aggressin) is, however, not the only substance that may be used to restore the virulence of washed black-quarter spores; v. Hibler in 1908 (31) ascribed the same effects to lactic acid and foreign substances in general, such as sterile sand, ground glass, etc. We used glycerine in our experiments, one of which may be given here:—

EXPERIMENT No. 14.

Guinea-pig No.	Injected Intramusc.	Result.
1 } Controls	1 c.c. of original culture	Died from black-quarter.
2 } Controls	2 c.c. ,, ,,	,, ,,
3 } Controls	3 c.c. ,, ,,	,, ,,
4 } Controls	2 c.c. of cult. washed three times, plus normal saline	Lived.
5 } Controls	4 c.c. ,, ,, ,,	,,
6 } Controls	6 c.c. ,, ,, ,,	,,
7 } Controls	8 c.c. ,, ,, ,,	,,
8............	2 c.c. of washed cult., plus normal saline, plus 60 % glycerine	Died from black-quarter.
9............	4 c.c. ,, ,, ,,	,, ,,
10............	6 c.c. ,, ,, ,,	,, ,,
11............	8 c.c. ,, ,, ,,	Died; no symptoms of black-quarter.
12 } Controls	2 c.c. of sterile glycerine	Lived.
13 } Controls	5 c.c. ,, ,,	,,

This shows clearly that the same effects can be produced by the addition of only a small quantity of glycerine (1.2 c.c.). In these cases it is believed that infection by the black-quarter organisms is favoured by mechanical injury to the tissues; that a considerable amount of injury or irritation is set up by the injection of glycerine is quite certain, since such small quantities as 1 or 2 c.c. injected subcutaneously into mice generally prove fatal within a few hours. To show that the organisms contained in a bacterial vaccine are not responsible for conferring immunity on animals, but that the immunity must be ascribed to a bacterial product, an ordinary powder vaccine was taken, made into an emulsion which was then filtered, and the filtrate injected into animals. The following two experiments show the results of our tests with the original powder vaccine and the filtrate obtained from the powder:—

(1) EXPERIMENT No. 15. *March*, 1924.
Single and Second of Double Powder Vaccine.

Sheep No.	Vaccine, 10.3.24.	Virus, 24·3·24.	Result.
6848	1 dose subcut.	0·7 c.c. vir. cult. intramusc.	Died (black-quarter).
6608	1 ,, ,,	1 c.c. ,, ,,	Lived.
6519	5 doses ,,	1 c.c. ,, ,,	Died (black-quarter).
6781	10 ,, ,,	1 c.c. ,, ,,	Lived.
6547	Controls	0·5 c.c. vir. cult. intramusc.	Died (black-quarter).
6562		0·7 c.c. ,, ,,	Lived.
6636		1 c.c. ,, ,,	,,

(2) EXPERIMENT No. 16. *July*, 1924.
Filtered Extract from Powder Vaccine.

Sheep No.	Vaccine Subcut., 2.7.24.	Virus Intramusc., 15.1.24.	Result.
6755	2 c.c. of filt. extract	0·7 c.c. of virus cult. I	Lived.
6875	10 c.c. ,, ,,	0·7 c.c. ,, ,,	Died (black-quarter).
7392	20 c.c. ,, ,,	0·7 c.c. ,, ,,	Lived.
7509	20 c.c. ,, ,,	1 c.c. ,, ,,	Died (black-quarter).
8683	Controls	0·7 c.c. of virus cult. I	Died (black-quarter).
7388		0·7 c.c. ,, ,,	,, ,,

When comparing the results obtained from these two experiments, one is struck by the fact that in both cases a certain amount of immunity had been produced, and further that the difference in the degree of immunity that had been conferred was not very marked. In any case it is proved very definitely that the immunizing substance is something that is water-soluble and filterable.

