Zschokke in 1922 (80) used the juice expressed from affected muscles, oedematous fluid, as well as fluid collected from the peritoneal cavity; in this way he was able to collect on an average about 8 litres from a calf. He added 0.5 per cent. phenol to his aggressin, which was collected in flasks. Filtering was carried out the same day, first through asbestos and then through either Berkefeld or Chamberland candles.

Our Own Methods.—Young cattle are injected intramuscularly in several places of the thick muscular masses with large quantities of virulent culture. Immediately after death the animal is taken to a specially clean room, washed, disinfected, and skinned under as aseptic conditions as possible. The oedematous fluid from the subcutaneous and intramuscular tissues is collected and strained through muslin into flasks. All the affected muscles are collected, cut into strips, and passed through a meat press. The muscle juice so obtained is added to the oedematous fluid and the mixture kept in cold storage for about twenty-four hours to allow of coagulation taking place.

During this process contamination with outside organisms is unavoidable, and this danger is particularly serious during the warm summer months. No matter what precautions are taken, one has to deal with a fluid containing not only black-quarter bacilli and spores, but also a mixture of other organisms.

To obtain a germ-free filtrate is not a simple matter when one has to deal with such a highly albuminous fluid. As a matter of fact, great difficulties were experienced at first when filtering through the ordinary Berkefeld candles was attempted. The process of filtering was extremely slow, and at the same time it was found difficult to get the candles properly cleaned after filtration.

The method of centrifugalization was then adopted, Sharples's laboratory centrifuge, driven by steam and running at 25,000 to 30,000 revolutions per minute, being used for the purpose. Large quantities of fluid could be passed through the centrifuge in a very short time, and it was found that after passing the fluid five times through this centrifuge it was practically germ-free. A few black-quarter organisms and cocci were still present, but it was hoped to prevent the multiplication of these by the addition of a fairly strong preservative agent. It was also thought that such an agent might after a little while be destructive to the few organisms present. In view of Foth's assertions, it was thought at the time that the presence of a few black-quarter organisms would rather be an advantage, and might lead to the production of a better immunizing preparation. It should be mentioned that at the time we had no special filtering apparatus to work with, and that now we have available a particularly suitable apparatus in the German Seitz filter the same difficulties would not be encountered, especially if filtration were preceded by centrifugalization.

Preservative Agents.

In order to obtain a preservative specially for aggressin prepared in this way, a large number of experiments with different preparations were carried out.

The main qualities to be looked for in this preservative agent were—

(1) it must not throw down too much deposit;
(2) it must not destroy the immunizing substances contained in the vaccine;

(3) it must with certainty prevent multiplication of the few organisms contained in the aggressin;

(4) it must have the tendency to kill off in time any organisms that are present;

(5) it must not be harmful to the animal injected with aggressin.

The bacterial-content of the aggressin was tested out by cultural methods and, where necessary, animal inoculation soon after it was made, and these tests were repeated at fixed intervals until the product was found to be absolutely germ-free.

Our observation in connexion with the different agents may be recorded briefly as follows:—

(*a*) *Glycerine* 60 *per cent.*—It may be mentioned that this concentration of glycerine is employed very successfully for the preservation of our anthrax spore vaccine.

Glycerine 60 per cent. was found to be eminently suitable for the preservation of aggressin prepared according to the method described above, for not only does it check all further growth, but it has also been found to destroy after varying periods the few organisms present. The following examples may be given:—

(1) *Aggressin Batch* 9 was found after preparation on the 13th September, 1923, to be completely sterile. Tests carried out at intervals up to 18th June, 1924, proved it to have remained sterile.

(2) *Aggressin Batch* 7 was found after preparation on the 4th July, 1923, to be infected with *B. chauvaei* and cocci. A further test carried out on the 10th August, 1923, proved it to be sterile and this sterile condition was maintained throughout until 18th June, 1924, when the last examination was carried out.

(3) *Aggressin Batch* 8 prepared on 16th July, 1923, was proved to be infected with *B. chauvaei* and cocci, but on 10th August, 1923, no black-quarter organisms could be demonstrated while cocci were still present. Finally, on 2nd January, 1924, the vaccine was shown to be completely sterile.

(4) In the case of Batch No. 4 infection remained for ten months, after which complete sterility was obtained and maintained.

(*b*) *Phenol and Glycerine* in the concentration of $\frac{1}{4}$ per cent. of the former and 25 per cent. of the latter have been found satisfactory as a preservative for blue-tongue vaccine, which, however, is drawn off from sheep under aseptic conditions.

This preparation only throws down a little precipitate, but its antiseptic properties are not sufficiently great to serve our purpose.

Tests showed that infected aggressin retained the infection for some time.

(*c*) *Phenol* was tried in concentrations of both $\frac{1}{2}$ and 1 per cent., but in both cases a considerable amount of deposit was thrown down. As far as the preservative action was concerned, 1 per cent. proved satisfactory, while $\frac{1}{2}$ per cent. did not prevent further infection of aggressin.

(*d*) *Toluol* in concentrations of ½, 1, and 2 per cent. did not prove to be satisfactory.

(*e*) *Chloroform* in 2 per cent. concentration throws down a pinkish precipitate, leaving a supernatant fluid, of a clear, faint pink colour, which shows no immunizing properties.

(*f*) *Chinosol* was tried in a concentration of 1 in 1,000; it threw down a brownish-red precipitate and did not exert a well-marked inhibitory influence on the growth of organisms.

(*g*) *Alcohol* in a 25 per cent. concentration throws down a brownish precipitate; the supernatant fluid, which has a rich red colour, passed readily through bacterial candles, but has no immunizing properties.

(*h*) *Ammonium Sulphate* was also tried, but had to be discarded owing to the fact that it proved to be extremely irritating when injected subcutaneously into guinea-pigs.

(*i*) *Chloramin T.* in dilutions of 1 in 1,000 throws down a brownish-red precipitate and has very little antiseptic value. Even concentrations of 1 in 500 failed to show a well-marked inhibitory effect on the organisms.

(*j*) *Oxy-chinoseptol* in concentrations of 0.05, 0.1, and 0.2 per cent. failed to preserve aggressin even after it had been centrifugalized six times.

(*k*) *Brillant Green* in a concentration of 1 in 1,000 might be suitable as a preservative agent, but had to be discarded on account of its toxic properties. Even 1 c.c. of aggressin containing the above agent was found to produce alarming symptoms in a guinea-pig, finally leading to extensive necrosis of the skin at the site of injection. The method of changing brilliant green into leuco-brilliant green by means of sodium hydro-sulphite, recommended by Coplans in 1922 (10), was also tried, but did not reduce the toxicity sufficiently to make the preparation safe for use in animals.

Conclusions.

Of the large number of preservative agents submitted to our tests, only one gave satisfactory results. 60 per cent. glycerine was found to be the ideal preservative for the aggressin under discussion for the following among other reasons:—

(1) It is not sufficiently irritating or toxic to render its use in animals unsafe and objectionable.
(2) It does not throw down any precipitate.
(3) It not only inhibits the growth of organisms, but also has the effect of gradually destroying them.
(4) It does not interfere with the immunizing properties, even after the vaccine has been preserved for over two years.

Immunity Tests.

Aggressin prepared in the manner described above confers a very good, lasting immunity on animals.

Small experimental lots of aggressin rendered germ-free by passing through filter candles have also been tested out, the filtrate so obtained giving the same protection as that resulting from the use of centrifugalized aggressin. In fact, no differences could be noted, nor would one expect any differences with the methods employed in their preparation. To avoid repetition no actual experiments will be recorded here, since examples of what may be expected are given in

the consideration of the keeping properties of the aggressin and of the duration of immunity.

Keeping Properties.

In this regard certain observations are considered to be of sufficient interest to be recorded here.

(*a*) Aggressin preserved in 60 per cent. glycerine and placed in cold storage has been tested at different periods; two of these tests are given, as follows:—

EXPERIMENT No. 24. *October*, 1924.

Test on Aggressin stored for 23 Months.

Sheep No.	Vaccine Injected, 20.10.24.	Vir. Cult. I Inj., 11.11.24.	Result.
8708	5 c.c. subcut.	0·7 c.c. intramusc.	Lived.
8781	5 c.c. ,,	1 c.c. ,,	,,
9614	5 c.c. ,,	0·7 c.c. ,,	,,
9627	5 c.c. ,,	1 c.c. ,,	,,
9544	Controls	1·7 c.c. intramusc.	Died (black-quarter).
9602		1 c.c. ,,	,, ,,
8940		1 c.c. ,,	,, ,,

The results show that the immunizing properties are still retained and that, in fact, quite a good immunity is still set up by such a vaccine.

EXPERIMENT No. 25. *March*, 1925.

Test on Aggressin stored for 2¼ Years.

Sheep No.	Vaccine Inj., 10.3.25.	Vir. Cult. I Inj., 24.3.24.	Result.
10993	5 c.c. subcut.	1 c.c. intramusc.	Lived.
11238	5 c.c. ,,	1 c.c. ,,	,,
11345	5 c.c. ,,	1 c.c. ,,	,,
11390	5 c.c. ,,	1 c.c. ,,	,,
11027	Controls	0·7 c.c. intramusc.	Died (black-quarter).
11139		1 c.c. ,,	,, ,,

Here again the results are striking, showing clearly that the keeping properties of the aggressin preserved in 60 per cent. glycerine are extremely good.

(*b*) To test the resistance against heat, samples of aggressins have been submitted to heating at different temperatures in a water-bath and then tested out for their immunizing value. During heating the following physical changes were observed:—At 60° C. for half an hour the aggressin remained practically unaltered, while at 70° C. it was slightly thickened, and at 80° C. it got so thick that it was injected through a hypodermic needle with difficulty. At 90° C. the aggressin was almost competely coagulated, appearing as a semi-solid mass of a brownish colour.

EXPERIMENT No. 26. *December*, 1924-*January*, 1925.

Test on Samples of Aggressin submitted to Various Temperatures.

Sheep No.	Vaccine Subcut.	Heated in Water-bath.	Vir. Cult. I, Intramusc.	Result.
8919	5 c.c., 6.1.25	60° C. for ½ hour	0·6 c.c., 20.1.25	Lived.
8962	5 c.c., „	70° C. „ ½ „	0·6 c.c., „	„
8980	5 c.c., „	80° C. „ ½ „	0·6 c.c., „	„
9554	Controls		0·4 c.c., 20.1.25	Lived.
9100			0·6 c.c., „	Died(black-quarter).
9077	5 c.c., 6.1.25	90° C. for ½ hour	0·8 c.c., 26.1.25	Died.
9100	5 c.c., „	95° C. „ ½ „	0·8 c.c., „	Lived.
9632	Controls		0·6 c.c., 26.1.25	Lived.
9628			0·8 c.c., „	Died(black-quarter)
8877	5 c.c., 17.12.24	95° C. for ½ hour	1 c.c., 31.12.24	Died(black-quarter).
8896	5 c.c., „	95° C. „ ½ „	2 c.c., „	„ „
8915	5 c.c., „	95° C. „ 1 „	1 c.c., „	„ „
8933	5 c.c., „	95° C. „ 1 „	2 c.c., „	„ „
8949	5 c.c., „	95° C. „ 2 hours	1 c.c., „	Lived.
8951	5 c.c., „	95° C. „ 2 „	2 c.c., „	Died(black-quarter
9433	Controls		0·5 c.c., 31.12. 24	Died(black-quarter).
9531			0·7 c.c., „	„ „
9579			1 c.c., „	„ „

The results show that heating op to 80° C. for half an hour does not appear to destroy the immunizing properties of the aggressin. Above this temperature, coagulation of the albuminous substances takes place to a marked extent, and the immunizing properties appear to be decidedly smaller. A relatively large amount of virus was used in the tests carried out in December, but since the vaccine heated to 90° C. failed to protect a sheep against one minimum lethal dose, we can accept that the immunizing properties had become smaller.

Duration of Immunity.—This matter is of importance, particularly since it has been claimed by some manufacturers that life-long immunity could be established after only one injection of aggressin.

Experiment No. 27.

To Test the Duration of Immunity.

Sheep No.	Vaccine Inj. Subcut.	Vir. Cult. I Inj. Intramusc.	Result.
6963	5 c.c., 2.4.24	1 c.c., 8.10.24	Lived.
7088	5 c.c., ,,	1·5 c.c., ,,	,,
7198	5 c.c., ,,	2 c.c., ,,	Died (black-quarter).
9598	Controls	0·5 c.c., 8.10.24	Lived.
9522		1 c.c., ,,	Died (black-quarter).
9174		2 c.c., ,,	,, ,,
7282	5 c.c., 2.4.24	1 c.c., 31.12.24	Lived.
7383	5 c.c., ,,	1·5 c.c., ,,	Died (black-quarter).
9433	Controls	0·5 c.c., 30.12.24	Died (black-quarter).
9531		0·7 c.c., ,,	,, ,,
9579		1 c.c., ,,	,, ,,
7659	5 c.c., 2.4.24	0·7 c.c., 10.3.25	Lived.
7743	5 c.c., ,,	1 c.c., ,,	,,
7747	5 c.c., ,,	1 c.c., ,,	,,
8954	Controls	0·7 c.c., 10.3.25	Died (black-quarter).
8958		1 c.c., ,,	,, ,,

Although some of the animals died in the tests, evidence is forthcoming that the immunity conferred on animals is a lasting one. In this case, a fairly strong immunity could still be demonstrated after over eleven months.

It seems to be possible that in many cases this immunity may be of sufficiently long duration to carry calves over the most susceptible age.

Imported Aggressins.—Quite a number of natural aggressins are imported into this country and most of these have been submitted to tests for safety and efficacy.

For the purpose of comparison with our own product, examples of tests carried out with two of the best imported aggressins are given here.

Experiment No. 28. *August*, 1923.

Test on Imported Aggressin A.

