

ranted against scabies; sheep against "rot," redwater, and scabies; swine against quinsy and strangles.¹ In the Anglo-Saxon work on medicine, yet to be mentioned, sudden death in sheep and swine, pestilence among cattle, pocks and skin eruption in sheep are referred to, and clearly indicate the serious nature of the maladies met with. Among the list of *Anomalous Laws* of Wales, given in the above work (p. 415), is one providing for payment for veterinary attendance. Finally, even the question of compensation for disease and injury was provided by a fixed scale. A man who borrowed a horse and gave it a sore back had to compensate the owner according to the severity of the injury.

Owen² believes the Briton to have been a better horse-master than the Anglo-Saxon, for it was he who was specially selected to take care of the horses of the King. We may suppose that the veterinary practitioners were also drawn from among the Britons, which may account for their practice being inferior to that of the relatively cultured Anglo-Saxon medical man.

We are now in a position to inspect the Saxon medical manuscripts, and will take the earliest which has survived destruction—the celebrated *Leech Book*.

(To be continued.)

RESEARCHES REGARDING EPIZOOTIC ABORTION OF CATTLE.³

(From the Research Institute for Animal Pathology, Royal
Veterinary College, London.)

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THE purpose of the experiments described in this article was: (1) to throw further light on the methods of infection in contagious abortion in cattle; (2) to ascertain what is the earliest date after infection at which a positive diagnosis can be made by the agglutination or complement tests; (3) to ascertain the extent to which animals infected by different methods and with varying doses react to the infection as indicated by the amount of agglutinin or sensitiser developed in the system; and (4) to enable one to compare the value of the agglutination and complement tests in the diagnosis of contagious abortion.

M'Fadyean and Stockman⁴ were the first to employ the agglutina-

¹ The term "strangles" is employed in the translation.

² *Op. cit.*

³ The expenses connected with the researches were defrayed from a grant received from the Board of Agriculture and Fisheries.

⁴ Report of the Departmental Committee appointed by the Board of Agriculture and Fisheries to inquire into epizootic abortion: Appendix to Report, Part I., June 1909.

tion and complement tests in the diagnosis of epizootic abortion, and the methods which we have followed are broadly those described by these authors in the Departmental Committee's Report and the later article which appeared in this Journal.¹ For the better understanding of the results, however, it appears to be desirable to describe in outline the method employed in the experiments dealt with in this article.

Agglutination Tests.—The bacillary emulsion has in all cases been prepared from surface growths on serum-agar, the tubes having been incubated at 38° C. for two or three days. The growth is washed off with water containing .5 per cent. carbolic acid and .8 per cent. sodium chloride, and, after thorough shaking, the rich emulsion is filtered through paper. By the gradual addition of more of the carbolised saline solution the emulsion is then diluted to the desired degree. The results of the agglutination test are most distinct when the emulsion is only faintly hazy, and after a little experience it is possible to secure practical uniformity in the richness of the emulsion by observing the appearance of the liquid in a tube of given calibre.

The combined emulsion and serum used in each tube measures 2 cc., and the small tubes used for the purpose have a total capacity of 3 cc.

After the addition of the serum the tubes are placed in an incubator at 38° C., and left there for twenty-four hours, at the end of which time the results are read off. It may here be noted, however, that the results are not sensibly affected if the tubes at the end of the twenty-four hours are removed from the incubator and left for another day at ordinary room temperature.

No animal's serum is ever tested by itself, and as a rule there is included in each series serum from a known infected animal. It is hardly necessary to state that a control tube of simple emulsion (without serum) is always included in the series, and serves as the standard for comparison when the result in any tube with serum appears doubtful.

At least two of the authors have always taken part in the reading off of the results; and in all cases of what appeared to be doubtful agglutination the result was recorded as negative unless two concurred in the verdict "recognisable."