All these considerations bring us to the conclusion that the principle on which the preparation of bacterial vaccines against black-quarter has been based must be regarded as wrong. The immunity which is conferred on animals by the use of vaccines containing living organisms is due, not to bacterial action, but to the presence of a bacterial product which is water-soluble and filterable, and which is generally referred to as "aggressin." There appears to be no doubt whatever that the little immunity that has been obtained after the use of bacterial vaccine has been due simply to the presence of aggressin. When luck would have it that a fairly large amount of aggressin was present in the powder or other bacterial vaccine, good results were obtained, whereas the reverse was the case when very little aggressin happened to be present. Since the bacterial vaccines are based on wrong principles, they may be ruled out of court for this reason alone. But there are many other weighty arguments that can be brought up against this class of vaccine. The main qualities of a good vaccine are:—

(1) *Its safety.*—The results of experiments given in this paper show clearly that the powder form of vaccine cannot be considered

safe in all circumstances; as a matter of fact, no vaccine can be considered quite safe so long as it contains fully virulent organisms.

(2) *Its efficacy.*—Because bacterial vaccines contain fully virulent organisms, it follows that the dosage must always be on the small side, otherwise the safety of the vaccine will be sacrificed. This being so, and because the immunizing properties of the vaccine are undoubtedly due to the presence of aggressin, it is not easy to prepare a bacterial vaccine with the maximum of immunizing properties. To obtain this desirable result more than a trace of immunizing substance or aggressin must be injected into the animal, and relatively large quantities of aggressin can be used only in the absence of the dangerous virulent black-quarter organisms.

(3) The ease and correctness with which it can be administered. Even when the greatest care is taken it is practically impossible to make certain that each animal receives the correct dose, when one has to deal with the powder vaccine. The organisms are not evenly distributed in the powder and when an emulsion is made in water it is extremely difficult to get an even suspension of the muscle particles. Under South African conditions the position becomes worse, because the farmer himself usually carries out the inoculation; when it is difficult for the experienced veterinarian to apply the correct dosage, it can readily be understood what is likely to happen if the farmer has to carry out the operation.

Practically the same remarks apply to other black-quarter vaccines which contain living organisms; it is as difficult or impossible in their case to obtain an even suspension of the organisms.

Conclusions.

(1) Powder vaccine and other vaccines containing living organisms have been of considerable assistance in the past in combating black-quarter in cattle, but may now definitely be discarded.

(2) Their preparation and application have been based on entirely wrong principles, namely, on the assumption that the organisms contained in them were directly responsible for the production of immunity in animals.

(3) The immunizing substances in a black-quarter vaccine are the so-called aggressins, which will be discussed more fully elsewhere in this report.

(4) Apart from this fact, most black-quarter vaccines containing virulent spores are either dangerous or else produce very little immunity in animals.

(5) Owing to the difficulty of carrying out correct dosage, the powder vaccine on this score alone often proves most unreliable in practice.

(6) Black-quarter organisms that have been freed of their products by thorough washing are no longer pathogenic. Their virulence may, however, be restored by the addition of small quantities of aggressin or of foreign substances, such as glycerine, etc.

II.—Immunization with Anti-serum, Sero-vaccine, and Toxin.

Passive immunity in black-quarter is considered at this stage simply because the use of hyperimmune sera is intimately bound up with the question of sero-vaccination or active immunity conferred by the action of anti-serum and vaccine injected simultaneously.

By means of experiments carried out by Kitt (37) during the period 1893-99 he was able to prove that an effective anti-serum against black-quarter could be obtained by the repeated injection of virulent muscle powder into sheep, goats, cattle, and horses. Since then the powder has been replaced by virulent culture. The use of anti-serum is of value when the disease has broken out in a herd and it is desired to put a stop to the mortality immediately. Since black-quarter is an enzootic disease, and cases most commonly occur only sporadically, the need of an anti-serum has never been felt so keenly as in some other infectious diseases, such as anthrax, hog cholera, etc. In South Africa, losses from black-quarter can be prevented entirely by timely inoculation of susceptible stock with an aggressin. For this reason there has been no necessity for putting into practice a method of conferring passive immunity on animals. For purely experimental purposes we have, however, undertaken the preparation of hyperimmune sera. That quite an efficient anti-serum can be produced against black-quarter is shown in the following experiments:—

(*a*) EXPERIMENT No. 17. *July*, 1924.

Hyperimmunization of Calf No. 825.