Sheep No.	Vaccine, 3.8.23.	Virul. Cult. I, 20.8.23.	Result.
3805	5 c.c. subcut.	0·5 c.c. intramusc.	Lived.
4607	5 c.c. ,,	0·5 c.c. ,,	,,
6075	5 c.c. ,,	0·5 c.c. ,,	,,
4971	5 c.c. ,,	0·5 c.c. ,,	,,
5407	5 c.c. ,,	0·5 c.c. ,,	Died (black-quarter).
4908	5 c.c. ,,	0·5 c.c. ,,	Lived.
6474	Controls	0·5 c.c. intramusc.	Died (black-quarter).
6510		0·5 c.c. ,,	,, ,,

The immunity was tested against only one minimum lethal dose of virus and five out of the six sheep survived, thus showing a fair degree of immunity to be present.

EXPERIMENT No. 29. *April*, 1924.

To Test Imported Aggressin B.

Sheep No.	Vaccine, 2.4.24.	Virul. Cult. 1, 14.4.24.	Result.
6967	5 c.c. subcut.	1 c.c. intramusc.	Lived.
6982	5 c.c. ,,	1 c.c. ,,	,,
7030	5 c.c. ,,	1 c.c. ,,	,,
7679	5 c.c. ,,	1 c.c. ,,	,,
6933	Controls	1 c.c. intramusc.	Died (black-quarter).
6935	Controls	1 c.c. ,,	,, ,,

It may be stated that this test was carried out a few months after the date of expiration given on the label of the bottle. The results of the test show, however, that its immunizing properties were still retained.

EXPERIMENT No. 30. *April*, 1924.

To Test Duration of Immunity conferred by Imported Vaccine B.

Sheep No.	Vaccine Inj. Subcut.	Vir. Cult.-I, Intramusc.	Result.
6198	5 c.c., 2.4.24	1 c.c., 8.10.24	Died (black-quarter).
6497	5 c.c., ,,	1·5 c.c., ,,	,, ,,
6501	5 c.c., ,,	2 c.c., ,,	,, ,,
9598	Controls	0·5 c.c., 8.10.24	Lived.
9174	Controls	1 c.c., ,,	Died (black-quarter).
9522	Controls	2 c.c., ,,	,, ,,
6956	5 c.c., 2.4.24	0·7 c.c., 11.11.24	Lived.
7060	5 c.c., ,,	1 c.c., ,,	Died (black-quarter).
9544	Controls	0·7 c.c., 11.11.24	Died.
9602	Controls	1 c.c., ,,	,,
8940	Controls	1 c.c., ,,	,,

These results are certainly suprising and difficult to explain. The same vaccine was used as in the previous experiment and the inoculations were made on the same day.

Apparently very little or no immunity was present after six months.

Discussion and Conclusions.

(1) The results of experiments carried out by the authors go to confirm those of other workers that germ-free natural aggressins confer a strong, lasting immunity on animals.

(2) By the methods described here, namely, centrifugalization with a special machine and the addition of 60 per cent. glycerine, a sterile aggressin can be obtained.

(3) Vaccines prepared in this way have been shown to retain their immunizing properties for at least 2¼ years.

(4) The aggressin can stand heating to 80° C. in a water-bath for half an hour without the immunizing properties being interfered with.

(5) Sheep injected with our aggressin still showed immunity when tested eleven months later. These animals had in the meantime not been exposed to natural infection.

(6) The main disadvantages connected with this method of vaccination are the suffering inflicted on animals, its costliness, and the extra work entailed in its preparation. The points referred to hardly require any further explanation; calves are used in the manufacture of the vaccine, and it is quite common for them to go through an acute attack of black-quarter lasting for many hours, during which they must suffer terrible agony; the period of suffering cannot be shortened and they must be allowed to die a natural death, otherwise the production of aggressin is interfered with considerably. According to Zschokke (80), an average of 8 litres aggressin could be obtained from a single calf, but in our experience the quantity was much smaller; this alone makes the vaccine expensive. Even the simplest technique that could be employed would involve a great deal of labour, and this further increases the cost of production. If this aggressin were greatly superior to any other form of black-quarter vaccine, the extra cost of production would be justifiable, but the authors are of opinion that an equally efficient vaccine could be produced in a much simpler and cheaper manner. The vaccine referred to is the so-called artificial aggressin to be discussed next.

(2) Artificial Aggressin.

Roux's work in connexion with filtrates obtained from black-quarter cultures has already been referred to. The following further reference to the literature will be found to have some bearing on the subject now under discussion:—

Saintefebré in 1893 (61) made the statement that he had been able to immunize against black-quarter by the subcutaneous injection of filtered broth cultures.

In 1911, Foth (17) reported that it was possible to obtain germ-free filtrates by repeated filtering of virulent cultures through bacterial filter candles, and that with an alcoholic precipitate of this filtrate guinea-pigs could be immunized against a lethal dose of virulent material. We have already referred to the fact that Foth now holds other views.

In discussing Nitta's and Eichhorn's filtrates in 1918, Kelser (35) suggested that they were more or less unstable, and therefore not likely to withstand such outside influences as heat, light, air, etc.; he further contended that a considerable variation in potency was found to be present in the case of the different media used for their preparation.

Gräub and Zschokke (22) prepared black-quarter filtrates in Switzerland and in 1920 their first report on the results of this work was published. They were able to immunize cattle against two minimum lethal doses, and stated that this immunity could be increased by subsequent injection of attenuated material which, if

used by itself, conferred only a weak and unsafe protection. With this combined method they were able to immunize guinea-pigs against five minimum lethal doses of virus.

In 1921, Gräub (23) published the results of field tests carried out during the previous year, and according to these only 3 out of 4,800 inoculated were lost.

Weissenrieder in 1921 (73) reported that out of 22,089 cattle inoculated in Kanton Bern, twenty-four deaths from black-quarter took place, while the losses in St. Gallen and Glarus amounted to only 11 out of 9,558 inoculated.

In 1922 the veterinary authorities in Switzerland (16) mentioned the loss of only 2 out of 3,065 cattle that were inoculated.

In 1924, Gräub (24) brought out a further report which showed that of 89,060 cattle vaccinated during 1922 and 1923, deaths from black-quarter amounted to only 87. Gräub recommended a dose of 2 c.c., since he was not able to see an appreciable difference in the degree of immunity resulting from this and the dose of 5 c.c. usually employed.

In Holland (65) a filtrate was prepared from equal parts of black-quarter cultures and muscle juice; out of 403 calves vaccinated with this material, the loss of only one could be recorded.

In 1922 (45) black-quarter filtrate was also made use of in Dutch Indies.

As mentioned earlier, the first attempts at preparing an efficient black-quarter vaccine of this type in South Africa were made by Meier, who followed the method of Gräub and Zschokke. The filtrate so obtained produced only a very weak immunity and, as stated earlier, these poor results led to special attention being paid to the study of natural aggressins. Work in connexion with artificial aggressin was, however, continued by Viljoen and Sheppard, who, following the technique suggested by Nitta, were able to bring out a considerably improved product which was issued for use in practice from 1922 onwards. Since then further efforts at improvement have been continued by the authors; that these efforts have been crowned with a considerable measure of success will be seen in the following pages.

Methods of Preparation and Testing.

The following references to previous work may be given:—

The reports of Roux in 1888 and Leclainche and Vallée in 1900 have already been referred to.

Grassberger and Schattenfroh in 1908 (21) used media containing a high percentage of chalk and carried out filtration through a layer of semi-solid chalk.

Kelser (1918) (35) reported that the best results were obtained from the use of a modification of Martin's peptone broth medium.

Nitta (1918) (52) used for his cultures tubes or flasks having a capacity of ½-1 litre. Into each flask or tube were placed small pieces of lean beef or calf's liver, sufficient to form a layer a few inches thick; ordinary broth was then added, the vessels plugged with cotton wool and sterilized in steam. A large loop full of black-quarter exudate or culture was used to inoculate each flask, which was then incubated at 37° C. for five to ten days. The filtrate was preserved by the addition of toluol.

Gräub and Zschokke (1920) (22) used broth to which organic substances had been added, according to the method of Tarozzi.

For filtering Chamberland candles were made use of. In Technical Bulletin No. 10 issued by the Kansas State Agricultural College (34) it is stated that the organisms are grown in brain-liver medium for nine days, the liquid removed, phenolized, and then filtered. Luitjens (1922) (45) grew his cultures in liver broth for three weeks, passed the liquid through sterile gauze, and then submitted it to centrifugalization; this was done to avoid getting the filter candles blocked; filtration was then carried out with coarse Berkefeld and fine Chamberland candles after 5 per cent. phenol had been added.

Berg in 1923 (8) strongly recommended " Thirty days blackleg filtrate." To obtain such a filtrate a special medium was employed, differing from the usual media in the following respects:—

(1) Sterilization is carried out by filtration (and not by heat) so that none of the nutrient material would be destroyed or the P.H. altered in any way.

(2) The constituents of the medium are selected in the light of the best available data on the food and mineral requirements of bacteria in general and the blackleg bacillus in particular. No details of these constituents were given. Berg claimed that a filtrate made from a seven days' culture would protect calves against enormous doses of blackleg virus. To obtain the maximum protective value the cultures must be allowed to grow for thirty days or longer.

Lourence and Te Hennepe, according to their report of 1924 (44), prepared their filtrate from cultures grown in ordinary broth containing pieces of meat or liver.

Tenhaeff (1924) (65), in referring to these cultures, mentioned that they were grown for eight days and three weeks.

Allen and Bosworth (1924) (1) employed as medium a tryptic-digest broth to which fresh minced muscle had been added, the final P.H. being approximately 7.8. Each bottle was inoculated with 30 c.c. of a 24-48 hours' broth culture and at the same time sufficient sterile glucose solution was added to give a concentration of 0.2 per cent. A very active growth resulted and was allowed to go on for five days, when filtering through Berkefeld candles was carried out. To stimulate the production of a higher degree of immunity, double vaccination at an interval of fourteen days was recommended.

Berg (8) made a further statement in 1924, when he claimed that prolonged anaerobic growth could be obtained by utilizing media containing 12 per cent. dextrose; in the case of black-quarter vigorous growth continued for thirty days and longer. The composition of the medium was given as follows:—

Water	7,000 c.c.
Liver (nog)	2,500 grm.
Peptone	80 grm.
Ringer salt	40 grm.
Chalk...	350 grm.
Dextrose...	1,000 grm.

These quantities were intended for a 12-litre flask. Special stress is laid on the fact that the liver must be fresh, not more than twenty-four hours old. Sterilization was carried out at 15 lb. pressure for forty-five minutes, which was found not to be injurious to the medium. It is claimed that the medium keeps well and that no readjustment of the P.H. was necessary.

Dalling (1924) (12) obtained the best results when the filtrate was prepared from cultures grown for four days, the immunity being such that in 80 per cent. of cases 2.5 c.c. vaccine would afford protection against at least one minimum lethal dose. A higher degree of immunity, up to 100 per cent., could be obtained by carrying out a second inoculation after an interval of fourteen days.

Our Methods.—The discussion of these may be taken up from the time (1922) Viljoen and Sheppard were able to bring about such improvements that the vaccine could be issued for use by farmers. Nitta's methods were followed, excepting for the following modifications:—

Large flasks of 10 litres capacity were employed and the ordinary bouillon was replaced by liver broth. Still later the medium was made up of boiled pieces of liver placed in the liver broth, which contained 1 per cent. glucose, 1 per cent. peptone, .3 per cent. NaCl, and 12 per cent. $NaHPO_4$. Flasks containing the medium were sterilized in the autoclave at 110-115° C. for an hour on each of three successive days. The P.H. was adjusted to 8.4 to allow for the subsequent change in reaction; this was not found to interfere with the initial growth in any way. Inoculation of the medium was done by means of a Pasteur pipette. Infection of the flasks was not uncommon and it was thought that this infection took place during the process of inoculation of the medium. To rule out infection derived from this source, a test-tube containing v. Hibler's medium was fixed in the neck of the flask, inoculated through a glass tube by means of a platinum loop or Pasteur pipette; as soon as it showed good growth the tube with its contents was pushed down into the liver broth.

Later on it was found that infection of the flasks did not occur during the process of inoculation, but was due to faulty sterilization. We therefore reverted to the use of the Pasteur pipette of 5-10 c.c. capacity, the seed material being obtained from cultures grown in v. Hibler brain medium which had been submitted to sterilization by live steam. With this method a growth considered fairly good at the time was obtained; the growth was indicated by the presence on the surface of the medium of gas bubbles varying in size up to that of a crown piece. Gas formation as an indication of growth was observed for different periods up to thirty-four days, but what we could now consider a good growth was not present. Tests carried out with filtrate prepared from these cultures showed that the immunity produced in animals was rather weak and very variable, thus confirming the experiences of Kelser, Zschokke, and others. While some batches of filtrate gave a good immunity against one minimum lethal dose of virus, with others only some of the test animals were protected against one minimum lethal dose.

Further Improvements.—It was realized that so long as the filtrate was variable in its immunizing value, it could not be considered a marked improvement on the powder vaccine previously discussed. Further attempts at improvement were, therefore, made, and the following is a short résumé of the work that has been done in this direction:—

Since quite good immunizing batches of vaccine had been obtained in some cases, it was clear that the fault lay in the technique employed, and particularly in the culture medium. To rectify the faults, numerous experiments were made.

Intermittent sterilization of the medium at 100° C. (instead of 110-115°) for an hour on each of three to four successive days brought about no real improvement; the growth obtained was slightly prolonged, but not more intensive; no better immunity could be demonstrated, while many flasks showed infection and had to be discarded.

Liver, brain, heart, spleen, and muscle obtained from different species of animals (horses, cattle, and sheep) were tried separately and in combination, but no appreciable improvement could be demonstrated.