The result was recorded as "complete" when, owing to agglutination and sedimentation, the whole of the liquid had become as clear as pure water; as "nearly complete" when the merest trace of haziness from suspended bacilli could still be detected in the liquid; as "advanced" when about half the bacilli appeared to have fallen to the bottom of the tube, with a corresponding degree of clearing in the liquid; as "distinct" when there was a lesser degree of agglutination and precipitation, but the difference as compared with the control tube (without serum) was easily recognisable; and as "recognisable" when a careful comparison with the control tube was necessary in order to determine that some agglutination had taken place.

In the tables the figures indicate the amount of the mixture which contained 1 of the serum, *e.g.*, 20 means 1 of serum in 20 of the

¹ Vol. XXV., p. 22.

mixture. The contractions have the following meanings: comp.=complete; n. comp.=nearly complete; adv.=advanced; dist.=distinct; recog.=recognisable; and neg. means that there was no agglutination.

Complement Tests.—As is well known, the complement test devised by Bordet and Gengou depends on the fact that, given a serum containing specific immune body or sensitiser, in a mixture containing that serum, the corresponding bacteria, and complement, (guinea-pig serum), the bacteria after combining with the sensitiser absorb and retain the complement. Provided the amounts of the above ingredients have been properly chosen, the result will be the complete disappearance of free complement from the mixture, and whether this has happened or not can be determined by adding to the mixture a small quantity of sensitised red corpuscles. Should complement still remain in the mixture, complete or partial hæmolysis of the corpuscles will take place, with consequent tingeing of the liquid by the liberated hæmoglobin. In the contrary case the red corpuscles will remain intact, and consequently there will be no alteration in the colour of the liquid.

The easiest application of the complement test is to demonstrate the presence of sensitiser in the serum of a hyperimmunised animal, because, owing to the richness of the serum in sensitiser, a small amount of it can sensitise highly a large number of bacteria and determine the complete absorption of a considerable amount of complement. Hence in such a case some latitude is allowable in the relative amounts of serum, bacteria, and complement employed in the mixture, but much more circumspection is necessary when the test is applied to naturally infected animals, or to animals at an early stage of disease, in order to determine whether any sensitiser has made its appearance in the blood. In these cases it is obvious that if the blood is poor in sensitiser the latter is likely to be missed if one employs more than a very small quantity of complement in the test mixture.

In order to make the test work with delicacy, so as to be reliable for the detection of very small amounts of sensitiser, and to enable one to recognise the relative richness of different samples of serum in sensitiser, one must employ a series of tubes containing different amounts of complement, ranging upwards from the smallest amount necessary to produce visible hæmolysis of the added sensitised red corpuscles.

In our experiments we have generally used a series of five tubes, with the ingredients in the proportions shown below. The numbers used to distinguish the tubes are selected for the purpose because they correspond with the number of units of complement present in the several tubes, '01 cc. of fresh guinea-pig serum being regarded as the unit.

	No. 2.	No. 3.	No. 5.	No. 7.	No. 10.
Serum . . .	'5	'5	'5	'5	'5
Emulsion . . .	'2	'2	'2	'2	'2
Complement . . .	'02	'03	'05	'07	'1
'8 % Salt Sol. . .	'18	'17	'15	'13	'1
	'9 cc.	'9 cc.	'9 cc.	'9 cc.	'9 cc.

The control tubes employed in all cases were the following :—

1. A series to show what amount of the complement was necessary to bring about complete hæmolysis of the amount of sensitised corpuscles employed in the test. This series comprises the following four tubes, each of which is distinguished by a number indicating the number of units of complement present in it.

A Control.

	<i>No. 1.</i>	<i>No. 2.</i>	<i>No. 3.</i>	<i>No. 4.</i>
Emulsion	'2	'2	'2	'2
Complement	'01	'02	'03	'04
'8 % Salt Sol. . . .	'69	'68	'67	'66
	<hr/>	<hr/>	<hr/>	<hr/>
	'9 cc.	'9 cc.	'9 cc.	'9 cc.