14. 7.24	....	20 c.c. of black-quarter filtrate subcutaneously.			
29. 7.24	....	5 c.c. of	vir. black-quarter	culture I	subcutaneously.
12. 8.24	....	10 c.c.	,,	,,	,,
28. 8.24	....	10 c.c.	,,	,,	,,
3. 9.24	....	15 c.c.	,,	,,	,,
11. 9.24	....	20 c.c.	,,	,,	,,
16. 9.24	....	40 c.c.	,,	,,	,,
1.10.24	....	80 c.c.	,,	,,	,,
11.10.24	....	150 c.c.	,,	,,	,,
21.10.24	....	200 c.c.	,,	,,	,,
31.10.24	....	250 c.c.	,,	,,	,,
13.11.24	....	600 c.c.	,,	,,	,,

Serum was taken from this calf on 3rd October, 1924, and again on 12th November, 1924. It is interesting to note that this calf was able to tolerate quite easily such an enormous amount of virulent culture as 600 c.c.

(*b*) EXPERIMENT No. 18. *November*, 1924.

To Test Anti-serum obtained from Calf 826.

Sheep No.	Anti-serum Injected.	Virulent Cult. I Injected.	Result.
9319	5 c.c. subcut., 7.11.24	1 c.c. intramusc., 11.11.24	Lived.
9392	10 c.c. ,, ,,	1·5 c.c. ,, ,,	,,
9544	Controls	0·7 c.c. intramusc., 11.11.24	Died (black-quarter).
9602		1 c.c. ,, ,,	,, ,,
8940		1 c.c. ,, ,,	,, ,,

It will be seen that the serum was quite efficient, protecting sheep against at least two minimum lethal doses of virus.

(*c*) EXPERIMENT No. 18. *December*, 1924.

To Test Anti-serum of Calf 926.

Sheep No.	Anti-serum Injected.	Virulent Cult. I Injected.	Result.
8726	5 c.c. subcut., 17.12.24	1 c.c. intramusc., 31.12.24	Died (black-quarter).
8739	5 c.c. subcut., 17.12.24	0·5 c.c. intramusc., 7.1.25	Lived.
9142	5 c.c. „ „	0·7 c.c. „ „	Died (black-quarter).
9400	5 c.c. „ „	1 c.c. „ „	„ „
9433	Controls, first lot	0·5 c.c. intramusc., 31.12.24	Died (black-quarter).
9531		0·7 c.c. „ „	„ „
9579		1 c.c. „ „	„ „
9255	Controls, second lot	0·4 c.c. intramusc., 7.1.25	Died (black-quarter).
9297		0·6 c.c. „ „	„ „
9302		0·8 c.c. „ „	„ „

In this case the immunity test was carried out at intervals of fourteen and twenty-one days respectively, after the animals received the serum.

The results show that the immunity rapidly declines and that very little is present after fourteen days. It is true that one sheep survived the test even after three weeks, but this animal may have possessed natural resistance against the disease.

In the previous chapter the conclusion was arrived at that the immunizing agent was a bacterial product (aggressin) and that the organisms themselves had very little to do with this. To find out whether the same observation held good in the case of passive immunity, filtrates were used for the production of anti-serum. The results are shown in the following experiments:—

(*a*) EXPERIMENT No. 19. *March*, 1925.

Hyperimmunization of Sheep 11010 *with Filtrate.*

17.3.25	....	50 cc.	of black-quarter filtrate intraperitoneally.
24.3.25	....	100 c.c.	„ „ „
31.3.25	....	200 c.c.	„ „ „
6.4.25	....	400 c.c.	„ „ „
15.4.25	....	500 c.c.	„ „ „
5.5.25	....	200 c.c.	„ „ „
13.5.25	....	300 c.c.	„ „ „

Serum was obtained from the sheep on 11th May, 1925, and tested as follows:—

(*b*) EXPERIMENT No. 20. *May*, 1925.
To Test Anti-serum of Sheep 11010.