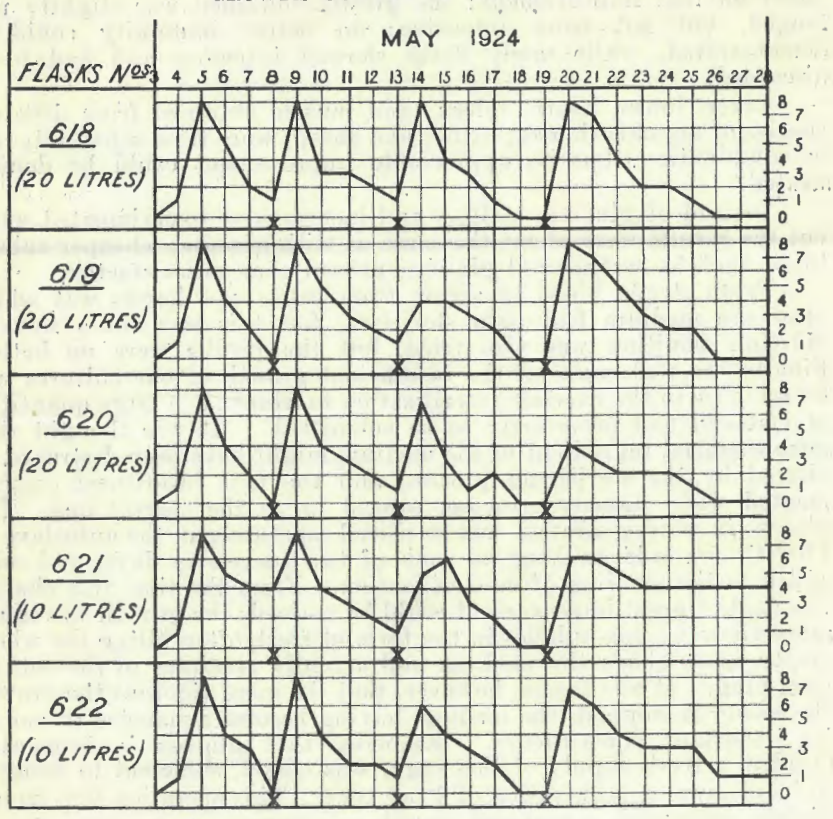
Instead of glucose, maltose and lactose were experimented with, but the results were about the same as with glucose; cheaper substitutes, such as commercial glucose, proved very unsatisfactory.

Fresh sterile blood or serum from cattle and horses was added after the medium had been sterilized, but this was not a success. Martin's bouillon was also tried, but the results were no better. Finally the real cause of the insufficient growth of our cultures was found to lie in the excessive sterilization to which such large quantities of material had necessarily to be submitted. It was thought that some essential ingredient of the medium might have been destroyed or altered by the sterilizing process, and the first constituent experimented with, namely, glucose, proved to be the correct one. The glucose in watery solution was sterilized separately in the autoclave at 116° C. for half an hour on each of two successive days, and only added to the medium after sterilization. From the time this change was made a great improvement could be noticed; the growth was much more vigorous, gas-bubbles in the form of froth often filling the whole empty space above the medium and actually reaching to the cotton-wool plug. It was found, however, that the more vigorous the growth the sooner it stopped, the medium having become exhausted of one of its important constituents. Knowing that glucose is fermented rapidly, a fresh supply of this sugar was added, sufficient to bring it to its original concentration of 1 per cent. This operation was immediately successful, a renewed growth as vigorous as, or even more so than, the initial one following very soon after. When this second period of growth has stopped, the operation may be repeated with the same good results. In fact, the cultures can be kept growing for a month or longer by repeating this simple operation.

At one time we thought that the P.H. had a great deal to do with the growth of black-quarter organisms, and, as a matter of fact, in cases where growth had ceased it was found to be as low as 5.3. Adjustment to about 8, without addition of glucose, did not, however, have the desired results.

Since the addition of glucose has to be made to growing flasks after every few days, we have made it a practice of adjusting the reaction of the medium at the same time. The important question of how long the cultures should be kept growing now arises. To answer this, many observations and experiments have been made. It is clear that the growth in each flask should be observed separately; this growth may be represented by curves, as shown in the following chart.—

GROWTH CURVES OF BATCH 62.



EXPLANATION.

All flasks injected with virulent B. O. culture 3rd May, 1924.

All flasks removed from incubator 28th May, 1924.

Medium readjusted (×) with Glucose and NaOH three times as marked.

Growth : 0—No growth ... ... No gas development.
1—Very weak growth ... ... A few small gas bubbles.
2—Weak growth ... ... Less than $\frac{1}{10}$ of surface covered by gas.
3—Rather weak growth ... ... $\frac{1}{10}$ to $\frac{1}{5}$ " " " "
4—Fair (sufficient) growth ... $\frac{1}{5}$ to $\frac{1}{2}$ " " " "
5—Fairly good growth ... ... $\frac{1}{2}$ to $\frac{3}{4}$ " " " "
6—Good growth ... ... $\frac{3}{4}$ to whole of surface covered by gas.
7—Very good growth ... ... Gas layer up to 1" thick.
8—Vigorous growth ... ... Gas layer over 1" thick.

Regarding the optimum period of growth required for the production of a good vaccine, it may be stated that in the routine method followed by us an average period of about three weeks is usually allowed; during this time the medium will have to be "readjusted" about three times.

It should be made perfectly clear, however, that the quality of the growth is perhaps even more important than the period. For instance, a few days' vigorous growth has been found of much greater value than a few weeks' weak growth. To illustrate this a few examples may be given:—

(*a*) EXPERIMENT No. 31. *July*. 1924.

Test of Vaccine Grown Four Days.

Sheep No.	Vaccinated, 15.7.24.	Vir. Cult. I, 29.7.24.	Result.
8617	5 c.c. subcut.	0·7 c.c. intramusc.	Lived.
8618	5 c.c. „	1 c.c. „	„
8224	20 c.c. „	0·7 c.c. „	„
8638	20 c.c. „	1 c.c. „	„
8723	Controls	0·7 c.c. intramusc.	Died (black-quarter).
8737		1 c.c. „	„ „

The results show a well-marked immunity to be present, sufficient to protect against more than one minimum lethal dose of virus. This happened to be an exceptionally good vaccine, but the results show clearly that after a few days' vigorous growth a good immunizing filtrate could be obtained in some cases.

(*b*) EXPERIMENT No. 32. *March*, 1923.

Test of Vaccine Grown for Twenty-three Days.

Sheep No.	Vaccinated, 26.3.23.	Vir. Cult. I Inj., 10.4.23.	Result.
5014	5 c.c. subcut.	0·5 c.c. intramusc.	Lived.
5117	5 c.c. „	0·5 c.c. „	Died (black-quarter).
5095	20 c.c. „	0·5 c.c. „	Lived.
5119	20 c.c. „	0·5 c.c. „	Died (black-quarter).
5083	Control	0·5 c.c. intramusc.	Died (black-quarter).

In this case the growth was weak throughout. The results showed some immunity to be present, but not nearly sufficient to allow the vaccine to be passed for issue.

(*c*) EXPERIMENT No. 33. *March*, 1923.

Test of Vaccine Grown for Forty-five Days.

Sheep No.	Vaccinated, 26.3.23.	Vir. Inj. Intramusc., 10.4.23.	Result.
5098	5 c.c. subcut.	0·5 c.c. vir. cult. I	Died (black-quarter).
4977	5 c.c. „	10 mg. vir. muscle powder	„ „
5057	20 c.c. „	10 mg. „ „	Lived.
5025	20 c.c. „	0·5 c.c. vir. cult. I	„
5133	Controls	0·5 c.c. vir. cult. I	Lived.
5034		10 mg. vir. muscle powder	Died (black-quarter).

This is a vaccine in which a weak growth continued for as long as forty-five days. The results obtained in the above test are much the same as were recorded in the previous experiment.

Summary of Improved Technique.

Having described the method of growing cultures intended for the preparation of artificial aggressin, we may now give a short summary of the whole process of manufacture:—

(1) About six to twelve 10-litre flasks containing the medium previously described are taken and incubated for at least forty-eight hours to make certain that no accidental infection had occurred.

(2) Inoculation of the flasks is carried out with pure cultures grown in v. Hibler's medium for twenty-four hours, by means of Pasteur pipettes. This operation is done under the flame, every precaution against contamination being taken.

(3) The flasks are now placed in the incubating-room for an average period of about three weeks, during which time the growth in each flask is observed daily and readjustment of the medium made when necessary (usually about three times).

(4) Flasks which have shown good growth are selected for the vaccine. They are first of all examined for purity (cross infection can be detected by the odour and change in colour), and if apparently pure, subcultures from each flask are made on ordinary agar slants and incubated both aerobically and anaerobically for three days. In the meantime the flasks are kept in cold storage. This cultural test is of course not absolutely reliable, but serves the purpose. Flasks which pass this test are selected for filtration, being taken out of the cold storage in the evening. The liquid is drawn off and kept at room-temperature during the night so as to allow sedimentation of suspended particles. It will be found that a considerable amount of deposit is thrown down during this time.

(5) Prefiltration or a preliminary filtration is now carried out as follows:—

The clear supernatant liquid is syphoned off from the flasks and passed through a layer of paper-mash, which is prepared by mixing small pieces of filter-paper in water contained in a bucket until a mixture of the consistence of paste is obtained. A round piece of wire-gauze is fitted into a funnel and then covered with a thin layer of cotton-wool; a layer of paper-mash, 1½-2 inches thick, is now made on top of the cotton-wool. Filtration is assisted by means of suction applied to a pressure flask. A fine deposit forming on the paper-mash is likely to retard the passage of the fluid, but this may be scraped off or, when necessary, the paper-mash has to be changed. When large quantities of vaccine have to be made, several funnels fitted out as above can be brought into operation simultaneously. The filtrate so obtained will pass readily through any bacterial filter.

(6) Final filtration: For this purpose we have found one of the larger asbestos filters, manufactured by Seitz Werke, Kreuznach, far superior to any other filtering apparatus. With this filter, fully fitted out with its eight asbestos plates, 30 litres of fluid can be filtered within half an hour. The fluid contained in a large glass vessel is placed about 3 feet higher than the Seitz filter, rubber-tubing being used to syphon off the fluid to the inlet of the filter. The outlet-pipe is connected with rubber-tubing to a sterile flask of 40 litres capacity.

Once the inlet-tubing is filled the liquid will by gravitation fill the chambers of the filter and pass through the asbestos plates to the outlet-pipe.

(7) Test for sterility: From 30 to 50 c.c. of the filtrate are syphoned off, under aseptic conditions, into several tubes containing v. Hibler's brain medium. These are incubated for three days, when, if no growth is observed, the filtrate may be considered sterile. If by any chance organisms are detected in the filtrate, refiltration may be carried out without harming the vaccine in any way.

(8) As a preservative agent, from ¼ to ½ per cent. phenol may be added to the sterile filtrate.

(9) Animal inoculations for the purpose of determining the safety and immunizing value of the vaccine are carried out as a routine measure.

To test the safety, two sheep are each injected with one dose of vaccine, while two further sheep receive each four doses (20 c.c.). These animals receive an injection of virus about a fortnight later, and the vaccine is passed for issue only if its immunizing value is satisfactory. Details of the tests for safety and immunity will be found in the following pages.

In order to show that a real improvement has been effected in the immunizing value of our artificial aggressin, it is necessary to give a few examples of tests carried out with some of the earlier products.

Immunity Tests.

EXPERIMENT No. 34. *February*, 1923.

To Test the Safety and Efficacy of Vaccine No. 10.

Sheep No.	Vaccinated, 26.2.23.	Vir. Cult. I, 13.3.23.	Result.
5020	5 c.c. subcut.	0·5 c.c. intramusc.	Died (black-quarter).
5089	5 c.c. ,,	0·5 c.c. ,,	Lived.
4980	20 c.c. ,,	0·5 c.c. ,,	,,
2281	20 c.c. ,,	0·5 c.c. ,,	Died (black-quarter).
5083	Control	0·5 c.c. ,,	Died (black-quarter).

The results show that only two out of the four sheep were protected against one minimum lethal dose of virus, and incidentally that the immunity derived from 20 c.c. was no better than that which followed after the injection of 5 c.c.

EXPERIMENT No. 35. *May*, 1923.

To Test the Safety and Efficacy of Vaccine No. 17.

Sheep No.	Vaccinated, 15.5.23.	Vir. Musc. Powder Inj., 28.5.23.	Result.
4982	5 c.c. subcut.	15 mg. intramusc.	Lived.
5044	5 c.c. ,,	15 mg. ,,	,,
5006	20 c.c. ,,	15 mg. ,,	,,
5066	20 c.c. ,,	15 mg. ,,	,,
5332	Controls	15 mg. intramusc.	Died (black-quarter).
5046	Controls	15 mg. ,,	,, ,,

In this case a more reliable immunity was obtained, all four sheep being protected against one minimum lethal dose of virulent black-quarter powder.

After the improved methods of preparation described in this paper had been adopted, no further losses in the immunity test with one to one and a half minimum lethal dose of virus were experienced. Consequently the amount of virus used in these tests was gradually increased, as will be seen in the following further experiments:—

EXPERIMENT No. 36. *June*, 1924.

To Test the Safety and Efficacy of Vaccine No. 62.

Sheep No.	Vaccine Inj., 9.6.24.	Vir. Cult. I Inj., 24.6.24.	Result.
7100	5 c.c. subcut.	1 c.c. intramusc.	Lived.
7301	5 c.c. „	2 c.c. „	„
7316	20 c.c. „	1 c.c. „	„
7742	20 c.c. „	2 c.c. „	„
8598	Controls	1 c.c. intramusc.	Died (black-quarter).
8600	Controls	0·7 c.c. „	„ „

In this case a fairly strong immunity could be demonstrated, all four sheep surviving the test, even when about three minimum lethal doses were employed.

EXPERIMENT No. 37. *July*, 1924.

To Test Safety and Efficacy of Vaccine Batch No. 64.

Sheep No.	Vaccinated, 15.7.24.	Immunity Test, 29.7.24.	Result.
6350	5 c.c. subcut.	1 c.c. vir. cult. I, intramusc.	Lived.
6543	5 c.c. „	2 c.c. „ „	„
6812	20 c.c. „	3 c.c. „ „	„
7350	20 c.c. „	5 c.c. „ „	„
8723	Controls	0·7 c.c. vir. cult. I, intramusc.	Died (black-quarter).
8737	Controls	1 c.c. „ „	„ „

The results of this test show the immunizing value of the vaccine to be very high, all four sheep surviving in spite of the fact that the quantity of virus was increased to seven minimum lethal doses.