Sheep No.	Anti-serum Injected.	Vir. Cult. I Injected.	Result.
10482	5 c.c. subcut., 12.5.25	1 c.c. intramusc., 13.5.25	Lived.
10517	5 c.c. ,, ,,	2 c.c. ,, ,,	,,
10669	5 c.c. ,, ,,	1 c.c. ,, ,,	,,
10682	5 c.c. ,, ,,	2 c.c. ,, ,,	,,
10929	5 c.c. normal sheep serum subcut., 12.5.25	1 c.c. intramusc., 13.5.25	Died(black-quarter), 15.5.25.
11221	5 c.c. ,, ,,	2 c.c. ,, ,,	,, ,,
11216	Controls	0·7 c.c. intramusc., 13.5.25	Died(black-quarter). 15.5.25.
11225	Controls	1 c.c. ,, ,,	,, ,,

The results show that quite an effective anti-serum can be prepared in this way, a dose of 5 c.c. protecting sheep against nearly three minimum lethal doses of virus.

Needless to say, it was ascertained by cultural and other tests that the filtrate was definitely germ-free.

Summary and Conclusions.—An efficient anti-serum against black-quarter can be obtained by hyperimmunizing animals with either virulent cultures or germ-free filtrates.

The properties of this anti-serum are similar to those of other well-known anti-sera; a typical passive immunity can be set up by its use, protection being afforded immediately, but disappearing again within two weeks.

The Use of Anti-serum Combined with Bacterial Vaccines.

The principle involved here is the same as that on which sero-vaccination in the case of some other diseases is based; the method is indicated where it is desirable to confer immediate protection on animals exposed to danger of infection. As mentioned earlier in this report, the necessity for conferring passive immunity is not so great in the case of black-quarter, which ordinarily occurs only in sporadic form.

Moreover, since perfectly safe and reliable vaccines in the form of aggressins are now available, it is unnecessary to combine serum with vaccine in order to obtain a safe method of immunization. Leclainche and Vallée were the first workers to make use of a combination of anti-serum and vaccines against black-quarter, but apparently the method has not found an extended application. To our knowledge only one vaccine of this kind has been imported into South Africa. A sample of it was obtained for experimental purposes, but the results of our experiments were rather interfered with by the fact that the makers confused *V. septique* with *B. chauvaei.* Our cultural and animal tests proved clearly that no black-quarter organisms were present, and, as a matter of fact, Wagner in 1924 (71) established the same fact after examination of a sample of similar vaccine prepared by the same manufacturers. The following experiments have been carried out in connexion with this vaccine and are perhaps worth recording:—

(1) Experiment No. 21. *October*, 1924.

To Test a Serum-culture Mixture (imported).

Sheep No.	Vaccinated, 31.10.24.	Virus Injected, 11.11.24.	Result.
8855	1·5 c.c. subcut.	0·7 c.c. vir. cult. I intramusc.	Lived.
8956	1·5 c.c. ,,	1 c.c. ,, ,,	,,
9044	1·5 c.c. ,,	1·5 c.c. ,, ,,	,,
9139	2·5 c.c. ,,	1 c.c. ,, ,,	,,
9147	2·5 c.c. ,,	1·5 c.c. ,, ,,	Died (black-quarter).
9287	2·5 c.c. ,,	2 c.c. ,, ,,	,, ,,
9544	Controls	0·7 c.c. vir. cult. I intramusc.	Died (black-quarter).
9602	Controls	1 c.c. ,, ,,	,, ,,
8940	Controls	1 c.c. ,, ,,	,, ,,

As will be seen from the results, a fairly strong protection against black-quarter was obtained, but it must be made clear that the immunity test was carried out on the eleventh day after the animals had received the vaccine. The immunity that was present could therefore quite well have been produced by the anti-serum, and not by the vaccine part of the mixture. To settle this point the following further experiment was carried out:—

(2) Experiment No. 22. *December*, 1924.

Sheep No.	Vaccine Injected, 17.12.24.	Virus Injected Intramusc.	Result.
8643	1·5 c.c. subcut.	0·5 c.c. vir. black-quarter cult. I, 7.1.25	Died.
8694	1·5 c.c. ,,	0·7 c.c. ,, ,, ,,	,,
8815	1·5 c.c. ,,	1 c.c. ,, ,, ,,	,,
9255	Controls for black-quarter	0·4 c.c. vir. black-quarter cult. I, 7.1.25	Died.
9297	Controls for black-quarter	0·6 c.c. ,, ,, ,,	,,
9302	Controls for black-quarter	0·8 c.c. ,, ,, ,,	,,
8846	1·5 c.c. subcut.	0·2 c.c. vir. *V. sept.* cult. XXVII, 13.1.25	Died.
8879	1·5 c.c. ,,	0·3 c.c. ,, ,, ,,	,,
9577	1·5 c.c. ,,	0·4 c.c. ,, ,, ,,	Lived.
9515	Controls for *V. septique.*	0·1 c.c. vir. *V. sept.* cult. XXVII, 13.1.25	Died.
9516	Controls for *V. septique.*	0·3 c.c. ,, ,, ,,	,,