By this time it was realized that a vaccine of high protective value had been obtained. Experience in the field at the same time had shown that vaccines which protected sheep with certainty against at least one minimum lethal dose of virus were quite satisfactory. In this connexion it must be pointed out that satisfactory results had been claimed for a number of years from the use of the powder vaccine, which in our experience practically never reached this standard of efficacy. In order to satisfy ourselves completely concerning the high state of efficiency reached by the vaccine, it was decided to determine

the highest degree of protection afforded by the filtrate. The following are the details of the test carried out in connexion with two separate batches:—

EXPERIMENT No. 38. *July and September*, 1924.
To Test Highest Amount of Protection.

Sheep No.	Vaccinated Subcut.	Vir. Cult. I, Intramusc.	Result.
7493	5 c.c., batch 72, 15.7.24	2 c.c., 29.7.24	Lived.
8703	5 c.c., „ „	5 c.c., „	„
8734	5 c.c., „ „	10 c.c., „	„
8735	5 c.c., „ „	20 c.c., „	„
8723	Controls	0·7 c.c., 29.7.24	Died (black-quarter).
8737		0·7 c.c., „	„ „
9498	5 c.c., batch 65., 1.9.24	2 c.c., 16.9.24	Lived.
9508	5 c.c., „ „	5 c.c., „	„
9518	5 c.c., „ „	10 c.c., „	„
9591	5 c.c., „ „	20 c.c., „	„
9600	5 c.c., „ „	30 c.c., „	„
9607	5 c.c., „ „	50 c.c., „	Died (black-quarter).
9523	Controls	0·7 c.c., 16.9.24	Lived.
9557		1 c.c., „	Died (black-quarter).

The results were certainly surprising, since we did not think it possible for such a high degree of immunity to be conferred on animals by only one injection of vaccine. In the case of both batches it will be noted that the ordinary dose of vaccine protected sheep against up to thirty minimum lethal doses of black-quarter virus. Stress must, however, be laid on the fact that these were exceptionally good batches of vaccine, and that even when the same technique is employed it is not always possible to reach the same high standard of efficiency. As a matter of fact, in the routine preparation of vaccine several batches have been encountered where all the sheep were not protected against two minimum lethal doses and more of virus. In these cases optimum growth was probably not obtained in all flasks, with the result that the immunizing value of the vaccine suffered to some extent. Examples of two such vaccine batches are given here:—

(*a*) EXPERIMENT No. 39. *March*, 1925.
Test of Vaccine Batch No. 82.

Sheep No.	Vaccinated, 10.3.25.	Vir. Cult. I, 24.3.25.	Result.
10587	2 c.c. subcut.	1 c.c. intramusc.	Lived.
10951	2 c.c. „	1 c.c. „	„
11401	5 c.c. „	1 c.c. „	„
11406	5 c.c. „	1 c.c. „	„
10619	20 c.c. „	2 c.c. „	Died (black-quarter).
10968	20 c.c. „	2 c.c. „	Lived.
11027	Controls	0·7 c.c. intramusc.	Lived.
11139		1 c.c. „	Died (black-quarter).

One out of the six sheep died in the immunity test when at least two minimum lethal doses were used. This sheep also suffered from pneumonia at the time, so that probably this was partly responsible for its death.

It will be noted that two sheep received only 2 c.c. of vaccine. This was to find out whether there was any marked difference in the degree of immunity resulting from the injection of different quantities of vaccine. The results show that at least as much protection was derived from 2 c.c. as from 5 or 20 c.c., thus confirming Gräub's statement previously referred to.

(*b*) Experiment No. 40. *April*, 1925.

To Test Vaccine Batch No. 85.

Sheep No.	Vaccinated. 15.4.25.	Vir. Cult. I, 28.4.25.	Results.
10260	2 c.c subcut.	1 c.c. intramusc.	Lived
10746	2 c.c. ,,	2 c.c. ,,	,,
10955	5 c.c. ,,	1 c.c. ,,	,,
11446	5 c.c. ,,	2 c.c. ,,	,,
10712	20 c.c. ,,	1 c.c. ,,	,,
10937	20 c.c. ,,	2 c.c. ,,	Died (black-quarter)
10605	Controls	0·7 c.c. intramusc.	Lived.
10636	Controls	1 c.c. ,,	Died (black-quarter).

The results obtained from this test are very similar to those of the experiment just discussed. Here, also, it happened to be one of the sheep which received 20 c.c. of vaccine that died in the immunity test.

Keeping Properties.

Under this heading will be discussed the keeping properties of artificial aggressin submitted to different temperatures and stored for varying periods.

(1) The first observations to be recorded were made in connexion with vaccines kept under the following conditions:—

(*a*) In cold storage at a temperature varying between 0° C. and 2° C.

(*b*) In a cellar where the light was fairly dim and where the temperature varied from 15° C. to 27° C.

(*c*) In the incubator at a temperature of 37° C. to 38° C.

Comparative tests with vaccines kept under these conditions were made as follows:—

EXPERIMENT No. 41. *May*, 1924.

Test on Vaccines Stored for Two and a Half Months.

Sheep No.	Vaccine Injected Subcut.	Vir. Cult. I, Intramusc.	Result.
7331	5 c.c., 13.5.24 (kept in incubator)	0·7 c.c., 14.6.24	Lived.
7440	5 c.c., ,, ,,	1 c.c., ,,	Died (black-quarter).
7484	5 c.c., ,, ,,	1·4 c.c., ,,	Lived.
7519	5 c.c., 13.5.24 (kept in cellar)	1 c.c., 14.6.24	Lived.
7530	5 c.c., ,, ,,	1·4 c.c., ,,	,,
7540	5 c.c., 13.5.24 (kept in cold storage)	1 c.c., 14.6.24	Lived.
7728	5 c.c., ,, ,,	1·4 c.c., ,,	,,
8589	Controls.	0·5 c.c., 14.6.24	Died (black-quarter).
7091		0·7 c.c., ,,	Lived.
7124		0·7 c.c., ,,	Died (black-quarter).
7284		1 c.c., ,,	,, ,,

The results show that the immunizing value of the vaccine was retained in all cases, excepting that the sample kept in the incubator seemed to have decreased in value slightly.

EXPERIMENT No. 42. *August*, 1924.

Test on Vaccines Stored for Five Months Twenty Days.

Sheep No.	Vaccine Injected Subcut.	Vir. Cult. I, Intramusc.	Result.
9179	5 c.c., 20.8.24 (kept in incubator)	0 7 c.c., 2.9.24	Lived.
9202	5 c.c., ,, ,,	1 c.c., ,,	,,
9229	5 c.c., 20.8.24 (kept in cellar)	0·7 c.c., 2.9.24	Lived.
9305	5 c.c., ,, ,,	1 c.c., ,,	,,
9430	5 c.c., 20.8.24 (kept in cold storage)	0·7 c.c., 2.9.24	Lived.
9533	5 c.c., ,, ,,	1 c.c., ,,	,,
9269	Controls	0·7 c.c., 2.9.24	Died (black-quarter).
9289		1 c.c., ,,	,, ,,
9368		0·7 c.c., 2.9.24	Lived.
9463		1 c.c., ,,	Died (black-quarter).

The results show that the immunizing properties were still retained in all cases. The virulent test was not so strong as in the previous case, and this may account for the fact that no breakdown in immunity was observed in the sheep which had been injected with vaccine kept in the incubator.

EXPERIMENT No. 43. *December*, 1924.

Test on Vaccine Kept for Nine Months Twenty-two Days.

Sheep No.	Vaccine B. 49 Inj. Subcut.	Vir. Cult. I, Intramusc.	Result.
8615	5 c.c., 22.12.24 (kept in incubator)	0·6 c.c., 7.1.25	Died (black-quarter).
8636	5 c.c., ,, ,,	1 c.c., ,,	,, ,,
9203	5 c.c., 22.12.24 (kept in cellar)	0·6 c.c., 7.1.25	Died (black-quarter).
9204	5 c.c., ,, ,,	1 c.c., ,,	Lived.
9217	5 c.c., 22.12.24 (kept in cold storage)	0·6 c.c., 7.1.25	Lived.
9227	5 c.c., ,, ,,	1 c.c., ,,	,,
9255	Controls	0·4 c.c., 7.1.25	Died (black-quarter).
9297		0·6 c.c., ,,	,, ,,

In this case both sheep injected with vaccine that had been kept in the incubator died in the immunity test, while one that had been done with vaccine stored in the cellar also succumbed to one and a half minimum lethal dose of virus.

Other observations had given the indication that vaccine kept in the cellar retained its immunizing properties as well as, or even better than, that stored in the cool chamber, and hence a further test was carried out in connexion with the first named.

EXPERIMENT No. 44. *January*, 1925.

To Test Vaccine Kept in the Cellar for Eleven Months Seven Days.

Sheep No.	Vaccinated, 5.1.25.	Vir. Cult. I Inj., 20.1.25.	Result.
8806	5 c.c. subcut.	0·4 c.c. intramusc.	Lived.
8861	5 c.c. ,,	0·6 c.c. ,,	,,
9585	5 c.c. ,,	0·8 c.c. ,,	,,
9631	5 c.c. ,,	1 c.c. ,,	,,
9554	Controls	0·4 c.c. intramusc.	Lived.
9558		0·6 c.c. ,,	Died (black-quarter).

The results show clearly that artificial aggressin kept at a reasonably low temperature would retain its immunizing properties for at least eleven months.

EXPERIMENT No. 45. *March*, 1925.

Comparative Tests with Vaccine Kept for One Year Nineteen Days.

Sheep No.	Vaccinated Subcut., 19.3.25.	Vir. Cult. I, Intramusc., 31.3.25.	Result.
10486	5 c.c. (kept in incubator)	1 c.c., strain 1	Died (black-quarter).
10492	5 c.c. ,,	1 c.c., ,,	,, ,,
10499	5 c c. (kept in cellar)	1 c.c., ,,	Lived.
10761	5 c.c. ,,	1 c.c., ,,	,,
10822	5 c.c. (kept in cold storage)	1 c.c., ,,	Died (black-quarter).
10835	5 c.c. ,,	1 c.c., ,,	Lived.
11388	Controls	0·7 c.c., strain 1	Died (black-quarter).
11400		1 c.c., ,,	,, ,,

The results confirmed those obtained from previous tests, namely, that vaccine kept in the incubator loses its immunizing properties after a time, while that kept at reasonably low temperatures retain these properties for long periods, in the present case for over a year.

This result is of great importance in view of the fact that some workers have thrown doubt on the keeping properties of artificial aggressin.

(2) To test the keeping properties of artificial aggressin when exposed to excessive heat and strong light, a number of experiments has been carried out.

(*a*) Heating carried out in a water-bath at different temperatures.

EXPERIMENT No. 46. *August*, 1924.

Sheep No.	Vaccine Injected Subcut., 20.8.24.	Vir. Cult. I, 2.9.24.	Result.
8875	5 c.c., batch 64, heated to 60° C. for ½ hour	1 c.c., intramusc.	Lived.
9000	5 c.c., „ „ 70° C. „ ½ „	1 c.c. „	„
9007	5 c.c., „ „ 80° C. „ ½ „	1 c.c. „	„
9063	5 c.c., „ „ 90° C. „ ½ „	1 c.c. „	„
9066	5 c.c., „ „ 95° C. „ ½ „	1 c.c. „	„
9269	Controls	0·7 c.c. intramusc.	Died (black quarter).
6989		1 c.c. „	„
9368		0·7 c.c. „	Lived.
9463		1 c.c. „	Died (black-quarter).

It is apparent from the results that the heat resistance of the aggressin is relatively great, its immunizing properties being fully retained even after exposure to heat at 95° C. for half an hour.

EXPERIMENT No. 47. *November*, 1924.

Sheep No.	Vaccine Injected Subcut., 11.11.24.	Vir. Cult. I, 3.12.24.	Result.
9038	5 c.c., batch 72, heated to 95° C. for ½ hour	1 c.c. intramusc.	Lived.
9135	5 c.c., „ „ 95° C. „ ½ „	2 c.c. „	Died (black-quarter).
9253	5 c.c., „ „ 95° C. „ 1 „	1 c.c. „	Lived.
9346	5 c.c., „ „ 95° C. „ 1 „	2 c.c. „	Died (black-quarter).
9580	Controls	1 c.c. intramusc.	Died (black-quarter).
9621		0·7 c.c. „	„
8633		0·5 c.c. „	„

In this case the vaccine, after heating for half an hour at 95° C., did not protect a sheep against four minimum lethal doses, although sufficient immunity was present to protect against two minimum lethal doses.

The same remarks apply to vaccine submitted to heating at 95° C. for one hour.

EXPERIMENT No. 48. *December*, 1924.

Sheep No.	Vaccine Inj. Subcut., 17.12.24.	Vir. Cult. I, 31.12.24.	Result.
8976	5 c.c., batch 73, heated to 95° C. for ½ hour	1 c.c. intramusc.	Died (black-quarter).
8988	5 c.c., " " 95° C. " ½ "	2 c.c. "	"
8989	5 c.c., " " 95° C. " 1 "	1 c.c. "	"
9025	5 c.c., " " 95° C. " 1 "	2 c.c. "	"
9157	5 c.c., " " 95° C. " 2 "	1 c.c. "	"
9196	5 c.c., " " 95° C. " 2 "	2 c.c. "	Lived.
9433	Controls	0·5 c.c. intramusc.	Died (black-quarter).
9531		0·7 c.c. "	"
9579		1 c.c. "	"

In this case the results show that aggressin heated at 95° C. for half an hour or longer did not protect sheep against two minimum lethal doses or more of virus.

One is forced to conclude therefore that heating aggressin to 95° C. for a period of half an hour or longer is sufficient to destroy some of its immunizing properties. Submitting the aggressin to lower temperatures (90° C. and under) does not seem to interfere with its immunizing value.