In this case the virus was injected three weeks after the vaccine, at a time when it was reasonable to expect all traces of passive immunity to have disappeared. The results are striking, no immunity against black-quarter being present. Since the vaccine portion of the mixture had been proved to contain *Vibrion septique*, an immunity test was also applied against malignant oedema. In this case one sheep survived the test, showing that there may have been some protection afforded against this disease by the vaccine.

Black-quarter Toxins and Anti-toxic Immunity.

Different workers have held the opinion that active toxins can be demonstrated in black-quarter cultures. Thus, according to Leclainche and Vallée's report of 1900 (41), a strong toxin could be obtained by growing the organisms in Martin's broth, the maximum quantity being present on the fifth day, after which it would decrease rapidly. Again, in 1908, Grassberger and Schattenfroh (21) published the results of experiments carried out with toxic culture filtrates. The filtrate was injected into animals for the purpose of immunization, but proved to be too dangerous.

The same workers also prepared an anti-toxic serum in cattle, and used it in combination with toxin for immunizing purposes. They claimed good results, but apparently this method of vaccination has now been discarded.

The idea that toxins play an important part in the immunization process against black-quarter has been prevalent for some time, but now that the value of aggressins as immunizing agents is becoming definitely established, most workers no longer believe in the existence of an anti-toxic immunity.

There may be a few exceptions; for example, Kelser in 1918 (35) made the statement that there seemed to be some definite relation between the toxicity and the potency of culture filtrates. According to him, no immunizing properties could be demonstrated in non-toxic culture filtrates.

During the same year Nitta (52), in discussing culture filtrates, made no mention of their possible toxicity, but states that the injection of his filtrate was not accompanied by the least danger. Practically all workers since then speak of the harmlessness of their products. Thus, Haslam and Lamb in 1919 (27) stated that the immunizing power of black-quarter filtrates was not dependent on toxicity.

Gräub and Zschokke in 1920 (22) emphasized that their filtrate was practically non-toxic and that immunizing properties and toxicity do not run parallel. In Technical Bulletin No. 10, Kansas State Agricultural College, United States of America (34), the statement appears that black-quarter filtrates obtained from brain and liver medium are non-toxic.

The results of numerous experiments carried out by the writers have proved conclusively that black-quarter filtrates are, for all practical purposes, non-toxic. To give an example of the numerous observations that have been made, the following experiment may be recorded here:—

EXPERIMENT No. 23.

Guinea-pig No.	Black-quarter Filtrate Injected.	Result.
1	2 c.c. subcutaneously	Lived.
2	5 c.c. ,,	,,
3	10 c.c. ,,	,,
4	10 c.c. intraperitoneally	,,
5	20 c.c. ,,	,,
6	40 c.c. ,,	,,

It will be seen that even such a large quantity as 40 c.c. injected intraperitoneally could be tolerated by a guinea-pig. Naturally with such large quantities of fluid injected intraperitoneally a certain amount of physical discomfort must be expected, but no sign of a reaction to toxin could ever be observed in any of the guinea-pigs.

We may also refer back to sheep 11010 which during hyper-immunization experiments received intraperitoneally as much as 500 c.c. filtrate without any signs of a reaction to toxin being observed.

Summary and Conclusions.

(1) With the ordinary technique employed for the preparation of black-quarter culture filtrates, the authors have not been able to demonstrate the presence of any toxic substance, and are therefore unable to support the findings of some other workers who have described the presence of an active toxin in their black-quarter cultures. A few years ago some considerable difficulty was experienced in differentiating between the pathogenic anaerobes, and it seems to be just possible that some of these workers had to deal with an organism that was not *B. chauvaei*. That this is not out of question can be accepted when it is remembered that even now well-known manufacturers issue for use against black-quarter a vaccine which has been proved to contain *Vibrion septique* and no black-quarter organisms.