(*b*) Exposure of aggressin to strong sunlight has also been tried, with the following results:—

EXPERIMENT No. 49. *December*, 1924.

Test on Aggressin Exposed to Strong Continuous Sunlight for Seven and a Half Hours.

Sheep No.	Vaccine Inj., 4.12.24.	Vir. Cult. I, 17.12.24.	Result.
9370	5 c.c., batch 72, subcut.	1 c.c. intramusc.	Lived.
9442	5 c.c., " "	2 c.c. "	"
9455	20 c.c., " "	1 c.c. "	"
9552	20 c.c., " "	2 c.c. "	"
9403	Controls	0·5 c.c. intramusc.	Died (black-quarter).
9479		0·7 c.c. "	Lived.
9569		1 c.c. "	Died (black-quarter).

The results show clearly that the immunizing value of the aggressin was not interfered with, all sheep surviving the immunity test carried out with as much as four minimum lethal doses of virus.

EXPERIMENT No. 50. *March*, 1925.

Test on Aggressin Exposed to Natural Climatic Conditions for Ten Days, during which 100 *Hours' Sunshine with a Maximum Temperature of* 105° *to* 117° *F. was registered.*

Sheep No.	Vaccine Inj., 11.3.25.	Vir. Cult. I, 31.3.25.	Result.
10528	5 c.c. subcut.	1 c.c. intramusc.	Lived.
10551	5 c.c. ,,	1 c.c. ,,	,,
11388	Controls	0·7 c.c. intramusc.	Died (black-quarter).
11400		1 c.c. ,,	,, ,,

In spite of this severe test, the immunizing properties of the vaccine seem to have been retained. The results of these two experiments are very striking and provide proof of the sound keeping properties possessed by artificial aggressin.

Duration of Immunity.—In the case of natural aggressin it was shown that sheep still possessed well-marked immunity as long as eleven months after they had received an injection of vaccine, and it now remains to see how this compares with immunity which follows on the injection of artificial aggressin. The following experiment will supply the required information on this point:—

EXPERIMENT No. 51. *March*, 1924.

Sheep No.	Vac., Batch 50, Inj. Subcut.	Vir. Cult. I, intramusc.	Result.
6756	5 c.c., 20.3.24	1 c.c., 8.10.24	Died (black-quarter).
6961	5 c.c., ,,	1·5 c.c., ,,	Lived.
6993	5 c.c., ,,	2 c.c., ,,	,,
9598	Controls	0·5 c.c., 8.10.14	Lived.
9174		1 c.c., ,,	Died (black-quarter).
9522		2 c.c., ,,	,, ,,
7023	5 c.c., 20.3.24	0·7 c.c., 31.12.24	Lived.
7063	5 c.c., ,,	1 c.c., ,,	,,
7092	5 c.c., ,,	1·5 c.c., ,,	Died (black-quarter).
7433	Controls	0·5 c.c., 31.12.24	Died (black-quarter).
7531		0·7 c.c., ,,	,, ,,
7579		1 c.c., ,,	,, ,,
7187	5 c.c., 20.3.24	0·7 c.c., 10.3.25	Lived.
7303	5 c.c., ,,	1 c.c., ,,	,,
7662	5 c.c., ,,	1 c.c., ,,	,,
8954	Controls	0·7 c.c., 10.3.25	Died (black-quarter).
8958		1 c.c., ,,	,, ,,

The results show that close on twelve months after the sheep received the aggressin sufficient immunity was still present to protect them against over one minimum lethal dose of virus.

It is true that a few sheep died in the earlier experiments, but this only goes to show that the protective value of the particular vaccine employed was not exceptionally high. At any rate, in the course of the experiments no appreciable decrease in the immunizing value of the aggressin could be demonstrated. It appears safe to conclude that immunity conferred by artificial aggressin is of relatively long duration, at least twelve months.

Black-quarter Filtrates Imported or Prepared by other Methods.

Apparently very few or no artificial aggressins are imported into this country: at any rate, none have been available to us for testing.

The only filtrates that come up for consideration under this heading are one which we prepared according to the recommendation of Berg, and two others, the preparation of which was carried out with modifications of his method. A comparative test was carried out with vaccine prepared according to our routine method (lot d), and with vaccines made according to Berg's formula (lot a), Berg's formula but with glucose sterilized separately (lot b), and our method, but with 12 per cent. glucose added at the beginning and no further adjustments made (lot c).

The following are the results:—

EXPERIMENT No. 52. *March*, 1925.

Sheep No.	Vac. Subcut., 10.3.25.	Vir. Cult. I, 24.3.25.	Result.
11213	5 c.c. of lot a	1 c.c. intramusc.	Lived.
11248	5 c.c. „	1 c.c. „	Died (black-quarter).
11309	5 c.c. of lot b	1 c.c. intramusc.	Lived.
11325	5 c.c. „	1 c.c. „	„
11450	5 c.c. of lot c	1 c.c. intramusc.	Died (black-quarter).
11346	5 c.c. „	1 c.c. „	Lived.
11389	5 c.c. of control lot d	1 c.c. intramusc.	Lived.
11443	5 c.c. „	1 c.c. „	„
11027	Controls	0·7 c.c. intramusc.	Lived.
11139		1 c.c. „	Died (black-quarter).

These show that vaccine prepared according to Berg's method gives only a doubtful immunity if the sugar is not sterilized separately. They further demonstrate that even such a large amount of

sugar as 12 per cent. added at once without readjustment during growth of the cultures does not give as good results as 3 or 4 per cent. glucose added at the rate of 1 per cent. at different periods.

Summary and Conclusions.

By the improved method described in this paper, an artificial aggressin can be obtained answering to all requirements of a good vaccine, namely:—

(*a*) Complete safety in use; the aggressin is absolutely harmless and non-toxic.

(*b*) High immunizing value; all routine batches have protected animals with certainty against at least one minimum lethal dose of virus; the majority of batches gave a much stronger immunity (5-10 minimum lethal doses), while in a few cases the immunizing value was sufficiently high to protect against thirty minimum lethal doses.

(*c*) A lasting immunity which has been found to protect sheep for at least twelve months is set up; further tests may prove this immunity to last even longer.

(*d*) Excellent keeping properties; vaccine stored in a comparatively dark, cool place will retain its immunizing properties for at least a year; when submitted to heat at boiling point (95° C.) for half an hour or longer, some of the immunizing properties disappear, but it still has some protective value; exposure to strong sunlight for seven and a half hours or to ordinary atmospheric conditions for ten days, during which there was 100 hours' sunshine, did not reduce the protective value in any way.

(*e*) Simplicity and cheapness of production; in the possession of these qualities, which need not be elaborated, artificial aggressin has an enormous advantage over natural aggressin; not only that, but there is a complete absence of the cruel suffering which has necessarily to be inflicted on animals in the course of the preparation of natural aggressin.

(3) The Nature of Immunity Conferred by Aggressins.

As we have seen earlier in this report, aggressins are aggressive substances which assist certain bacteria to overcome the natural defences of the body. They are abundantly present in the oedematous fluid and muscle juice of animals that have died of black-quarter and can be produced artificially by growing the organisms in suitable culture media. There is no reason to believe that the aggressins produced in these two different ways (natural and artificial) differ in any essential character; at any rate, in the course of our experiments, we have not been able to detect any well-marked differences. We are of the opinion that in the case of black-quarter the so-called natural and artificial aggressins are the same substance produced under different conditions and of course contained in different media, in the one case a liquid rich in albuminous substances (blood, serum) and the other a clear liquid (meat or liver extract) of much smaller density. Any minor differences, such as, for instance, in the keeping properties, can be attributed to the differences in the medium in which the aggressins are contained. In this connexion it may be mentioned that Gräub in 1924 (25) also expressed the opinion that natural and artificial aggressins develop the same protective action. Aggressins are a product of the living and multiplying bacteria, and in artificial cultures it

has been shown that quantitatively and qualitatively they are in direct proportion to the intensity of the growth obtained. Moreover, it can be shown that their production does not go on after active multiplication of the organisms has stopped. This point is illustrated in the following experiment with filtrates prepared from the same culture at different intervals after all growth had stopped:—

EXPERIMENT No. 53. *September*, 1923.

Sheep No.	Vaccine Inj. Subcut., 10.9.23.	Vir. Cult. I, 25.9.23.	Result.
7850	5 c.c., lot 1, filtered, 8.8.23	0·5 c.c. intramusc.	Lived.
7322	5 c.c., ,, ,, ,,	0·5 c.c. ,,	,,
6943	5 c.c., ,, ,, ,,	0·5 c.c. ,,	Died (black-quarter).
7789	5 c.c., lot 2, filtered, 15.8.23	0·5 c.c. intramusc.	Died (black-quarter).
7366	5 c.c., ,, ,, ,,	0·5 c.c. ,,	Lived.
7031	5 c.c., ,, ,, ,,	0·5 c.c. ,,	,,
7669	5 c.c., lot 3, filtered, 22.8.23	0·5 c.c. intramusc.	Lived.
7744	5 c.c., ,, ,, ,,	0·5 c.c. ,,	Died (black-quarter).
7364	5 c.c., ,, ,, ,,	0·5 c.c. ,,	,, ,,
7353	5 c.c., lot 4, filtered, 29.8.23	0·5 c.c. intramusc.	Lived.
6944	5 c.c., ,, ,, ,,	0·5 c.c. ,,	,,
7043	5 c.c., ,, ,, ,,	0·5 c.c. ,,	,,
6920	Controls	0·5 c.c. intramusc.	Died (black-quarter).
6922	Controls	0·5 c.c. ,,	Lived.

It should be noted that lot 1 vaccine was prepared on the day after all growth had stopped, while the others were obtained at weekly intervals. The results obtained from the first lot showed that the vaccine was not particularly effective, affording hardly sufficient protection against one doubtful minimum lethal dose of virus.

No better results were obtained with the following two lots of vaccine, showing there was no increased production of immunizing substances during a fortnight's incubation after the initial growth had stopped. With the last lot of vaccine all three sheep were protected, but this may be considered a mere coincidence; as already indicated 0.5 c.c. of virus was a doubtful lethal dose, so that the survival of three sheep in the last test compared with two in the first can easily be understood.

Regarding the immunity produced by aggressins, we are of opinion that their injection into animals calls forth the manufacture by the body tissues of specific anti-bodies, which may be called anti-aggressins, and that one is, therefore, justified in speaking of an anti-aggressin immunity in contradistinction to anti-toxic or anti-bacterial immunity.

The differences between aggressins and toxins have already been mentioned earlier in this report, but they may again be referred to briefly as follows:—

Aggressins differ from toxins mainly in the following respects:—

(*a*) They are non-toxic, and even when relatively large quantities are injected into animals no toxic reaction or any visible systemic disturbance is set up.

(*b*) They are remarkably stable substances, being influenced very little by outside influences, such as extremes of temperature, strong sunlight, etc. For instance, they resist heating in a water-bath at 95° C. for half an hour and exposure to direct sunlight for 100 hours. Most toxins are extremely sensitive to strong light and many are destroyed at temperatures between 60° and 80° C. In fact Grassberger and Schattenfroh (21) assert that the black-quarter toxin isolated by them was destroyed by heating at 50° C. for one hour. When stored in a relatively cool place they retain their immunizing properties for long periods; in the case of artificial aggressins for at least a year and natural aggressins over two years.

(*c*) When injected into animals they produce a solid lasting immunity which has so far been demonstrated to be at least a year. In the case of toxins the immunity set up is usually of short duration, the anti-toxins being eliminated from the body fairly rapidly.

Concerning anti-bacterial immunity, very little need be said, excepting that so far as our aggressins are concerned, we are satisfied they have been absolutely germ-free, so that any immunity that may have been derived from bacterial action does not call for any consideration.

IV.—Variation in Black-quarter Strains as regards Pathogenic Properties and Immunizing Power.

Since this question is of such great importance when the selection of black-quarter strains for vaccine preparation comes up for consideration, it merits separate and special discussion. The existence of black-quarter strains in different localities varying in their virulence has been suspected for a long time, and, as a matter of fact, it was made use of for explaining away unsatisfactory results obtained from the use of the older vaccines. In South Africa, attempts were made to overcome this difficulty by mixing a large number of strains obtained from different localities for the preparation of the powder vaccine. In some cases as many as nineteen such strains were mixed, but the resulting polyvalent vaccine gave no better results than had been obtained previously with a vaccine prepared from only one strain.

At first complete bacteriological investigations were not made to arrive at a correct classification of these strains, nor was their relative virulence determined by animal experimentation. When this was done at a later stage, it was found that a few strains isolated from cases of suspected black-quarter actually fell in the *Vibrion septique* group, and that an efficient vaccine (aggressin) prepared from only one strain of true black-quarter would protect against all other strains of true black-quarter. In connexion with these points a few references to recent literature may be inserted.

(*a*) Concerning the immunizing value of one black-quarter strain against another, the following references are of considerable interest:—

In 1921 Uchimura (69) published the results of experiments carried out in guinea-pigs with black-quarter filtrate prepared from strain P; he found that this vaccine protected guinea-pigs against eleven other strains of true black-quarter, but that no immunity was produced against five other anaerobes (*V. septique*, *B. oedematiens*, and *putrificus* type).

In 1922 Zschokke (80) drew the conclusion that the immunity produced by black-quarter aggressin was specific for black-quarter, and that no protection was afforded against infection with organisms related to black-quarter, such as *Vibrion septique*. On the other hand, Gräub, in 1924 (25), suspected that variation in virulence of local strains might be responsible for breakdowns in immunity that had been observed in some cases. He prepared special vaccines for use in those parts where losses in vaccinated animals had occurred, and apparently after that more reliable results were obtained. Against this, however, he mentioned the comparatively heavy losses experienced in Berne during 1923.

Lourence and Te Hennepe, in 1924 (44), prepared their mixed vaccine (previously referred to) from no fewer than twenty-five different black-quarter strains.