(2) Since no toxic substances could be demonstrated in our filtrates, it follows that we cannot accept the existence of an anti-toxic immunity, always provided of course the term toxin is used in the correct sense and is not meant to include the so-called aggressins, which are absolutely harmless and non-toxic substances.

Even in cases where toxins had been alleged to be present, no proof was advanced to show that they were responsible for the production of immunity. In order to obtain conclusive evidence it would be necessary to exclude the presence of aggressins, which are now accepted to be the immunizing substances in the case of black-quarter.

III.—Aggressins and Anti-aggressin Immunity.

The term "aggressin" was first introduced by Bail (6) to denote certain bacterial products which actively assist the invasive powers of bacteria by neutralizing the action of the defensive mechanism of the body (leucocytes, serum, etc.). This name is now generally accepted, and a great deal more has been learnt regarding the nature of these substances, as will be seen elsewhere in this report.

Aggressins are produced as the result of bacterial activity either in the animal body or in suitable culture media; in the former case the name "natural aggressin" has been applied, while in the latter the term "artificial aggressin" is commonly employed.

As long ago as 1888 Roux (60) was able to show that guinea-pigs could be immunized against black-quarter by means of filtered exudate. He could also demonstrate that filtrate obtained from broth cultures, killed by heating to 115° C., produced immunity in animals against black-quarter. Roux was therefore the first worker to employ against black-quarter vaccines which are now commonly referred to as natural and artificial aggressins. There is no reason to believe that any real differences exist between the so-called artificial and natural aggressins, but for the sake of convenience they will be discussed separately in this chapter.

(1) NATURAL AGGRESSINS.

Apart from the reference to Roux's work given above, the following further historical data are of considerable interest:—Duennhmann in 1894 (13) made the statement that injection into guinea-pigs of filtered muscle juice from affected animals conferred immunity on these animals.

Schoebl in 1910-11 (62) demonstrated that it was possible to produce immunity by means of germ-free black-quarter aggressin obtained by centrifugalizing oedematous fluid collected from guinea-pigs that had died of the disease. Similar material obtained from cattle was used successfully in the immunization of calves.

Nitta (52) confirmed Schoebl's work, but he passed the fluid through Chamberland filters. This method of vaccination has received a good deal of attention in the United States of America, as is shown by the work done during 1913-16 by Haslam and Franklin.

Zschokke (80) was the first to introduce the method into Switzerland, and in March, 1922, made the statement that the immunity conferred by aggressin lasted for at least five months, while the vaccine itself retained its immunizing properties for at least four months, after which a gradual decline was noted.

According to Tenhaeff, 1924 (65), field experiments were made in Holland during 1919, filtrate prepared from oedematous fluid and muscle juice being used, but the results were not satisfactory. In 1923 these experiments were repeated and the results obtained were much more satisfactory, only one calf out of 225 inoculated being lost.

In South Africa this method of vaccination first received attention during 1921, when M. W. Sheppard was an assistant in the vaccine laboratories. Prior to this, Meier was engaged on black-quarter work and paid attention particularly to the production of artificial aggressins. It was mainly owing to failure to obtain a reliable artificial aggressin that our efforts were directed to the special study of natural aggressins. At the same time work in connexion with artificial aggressins was not abandoned, but, on the contrary, a great deal of success was achieved.

Methods of Preparation and Testing.

The methods employed by other workers may briefly be referred to here.

It has been mentioned earlier that Schoebl adopted centrifugalization as a method of freeing his aggressin from organisms. As a preservative he used toluol. Kelser in 1918 (35) recommended that the muscle tissue be finely ground and placed with the oedematous fluid in fruit jars. To facilitate filtering these are frozen in an ice-salt mixture for several hours, after which they are inverted over funnels containing a thin layer of cotton-wool. The fluid is collected in a pan, from which it is drawn off into a flask placed in ice. Finally, the clot is pressed out and the fluid so obtained filtered last.

The vaccine is filtered twice, first through a Berkefeld filter of the V. and then through one of N. porosity. Haslam in 1920 (28) also recommended centrifugalization, which was to be carried out after the oedematous fluid had collected; after that filtering through Berkefeld or Mäudler filters is carried out.