(*b*) As regards the different strains of black-quarter and the allied group of anaerobes, a great deal has been written recently; in this connexion a few of the more important publications may be referred to briefly:—

It has been accepted generally that *B. chauvaei* represents the standard type of black-quarter and that *B. sarcophysematos bovis* (Foth) is identical with it. Zeissler and Miessner have always held that these two represent the only true black-quarter organism. In 1922, Miessner (49) proposed the name of para-rauschbrand (para-black-quarter) for diseased conditions closely resembling black-quarter, but set up by allied anaerobes which can be distinguished bacteriologically from the true black-quarter organism. Among the group of para-rauschbrand organisms, Miessner (50) included the bacillus of malignant oedema (Koch), *Vibrion septique* (Pasteur), Kitt's black-quarter bacillus, Ghon-Sach's bacillus, and the bacillus of Bradsot (Jensen).

Sobernheim and Zurmaniki, in 1924 (63), took exception to the newly proposed name of para-rauschbrand, preferring the old term malignant oedema; they argued that old established names could not be changed for new ones in such an arbitrary manner.

Up to 1924, Kitt (38) protested against the strain of black-quarter organisms described by him being classified under para-rauschbrand, his contention being that in spite of it forming short chains and of the atypical growth on glucose-blood-agar, it still resembled black-quarter in its non-pathogenicity for rabbits and in that immunity would be set up by it against the true black-quarter bacillus (Foth).

Pfeiler and Goerttler (53, 54) supported Kitt, and in confirmation of their contention mentioned that in cross-immunity tests in guinea-pigs they were able to protect with vaccine made from Kitt's bacillus against virus of Foth's strain, and vice versa.

Even in 1923 Goerttler (20) still maintained that type 1*a* (Foth) and 1*b* (Kitt) could be shown to afford protection one against the other.

Finally, in 1924, Kitt (39) himself proposed to abolish the term black-quarter bacillus (Kitt) and agreed to accept that in black-quarter and allied diseases only three distinct organisms come up for consideration, namely, *B. chauvaei* (Foth's black-quarter bacillus), bacillus of malignant oedema, and Fränkel's bacillus.

In 1923, Katzumi Kojima (40) described a new toxin producing organisms belonging to the black-quarter group; it is said to form

chains in old cultures, but not filaments, and to be more toxic than the ordinary black-quarter bacillus. Heller, H. H. (1920) (30), used the expression "Black-quarter group," and so allowing for further differentiation of the organisms falling under this group. Leclainche, Vallée, Glover, and Vincemin (1924) (43) employed the term "Charbon para-symptomatique" for certain organisms bearing some resemblance to *Vibrion septique* and having particular characteristics typical for the classical black-quarter organism. They admit that such organisms are rare, and so far found only in Normandy and Persche. They suggest that these may be either transition forms or an association of the two organisms. Many of these so-called transition forms do not produce cross-immunity amongst themselves, but individual strains can be used to immunize against themselves. They recommend the use of a polyvalent vaccine against black-quarter and allied types; such a vaccine can easily be prepared according to the method described by them in 1913.

Our Own Experiments.—It has so far not been possible to undertake a detailed study of the various black-quarter strains that have come under observation, but this side of the black-quarter problem will receive our serious attention in the near future. The information which we are able to give has been obtained from preliminary tests that have been carried out with different strains in regard to their suitability as vaccine producers.

The first step was to prepare a vaccine (artificial aggressin) from strain 1, which has been used for this purpose for a number of years, and to test its immunizing value against a number of other strains. The latter had been obtained from different parts of South Africa, excepting No. 6, which was isolated from a vaccine imported from France. The results of these tests are given in the following experiments.—

EXPERIMENT No. 54. *December*, 1924.

Sheep No.	Vaccine Inj. Subcut., 22.12.24.	Vir. Cult. Intramusc., 7.1.25.	Result.
8847	5 c.c., strain 1	0·4 c.c., strain 1	Lived.
8820	5 c.c., ,,	0·6 c.c., ,,	,,
8639	5 c.c., ,,	0·8 c.c., ,,	,,
9255	Controls	0·4 c.c., strain 1	Died (black-quarter).
9297		0·6 c.c., ,,	,, ,,
9302		0·8 c.c., ,,	,, ,,
9640	5 c.c., strain 1	0·3 c.c., strain 3	Lived.
8646	5 c.c., ,,	0·5 c.c., ,,	,,
8649	5 c.c., ,,	0·7 c.c., ,,	Died (black-quarter).
9358	Controls	0·3 c.c., strain 3	Lived.
9375		0·5 c.c., ,,	Died (black-quarter).
8660	5 c.c., strain 1	0·4 c.c., strain 6	Lived.
8664	5 c.c., ,,	0·7 c.c., ,,	,,
8671	5 c.c., ,,	1 c.c., ,,	,,
9367	Controls	0·4 c.c., strain 6	Lived.
9393		0·7 c.c., ,,	Died (black-quarter).

Sheep No.	Vaccine Inj. Subcut., 22.12.24.	Vir. Cult. Intramusc., 7.1.25.	Remarks.
8695	5 c.c., strain 1	0·8 c.c., strain 18	Lived.
8717	5 c.c., ,,	1 c.c., ,,	,,
8767	5 c.c., ,,	1·5 c.c., ,,	,,
9397	Controls	0·8 c.c., strain 18	Lived.
9417	Controls	1 c.c., ,,	,,
8811	5 c.c., strain 1	0·4 c.c., strain 21	Lived.
8818	5 c.c., ,,	0·7 c.c., ,,	,,
8820	5 c.c., ,,	1 c.c., ,,	Died (black-quarter).
9419	Controls	0·4 c.c., strain 21	Died (black-quarter).
9427	Controls	0·7 c.c., ,,	,, ,,
8843	5 c.c., strain 1	0·7 c.c., strain 12	Lived.
8849	5 c.c., ,,	1 c.c., ,,	,,
8851	5 c.c., ,,	1·5 c.c., ,,	,,
9449	Controls	0·7 c.c., strain 12	Died (black-quarter).
9462	Controls	1 c.c., ,,	Lived.
8856	5 c.c., strain 1	0·5 c.c., strain 30	Lived.
8905	5 c.c., ,,	0·7 c.c., ,,	Died (black-quarter).
8994	5 c.c., ,,	1 c.c., ,,	,, ,,
9449	Controls	0·2 c.c., strain 30	Died (black-quarter).
9462	Controls	0·5 c.c., ,,	,, ,,

With the exception of strains 18 and 30, the results were conclusive, showing that vaccine prepared from one strain was effective in protecting against at least one minimum lethal dose of other strains.

The results obtained with strain 18 were unsatisfactory in that the control animals did not die from the virus, whereas with strain 30 two out of three animals died. It will be seen later on, however, that the dose of virus employed was far too large, the minimum lethal dose of strain 30 lying between 0.01 and 0.02 c.c. A further test with these two strains was carried out, the results being as follows: —

EXPERIMENT No. 55. *December*, 1924.

Sheep No.	Vaccine, Strain 1, Subcut., 22.12.24.	Vir. Cult Intramusc., 13.1.25.	Result.
9010	5 c.c., strain 1	1·5 c.c., strain 18	Lived.
9078	5 c.c., ,,	2 c.c., ,,	,,
9235	5 c.c., ,,	2·5 c.c., ,,	,,
9521	Controls	1·5 c.c., strain 18	Lived.
9526	Controls	2 c.c., ,,	,,
9276	5 c.c., strain 1	0·2 c.c., strain 30	Died (black-quarter).
9294	5 c.c., ,,	0·3 c.c., ,,	,, ,,
9334	5 c.c., ,,	0·5 c.c., ,,	,, ,,
9528	Controls	0·1 c.c., strain 30	Died (black-quarter).
9553	Controls	0·2 c.c., ,,	,, ,,

These were no more satisfactory, the reasons being the same as given before.

The next step was to prepare small batches of vaccines from the different strains and to test these against No. 1 strain. In order to find out minor differences in the immunizing value of the different strains, rather weak vaccines were employed; the cultures were only grown for about twelve days, and during this time no fresh glucose was added. A larger number of strains was employed, the tests being carried out under three experiments on different dates as follows:—

EXPERIMENT No. 56. *February*, 1924.

Sheep No.	Vaccine Subcut., 13.3.24.	Vir. Cult. Intramusc., 26.2.24.	Result.
6868	5 c.c., strain 1	0·7 c.c., strain 1	Lived.
7545	5 c.c., ,,	0·7 c.c., ,,	,,
5896	20 c.c., ,,	0·7 c.c., ,,	,,
6214	20 c.c., ,,	0·7 c.c., ,,	,,
5853	5 c.c., strain 18	0·7 c.c., strain 1	Lived.
7467	5 c.c., ,,	0·7 c.c., ,,	,,
6235	20 c.c., ,,	0·7 c.c., ,,	,,
7847	20 c.c., ,,	0·7 c.c., ,,	,,
7428	5 c.c., strain 19	0·7 c.c., strain 1	Lived.
7478	5 c.c., ,,	0·7 c.c., ,,	Died (black-quarter).
6186	20 c.c., ,,	0·7 c.c., ,,	Lived.
6927	20 c.c., ,,	0·7 c.c., ,,	,,
7438	5 c.c., strain 21	0·7 c.c., strain 1	Lived.
7527	5 c.c., ,,	0·7 c.c., ,,	Died (black-quarter).
5866	20 c.c., ,,	0·7 c.c., ,,	Lived.
7498	20 c.c., ,,	0·7 c.c., ,,	Died (black-quarter).
6534	5 c.c., strain 22	0·7 c.c., strain 1	Lived.
7837	5 c.c., ,,	0·7 c.c., ,,	,,
5859	20 c.c., ,,	0·7 c.c., ,,	Died (black-quarter).
6881	20 c.c., ,,	0·7 c.c., ,,	Lived.
7285	Controls	1 c.c., strain 1	Died (black-quarter).
6783		0·7 c.c., ,,	,, ,,
7263		0·5 c.c., ,,	Lived.

The results were that in all cases some protection was afforded against one minimum lethal dose of strain 1, while with strains 1 and 18 no animals succumbed to the injection of virulent material.

EXPERIMENT No. 57. *March*, 1924.

Sheep No.	Vaccine Subcut., 10.3.24.	Vir. Cult. Intramusc., 24.3.24.	Result.
7058	5 c.c., strain 6	0·7 c.c., strain 1	Lived.
7288	5 c.c., ,,	0·7 c.c., ,,	,,
7297	20 c.c., ,,	0·7 c.c., ,,	Died (black-quarter).
7718	20 c.c., ,,	0·7 c.c., ,,	Lived.
7659	5 c.c., strain 7	0·7 c.c., strain 1	Died (black-quarter).
7675	5 c.c., ,,	0·7 c.c., ,,	,, ,,
6540	20 c.c., ,,	0·7 c.c., ,,	Lived.
7421	20 c.c., ,,	0·7 c.c., ,,	Died (black-quarter).
6252	5 c.c., strain 8	0·7 c.c., strain 1	Died (black-quarter).
6716	5 c.c., ,,	0·7 c.c., ,,	,, ,,
5855	20 c.c., ,,	0·7 c.c., ,,	Lived.
6473	20 c.c., ,,	0·7 c.c., ,,	,,
6774	5 c.c., strain 13	0·7 c.c., strain 1	Died (black-quarter).
7709	5 c.c., ,,	0·7 c.c., ,,	,,
6749	20 c.c., ,,	0·7 c.c., ,,	Lived.
7661	20 c.c., ,,	0·7 c.c., ,,	Died (black-quarter).
6303	5 c.c., strain 14	0·7 c.c., strain 1	Lived.
6217	5 c.c., ,,	0·7 c.c., ,,	,,
6904	20 c.c., ,,	0·7 c.c., ,,	Died (black-quarter).
6222	20 c.c., ,,	0·7 c.c., ,,	Lived.
6547	Controls	0·5 c.c., strain 1	Died (black-quarter).
6262		0·7 c.c., ,,	Lived.

With these strains again some protection was afforded in all cases. Strain 13 appeared to be the weakest, only one out of four vaccinated sheep surviving the immunity test.

EXPERIMENT No. 58. *May*, 1924.

Sheep No.	Vaccine Subcut., 23.5.24.	Vir. Cult. Intramusc., 14.6.24.	Result.
6306	5 c.c., strain 3	0·7 c.c., strain 1	Lived.
6869	5 c.c., ,,	0·7 c.c., ,,	,,
6976	20 c.c., ,,	0·7 c.c., ,,	,,
7022	20 c.c., ,,	0·7 c.c., ,,	,,
7084	5 c.c., strain 26	0·7 c.c., strain 1	Lived.
7024	5 c.c., ,,	0·7 c.c., ,,	Died (black-quarter).
7518	20 c.c., ,,	0·7 c.c., ,,	Lived.
7797	20 c.c., ,,	0·7 e.c., ,,	,,
8589	Controls	0·5 c.c., strain 1	Died (black-quarter).
7154		0·7 c.c., ,,	,, ,,
7091		0·7 c.c., ,,	Lived.

In this case both strains afforded almost complete protection against virus of strain 1. Strain 3 appears to have good immunizing properties.

In order to test out further the protective value of strain 3, three lots of stronger vaccine were prepared, lot 1 being grown for eleven days, lot 2 for twenty-three days, and lot 3 for twenty-eight days; adjustment of the medium as regards reaction and sugar-content was of course made during growth of the cultures. The results of the immunity tests are given in the following experiment:—

EXPERIMENT No. 59. *July*, 1924–*January*, 1925.

Sheep. No.	Vaccine Subcut.	Vir. Cult. Intramusc.	Result.
	2.7.24.	15.7.24.	
6795	5 c.c., lot 1	1 c.c., strain 1	Lived.
8346	5 c.c., ,,	0·5 c.c., ,, 3	,,
7340	20 c.c., ,,	1 c.c., ,, 1	,,
9529	20 c.c., ,,	0·5 c.c., ,, 3	,,
7462	Controls	0·5 c.c., strain 3	Died (black-quarter).
7683		0·7 c.c., ,, 1	,, ,,
7388		0·7 c.c., ,,	,, ,,
	7.10.24.	21.10.24.	
9329	5 c.c., lot 2	1 c.c., strain 1	Lived.
9413	5 c.c., ,,	2 c.c., ,,	,,
9468	5 c.c., ,,	1 c.c., ,,	,,
9529	5 c.c., ,,	2 c.c., ,,	,,
9549	5 c.c., ,,	1 c.c., ,,	,,
9555	5 c.c., ,,	2 c.c., ,,	,,
8938	Controls	1 c.c., strain 1	Died (black-quarter).
9137		1·5 c.c., ,,	,, ,,
	13.1.25.	26.1.25.	
9475	5 c.c., lot 3 (batch 78)	0·8 c.c., strain 1	Lived.
9494	5 c.c., ,, ,,	1 c.c., ,,	,,
9501	20 c.c., ,, ,,	2 c.c., ,,	,,
9504	20 c.c., ,, ,,	3 c.c., ,,	,,
9632	Controls	0·6 c.c., strain 1	Lived.
9629		0·8 c.c., ,,	Died (black-quarter).

The results are convincing, a strong immunity, amounting to protection against nearly four minimum lethal doses, being obtained in some instances.

Another test was carried out with a strain (33) isolated from a vaccine imported from America. The immunity produced by a filtrate prepared from this strain is given in the following experiment:—

EXPERIMENT No. 60. *October*, 1924.

Sheep No.	Vaccine Subcut., 9.10.24.	Vir. Cult. Intramusc., 29.10.24.	Result.
9586	5 c.c., strain 33	1 c.c., strain 1	Lived.
9611	5 c.c., ,,	2 c.c., ,,	Died (black-quarter).
9052	20 c.c., ,,	1 c.c., ,,	Lived.
9160	20 c.c., ,,	2 c.c., ,,	,,
9391	Controls	0·5 c.c., strain 1	Lived.
9452		1 c.c., ,,	Died (black-quarter).
9499		1·5 c.c., ,,	,, ,,

The results show clearly that quite a good protection is afforded against one of our local strains.

In Experiment No. 56, carried out in February, 1924, it was seen that vaccine prepared from strain 18 gave a strong immunity against virus of strain 1. This vaccine was made when the strain was still virulent for guinea-pigs, but since then, for some unknown reason, it would seem to have lost its virulence. To test its immunizing value a further batch of vaccine was prepared, the immunity now produced being shown in the following experiment:—

EXPERIMENT No. 61. *March*, 1925.

Sheep No.	Vaccine Subcut., 10.3.25.	Vir. Cult. Intramusc., 24.3.25.	Result.
11445	5 c.c., strain 18	1 c.c., strain 1	Lived.
11458	5 c.c., ,,	1 c.c., ,,	Died (black-quarter).
11579	20 c.c., ,,	1 c.c., ,,	,, ,,
10661	20 c.c., ,,	1 c.c., ,,	Lived.
11027	Controls	0·7 c.c., strain 1	Lived.
11139		1 c.c., ,,	Died (black-quarter).

These results were rather surprising, showing that a strain which had partly lost its virulence no longer possessed the same immunizing value. Apparently in this case a decreased production of aggressin had taken place, and with the decrease in aggressive substances one would expect the organisms to become less virulent.

Strain 30, referred to previously, proved to be of such extreme interest, particularly in view of its exceptional virulence, that it had to be submitted to a number of further tests.

Its minimum lethal dose was found to be 0·01 c.c., and this is about thirty times greater than that observed in any other of our black-quarter strains. The strain was isolated from a heifer which had died about eight months after vaccination. Its morphological

and cultural characters are identical with those of typical black-quarter, while by animal tests it was shown to differ from *Vibrion septique* in that it failed to kill rabbits, even when such large doses of virus as 5 c.c. were used.

(*a*) EXPERIMENT No. 62. *February*, 1925.

Two lots of vaccine [lot (*a*), grown for twenty-seven days, and lot (*b*), grown for thirty-four days] were prepared from strain 30, and the immunizing value was tested against strains 1 and 3.

Sheep No.	Vaccine, Strain 30, Subcut.	Vir. Cult. Intramusc.	Result.
10570	5 c.c., lot 1, 10.2.25	1 c.c , strain 1, 24.2.25	Lived.
10705	5 c.c., „ „	1·5 c.c., „ „	Died (black-quarter).
10724	20 c.c., „ „	1 c.c., „ „	Lived.
10731	20 c.c., „ „	1·5 c.c., „ „	„
10962	Controls	0·7 c.c., strain 1, 24.2.25	Died (black-quarter).
10988	Controls	1 c.c., „ „	Lived.
8869	5 c.c., lot 2, 18.2.25	0·7 c.c., strain 3, 3.3.25	Lived.
8993	5 c.c., „ „	1 c.c., „ 1, „	Died (black-quarter).
9124	20 c.c., „ „	0·7 c.c., „ 3, „	„ „
9126	20 c.c., „ „	1 c.c., „ 1, „	Lived.
10821	Controls	0·3 c.c., strain 3, 3.3.25	Died (black-quarter).
10933	Controls	0·5 c.c., „ 3, „	„ „
11013	Controls	0·7 c.c., „ 1, „	Lived.
11378	Controls	1 c.c., „ 1, „	Died (black-quarter).

The results show that a considerable degree of immunity was present, but not sufficient to protect with certainty against anything over one minimum lethal dose of strains 1 and 3.

(*b*) In this case vaccines were prepared from strains 1 and 3, and used for immunizing against strain 30.

EXPERIMENT No. 63. *March*, 1925.

Sheep No.	Vaccine Subcut., 2.3.25.	Vir. Cult. Intramusc., 17.3.25.	Result.
11268	5 c.c., strain 1 (batch 81)	0·02 c.c., strain 30	Died (black-quarter).
11280	5 c.c., „ 1 „	0·03 c.c., „	Lived.
11308	5 c.c., strain 3 (batch 78)	0·02 c.c., „	Died (black-quarter).
11422	5 c.c., „ 3 „	0·03 c.c., „	„ „
10601	Controls	0·02 c.c. strain 30	Died (black-quarter).
10802	Controls	0·03 c.c., „	„ „

The results show that the immunity was not sufficient to protect against the quantities of virus employed, namely, two and three minimum lethal doses of strain 30.

(*c*) Experiment No. 64. *March*, 1925.

Cross-immunity Tests between Strains 1 *and* 30.

Sheep No.	Vaccine Subcut., 31.3.25.	Vir. Cult. Intramusc., 15.4.25.	Result.
10228	5 c.c. strain 1	0·02 c.c., strain 30	Lived.
10237	5 c.c., ,,	0·02 c.c., ,,	,,
10240	5 c.c., ,,	0·02 c.c., ,,	Died (black-quarter).
10247	5 c.c., ,,	1 c.c., strain 1	Lived.
10569	5 c.c., ,,	1 c.c., ,,	,,
10695	5 c.c., ,,	1 c.c., ,,	,,
10789	5 c.c., strain 30	1 c.c., strain 1	Died (black-quarter).
10927	5 c.c., ,,	1 c.c., ,,	Lived.
11049	5 c.c., ,,	1 c.c., ,,	,,
11071	5 c.c., ,,	0·02 c.c., strain 30	,,
11190	5 c.c., ,,	0·02 c.c., ,,	Died (black-quarter).
11286	5 c.c., ,,	0·02 c.c., ,,	Lived.
10253	Controls	0·01 c.c., strain 30	Died (black-quarter).
10255		0·02 c.c., ,, 30	,, ,,
10231		0·7 c.c., ,, 1	Lived.
10233		1 c.c., ,, 1	Died (black-quarter)

Here, again, the dose of virus used for strain 30 was rather high, but the results show that the protection against each other afforded by the two strains is very much the same, in each case two out of the three sheep surviving.

(*d*) Experiment No. 65. *May*, 1925.

Cross-immunity Test between Strain 30 *and* Vibrion septique *Strain* 27.

Sheep No.	Vaccine Subcut,, 5.5.25.	Vir. Cult. Intramusc., 19.5.25.	Result.
10654	5 c.c., strain 30	0·02 c.c., strain 30	Lived.
10667	5 c.c., ,,	0·02 c.c., ,,	Died (black-quarter).
10793	5 c.c., ,,	0·2 c.c., strain 27	,, (*Vibrion septique.*)
10971	5 c.c., ,,	0·2 c.c., ,,	,, ,,
10977	5 c.c., strain 27	0·02 c.c., strain 30	Died (black-quarter).
11056	5 c.c., ,,	0·02 c.c., ,,	Lived.
11063	5 c.c., ,,	0·2 c.c., strain 27	,,
11153	5 c.c., ,,	0·2 c.c., ,,	Died (*Vibrion septique*).
11356	Controls	0·02 c.c., strain 30	Lived.
11451		0·02 c.c., ,,	Died (black-quarter).
11278		0·2 c.c., strain 27	,, (*Vibrion septique*).
11290		0·2 c.c., ,,	,, ,,

The results obtained were neither satisfactory nor conclusive, because in the first place neither vaccine afforded protection against its own strain, and secondly the minimum lethal dose of strain 30 did not kill both control animals. The one animal which survived the

immunity test against strain 30 may have done so for this reason, and not because it received any protection from the vaccine. It seems clear, however, that vaccine prepared from strain 30 did not afford any protection against strain 27.

Summary and Conclusions.

The results of these experiments supply definite information in some directions.

(1) There exists great variation in the virulence of black-quarter strains obtained from different localities.

(2) All true black-quarter strains fall in the same group as far as their immunizing properties are concerned; vaccine prepared from one strain will afford protection against all others.

(3) The immunizing value of the different strains appears to vary considerably, so that not every black-quarter strain can be considered suitable for vaccine production.

(4) Avirulent strains such as No. 18 do not seem to have great immunizing value, their avirulent character being due probably to the absence of sufficient aggressin.

(5) One exceptionally virulent strain (No. 30) has come under observation. According to all the tests that have been applied, it appears to fall in the black-quarter group, but the indications are that it does not possess a great immunizing value, neither against itself nor against other black-quarter strains.

(6) Unsatisfactory results obtained from vaccination cannot be attributed to the presence of different black-quarter strains. If a good vaccine is prepared from a suitable immunizing strain, it will protect against all other black-quarter strains.

(7) Black-quarter aggressin will not protect against infection by allied organisms such as *Vibrion septique*.

V.—Vaccination against Malignant Oedema (*Vibrion septique*).

At one time the occurrence of malignant oedema in cattle was believed to be a complicating factor in the protective inoculation against black-quarter. Unsatisfactory results obtained from vaccination against the latter disease were thought to be attributable partly to the occurrence of malignant oedema in the vaccinated animals. Our more recent experience seems to point away from malignant oedema as a complicating factor of any great significance, because, ever since a more reliable black-quarter vaccine has been used in the field, complaints in connexion with losses from black-quarter or allied conditions are very few and far between. On the other hand, we recognize the possibility of it occurring on a sufficiently big scale to warrant protective inoculation being carried out. Chiefly for this reason some experiments in connexion with the preparation of an efficient vaccine have been undertaken.

Before discussing these experiments reference will be made to a few of the more important articles on this subject that have appeared in the literature.

In 1921 Uchimura (69) suggested the advisability of preparing a filtrate for use against *Vibrion septique*.

In 1924 Gräub (24) reported that in connexion with this matter experiments had actually been started by him in 1922.

In 1922 Pfeiler and Goerttler (54) carried out vaccination against *Vibrion septique* in connexion with certain cross-immunity tests undertaken by them.

Allen and Bosworth reported in 1924 (1) that they had been fairly successful in immunizing animals against *Vibrion septique* by two subcutaneous injections of filtrate or toxin, but that serious local effects were produced by this vaccine. Mixtures of under-neutralized toxin-anti-toxin gave good results, double inoculation at twelve days' interval being the more satisfactory. Dalling in 1924 (12) also reported that good results had been obtained by him with under-neutralized toxin. Sobernheim and Zurmaniki in 1924 (63) found that by means of germ-free filtrate it was difficult to confer a reliable immunity on guinea-pigs against *Vibrion septique*; in this respect this organism differs widely from *B. chauvaei*.

Our Experiments.—In the first instance, vaccines against *Vibrion septique* were prepared purely for experimental purposes, and especially for use in cross-immunity tests, but later a batch of this vaccine was actually issued for use in cattle. As mentioned earlier in this report, serious outbreaks of malignant oedema are practically unknown in this country, but what was believed to be an outbreak of this disease occurred in a herd of cattle in South-West Africa. The symptoms described resembled those seen in cases of black-quarter, but appearently the disease was not confined to any particular age of animals, and, moreover, it was stated that the use of black-quarter vaccines did not stop the mortality. Specimens of affected muscle from one case were received for examination by the writers, who were able to isolate *Vibrion septique* in pure culture.

In the following experiments carried out in connexion with the preparation of *Vibrion septique* filtrates, the same technique as used in the preparation of black-quarter aggressin was employed:—

(*a*) Experiment No. 66. *July*, 1924.

Sheep No.	*Vib. sept.* Cult. Filt., 14.7.24.	Vir. *V. sept.* Cult., 29.7.24.	Result.
6650	5 c.c., strain 27, subcut.	0·2 c.c., strain 31, intramusc.	Lived.
6722	5 c.c., ,, ,,	0·4 c.c., ,, ,,	,,
6741	20 c.c., ,, ,,	0·2 c.c., ,, ,,	,,
6826	20 c.c., ,, ,,	0·4 c.c., ,, ,,	,,
8881	Controls	0·2 c.c., strain 31, intramusc.	Lived.
8913	Controls	0·4 c.c., ,, ,,	Died (*Vibrion septique*).

The results show that with a dose of 5 c.c. it was possible to immunize sheep against at least one minimum lethal dose of *Vibrion septique*.

The filtrate was found, however, to possess toxic properties, a well-marked local reaction being present. This was particularly severe in the two sheep which had received the larger dose, there being marked oedematous swelling of the skin and subcutaneous tissues. In consequence of this the animals were very lame, but eventually made a good recovery.

(*b*) EXPERIMENT No. 67. *August*, 1924.

Sheep No.	*V. sept.* Vac. Subcut., 25.8.24.	Vir. *V. s.* Cult. Intramusc., 10.9.24.	Result.
9145	1 c.c., strain 27	0·2 c.c., strain 27	Lived.
9168	2 c.c., ,,	0·4 c.c., ,,	Died (*Vibrion septique*).
9238	3 c.c., ,,	0·2 c.c., ,,	,, ,,
9245	4 c.c., ,,	0·4 c.c., ,,	Lived.
9281	4 c.c., ,,	0·2 c.c., ,,	,,
9371	5 c.c., ,,	0·4 c.c., ,,	,,
9424	Controls	0·2 c.c., strain 27	Died (*Vibrion septique*).
9478	Controls	0·4 c.c., ,,	,, ,,

In this case a good immunity was obtained, doses of 4 and 5 c.c. protecting against at least two minimum lethal doses. It is interesting to note that a dose of 2 c.c. was not sufficient to protect against two minimum lethal doses, and that with a dose of 3 c.c. the sheep succumbed to one minimum lethal dose of virus.

In these cases a local reaction was also observed, but it was not nearly so severe as in the previous experiment. The vaccine had been kept a fortnight after filtration before the injections were made; whether this had anything to do with the smaller degree of toxicity is impossible to say at this stage.

(*c*) EXPERIMENT No. 68. *January*, 1925.

Test of Filtrate, same as used in Experiment No. 66, but kept in Cold Storage for Five and Three-quarter Months.

Sheep No.	*Vib. sept.* Cult. Filt.	Vir. Cult.	Result.
9103	5 c.c., strain 27	0·1 c.c., *Vibrion septique*, strain 27, 20.1.25	Lived.
9105	5 c.c., ,,	0·2 c.c., ,, ,, ,,	,,
9155	5 c.c., ,,	0·3 c.c., ,, ,, ,,	,,
9173	5 c.c., strain 27	0·8 c.c., black-quarter, strain 1, 26.1.25	Died (black-quarter).
9220	5 c.c., ,	1 c.c., ,, ,, ,,	,,
9231	5 c.c., ,,	1 c.c., ,, ,, ,,	,,
9560	Controls for *Vibrion septique*	0·1 c.c., *Vibrion septique*, strain 27, 20.1.25	Died (*Vibrion septique*).
9562	Controls for *Vibrion septique*	0·2 c.c., ,, ,, ,,	,,
9632	Controls for black-quarter	0·6 c.c., black-quarter, strain 1, 26.1.25	Lived.
9628	Controls for black-quarter	0·8 c.c., ,, ,, ,,	Died (black-quarter).

The results show that after this relatively long period the vaccine still retained its immunizing value. It showed no protection whatever against black-quarter.

Only a slight local reaction was present, so that apparently the toxic substances which were responsible for the marked reactions in the previous experiment had decreased considerably after storage for

five and three-quarter months. Whether the toxicity will always decrease on keeping of the vaccine is not possible to say at the present stage of our investigations.

One batch of vaccine was issued for use in cattle on the farm previously referred to, and, according to reports received from the farmers, marked local and systemic reactions were observed in some cases. The owner even believed that the vaccine was responsible for the death of a few cows and heifers in calf which were in rather poor condition. This vaccine was more than a month old before the farmer actually used it, and, moreover, reactions in our test animals were not exceptionally severe.

We are not able to draw any definite conclusions, excepting that when issuing filtrates prepared from *Vibrion septique* their toxicity will have to be borne in mind. Further experiments to clear up this point are called for.

(*d*) Experiment No. 69. *December*, 1924.

This strain of *Vibrion septique* was isolated from a serum-culture mixture imported from Europe and intended for use against black-quarter.

Sheep No.	Vac. Subcut., 22.12.24.	Vir. Cult. Intramusc., 7.1.25.	Result.
9002	5 c.c., strain 34	0·5 c.c., strain 1 (black-quarter)	Died (black-quarter).
9206	5 c.c., ,,	0·7 c.c., ,, ,,	,, ,,
9216	5 c.c., ,,	1 c.c., ,, ,,	,, ,,
9249	5 c.c., strain 34	0·2 c.c., strain 27 (*Vibrion septique*)	Killed 13.1.25, on account of necrosis leg.
9423	5 c.c., ,,	0·3 c.c., ,, ,,	Lived.
9540	5 c.c., ,,	0·4 c.c., ,, ,,	,,
9332	Controls for *Vibrion septique*	0·2 c.c.	Died (*Vibrion septique*).
9343	Controls for *Vibrion septique*	0·4 c.c.	Killed, 13.1.25, gangrene and necrosis on leg.
9255	Controls for black-quarter	0·4 c.c.	Died (black-quarter).
9597	Controls for black-quarter	0·6 c.c.	,, ,,
9302	Controls for black-quarter	0·8 c.c.	,, ,,

The results here show that an efficient filtrate can be prepared for use against *Vibrion septique* and that such a vaccine affords no protection against black-quarter.

They also show the severe local effect produced by virulent cultures of *Vibrion septique*; sheep 9249 apparently had a strong immunity against *Vibrion septique* and yet had to be destroyed on account of extensive necrosis of the injected limb. One of the control sheep also had to be destroyed for the same reason.

(*a*) Experiment No. 70. *December*, 1924.

This experiment was carried out at the same time as the previous one, with the object of proving definitely that no immunity is set up by black-quarter aggressin against *Vibrion septique*.

Sheep No.	Vac. Subcut., 17.12.24.	Vir. Cult. Intramusc., 7.1.25.	Result.
8924	5 c.c., black-quarter vac. (batch 73)	0·2 c.c., *Vibrion septique*, strain 27	Died (*Vibrion septique*).
8959	5 c.c., ,, ,,	0·2 c.c., ,, ,,	,,
9001	5 c.c., ,, ,,	0·3 c.c., ,, ,,	,,
9197	5 c.c., ,, ,,	0·3 c.c., ,, ,,	,,
9332	Controls	0·2 c.c., *Vibrion septique*, strain 27	Died (*Vibrion septique*).
9343	Controls	0·4 c.c., ,, ,,	Killed, see Exp. 69.

The results are conclusive, showing that no relationship exists between these two organisms, as far as the immunizing properties are concerned.

(*a*) *Summary and Conclusions.*

(1) An efficient filtrate vaccine against *Vibrion septique* can be obtained by following the same methods of preparations as are recommended in this paper for black-quarter vaccine.

(2) *Vibrion septique* cultures have markedly toxic properties which exert their effects generally and locally, the latter resulting in extensive necrosis of the tissues near the site of injection.

(3) Toxic substances are also present in filtrates prepared from the cultures, but separated from the organisms they do not appear to have such serious effects. The loss of cattle after injection of germ-free filtrate has been reported, but to what extent this is true cannot be stated. Makers of *Vibrion septique* filtrate would be well advised to keep in mind the possibility of their vaccines possessing markedly toxic properties.

(4) Owing to the undoubted toxicity of *Vibrion septique* filtrates, the writers are not prepared at this stage to express an opinion on the nature of the immunity produced, whether largely anti-aggressin or partly anti-toxic.

(5) Conclusive proof is advanced to show that black-quarter does not immunize against *Vibrion septique* and vice versa.

(*b*) *Combined Vaccination against Black-quarter and Malignant Oedema.*

The advisability of adopting a method of vaccination that would afford protection against both black-quarter and infection with allied organisms has often been referred to by different workers. Thus, Uchimura (69) raised this point in 1921, suggesting that a mixture of filtrates prepared from black-quarter, malignant oedema, and *B. putrificus* cultures might be tried.

With a combined injection of black-quarter filtrates and *Vibrion septique* toxin-anti-toxin, Allen and Bosworth (1) succeeded in 1924 to render guinea-pigs refractory to both organisms. Maenniger in 1924 (46) reported on the good results obtained from the use of a

bivalent filtrate vaccine against black-quarter and *Vibrion septique*. The two filtrates were prepared separately according to the method of Gräub and Zschokke, and afterwards mixed together. The mixture was said to have been non-toxic and to have proved satisfactory in practice against both diseases. To determine the possibility of using such a mixed vaccine, we carried out only one experiment, the filtrates being prepared from cultures of the two organisms grown separately. The results of the immunity test were as follows:—

EXPERIMENT No. 71. *March*, 1925.

Sheep No.	Vaccine Subcut., 10.3.25.	Vir. Intramusc., 24.3.25.	Result.
11059	10 c.c., bivalent vac.	1 c.c., black-quarter cult., strain 1	Lived.
11089	10 c.c., ,,	1 c.c., ,, ,, ,,	,,
11100	10 c.c., ,,	1 c.c., ,, ,, ,,	,,
13644	10 c.c., ,,	0·2 c.c., *Vibrion septique* cult., strain 27	,,
11398	10 c.c., ,,	0·3 c.c., ,, ,, ,,	,,
11420	10 c.c., ,,	0·4 c.c., ,, ,, ,,	,,
11027	Controls for black-quarter	0·7 c.c.	Lived.
11139	Controls for black-quarter	1 c.c.	Died (black-quarter).
11266	Controls for *Vibrion septique*	0·2 c.c.	Died (*Vibrion septique*.
11273	Controls for *Vibrion septique*	0·4 c.c.	,,

The results were very satisfactory, the mixed vaccine protecting all animals against at least one minimum lethal dose of both organisms. No well-marked toxic effect was observed to be present, but since only one experiment had been undertaken, one would not be justified in drawing definite conclusions. In localities where black-quarter is often complicated by the occurrence of malignant oedema, the use of such a mixed vaccine is indicated.

VI.—FINAL SUMMARY AND CONCLUSIONS.

(1) Black-quarter has been known to exist in South Africa ever since the first veterinarians came to this country. At the present time it is widespread, occurring as an enzootic in many parts of the country, and being particularly prevalent in low-lying areas.

(2) Natural infection occurs in young cattle and, to a smaller extent, in sheep. In the latter animals, the disease often makes its appearance on a large scale, infection taking place through wounds in the skin.

(3) It is not a scheduled disease under the Stock Diseases Act, the State departments concerned acting only in an advisory capacity. Control is left to the individual stockowners, who are given full advice concerning the best methods of prevention that are known to science.

Losses from the disease can be avoided by timely inoculation of susceptible stock with a reliable vaccine.

(4) Bacterial vaccines are based on the principle that the organisms themselves are largely responsible for the immunizing value of a vaccine. This has now been proved to be wrong, the "washed" organisms having neither pathogenic properties nor

immunizing value. To render such a vaccine safe for use, the number of organisms contained in it must always be kept at a minimum, and in doing this the immunizing value (aggressin-content) is often greatly reduced. Attempts on a big scale to find a method of preparation that would give a vaccine which could be considered both safe and efficient have ended in failure.

(5) Efficient anti-sera can be obtained easily by hyperimmunizing sheep with either virulent cultures or germ-free filtrates. Such sera may be used in cases where it is desired to obtain an immediate protection or in conjunction with virulent cultures or bacterial vaccines. Under the conditions prevailing in this country, there has so far been no need to resort to the use of anti-sera.

(6) The writers have never detected any sign of toxin-formation in their black-quarter cultures.

(7) The immunizing principle in black-quarter is a bacterial product which is formed during the active multiplication of the causal organism either in the animal tissues or in suitable artificial media. Owing to the aggressive character which it confers on the organism, this substance has been termed aggressin; when it is produced in the animal body it is called "natural aggressin," and when it is formed in artificial cultures it is usually referred to as "artificial aggressin."

The writers have not been able to detect any real differences between these two forms of aggressin and are forced to regard them as identical. Apparently the one produced in the animal body is formed under ideal conditions, and is therefore present with more certainty and in greater abundance; the other is produced artificially, and the degree or intensity of its production must, and undoubtedly does, depend on the nearness to which one is able to approach the artificial and natural conditions of bacterial growth. The efficiency of an artificial aggressin as an immunizing substance depends, therefore, on the perfection to which the artificial cultivation of the organisms can be brought.

The following properties possessed by aggressins are of considerable importance:—

(*a*) They are absolutely non-toxic and germ-free.

(*b*) They have excellent keeping properties; natural aggressin preserved in 60 per cent. glycerine has been kept for two and a quarter years, and artificial aggressin for over a year, without their immunizing properties being lost; natural aggressin resists heating at 80° C. for half an hour, artificial aggressin 95° C. for half an hour, and direct sunlight for 100 hours.

(*c*) They confer a strong, lasting immunity; vaccinated sheep still showed immunity twelve months after inoculation. Artificial aggressin has a great advantage over natural aggressin, in that it can be prepared much more cheaply and simply.

(8) A well-marked variation in the virulence and immunizing value of black-quarter strains from different localities has been established. The variation in virulence is not of great practical importance, since with an efficient vaccine it is possible to immunize animals against all other strains.

(9) A good immunizing vaccine (filtrate) against malignant oedema can be prepared by following the technique employed by us for the preparation of black-quarter filtrate.

(10) Vaccination against black-quarter affords no protection against infection with malignant oedema and vice versa.

(11) A mixed vaccine for use against both diseases can be obtained by growing the respective organisms separately and then mixing the two filtrates in equal proportions.

(12) During the last few years, black-quarter filtrates (artificial aggressins) have been used extensively in South Africa, the annual issues being well over a quarter million doses; for 1924 the figure was 268,040 doses. The results obtained have been extremely satisfactory, so much so that complaints in regard to the efficacy of the vaccine are practically unknown. Experience has shown that a black-quarter vaccine may be considered satisfactory so long as animal tests show it to be efficient against at least one minimum lethal dose of virus. This was the accepted standard with the first artificial aggressins that were issued, and we have on record cases where with this vaccine farmers were able to stop all mortality from black-quarter on extremely badly infected farms; in fact, in some of these cases repeated vaccination with other preparations had been tried unsuccessfully. We now use vaccines of a higher standard, protection being required against at least two or three minimum lethal doses of virus. Several thousand doses of natural aggressin have also been used with great success; this vaccine has not been used more extensively simply because the more easily prepared and cheaper artificial aggressin has given every satisfaction.

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