This series of controls is a very important one, because it furnishes information necessary for interpreting the results in the preceding series when there is evidence of absorption of complement in any of the tubes included in it. For example, it sometimes happens that the added corpuscles do not hæmolyse completely in A control tube No. 1, and in that case one ignores the results in tube No. 2 of the proper test series. Similarly, with incomplete hæmolysis in the A control tubes No. 1 and No. 2, one has to ignore results in tubes 2 and 3 of the proper test series. It may be observed, however, that we have seldom or never found hæmolysis to be incomplete in the second control tube.

2. B Control.

	<i>No. 1.</i>	<i>No. 2.</i>	<i>No. 3.</i>	<i>No. 4.</i>
Complement	'01	'02	'03	'04
'8 % Salt Sol. . . .	'89	'88	'87	'86
	<hr/>	<hr/>	<hr/>	<hr/>
	'9 cc.	'9 cc.	'9 cc.	'9 cc.

In this series also, the figures used to distinguish the tubes correspond with the number of units of complement present. These tubes are necessary to afford assurance that the complement used is active in the absence of the emulsion. The results are frequently identical in the two series, A and B, though not rarely the hæmolysis occurs more promptly in No. 1 tube of the B series than in the corresponding tube of the A series, and the difference indicates the amount of complement that appears to be directly absorbed by the bacteria, *i.e.*, independently of any sensitisation of these.

3. Control Tube X.

The tube which is thus designated contains—

Serum	'5
Complement	'02
'8 % Salt Sol.	'38
	<hr/>
	'9 cc.

This tube contains no emulsion, and the added corpuscles must always hæmolyse in it.

4. *Control Tube Y.*

This tube contains—

Serum	·5
Emulsion	·2
·8 % Salt Sol.	·2
	<hr/>
	·9 cc.

It contains no complement and the added corpuscles must remain unhamolysed in it.

When serum from a number of different animals is being tested at the same time the four A tubes and the four B tubes serve as controls for the whole series, but it is obvious that there must be a separate X and Y tube for the serum of each animal. In the following tables the results in the different control tubes are not given, but it is to be understood that the full series of controls was used in every case, and that the results indicated absence of the errors against which the controls are intended to guard.

Sensitising of the Corpuscles.—The corpuscles were always obtained from fresh ox blood, and nearly always from the same animal. They were washed with normal saline solution, and sensitised by mixing with not less than an equal volume (usually with double the volume) of fresh inactivated serum from a goat immunised against ox corpuscles. The sensitised corpuscles were again washed, and sufficient saline solution was finally added to bring the volume up to double that of the original blood. The volume of the sensitised corpuscles added to each of the tubes as a test for free complement was ·1 cc., or the equivalent of $\frac{1}{20}$ th cc. of the original blood.

In order to permit of absorption of complement the tubes were placed in an incubator at 38° C. for two hours, and after the addition of the sensitised corpuscles they were replaced in the incubator for one hour. The results were then read off, commencing with the A and B controls.

SERIES I.

On the 12th April 1912 blood was drawn from the jugular of each of ten yearling cattle, and on the following day the serum of each animal was tested as to its agglutinating effect on a suspension of abortion bacilli in dilutions of 1 in 20, 1 in 40, 1 in 100, and 1 in 200. No appreciable agglutination occurred in any of the tubes. As a control to the above, serum was simultaneously tested from a cow that had aborted in February 1911. This serum had been taken on the 17th February 1911 and preserved in a sterile condition. Agglutination was complete in 1 in 200, nearly complete in 1 in 100, and less in the other two tubes (1 in 20 and 1 in 40).¹

On the 17th April the complement test was applied to the same serum (taken on the 12th April) from each of the above ten yearlings and a cow which had aborted a fortnight previously. The result of

¹ It will be observed that the result of this test of the stored serum was paradoxal. The test was repeated on the following day and with exactly the same result. The fact is the more interesting as the serum had been preserved in the pure condition without the addition of any antiseptic.

the test was entirely negative in the case of all the tubes with the yearling sera, but positive in the case of the serum from the cow.

The agglutination and the complement tests thus agreed in indicating that the ten yearlings in question were free from infection with the bacillus of cattle abortion.

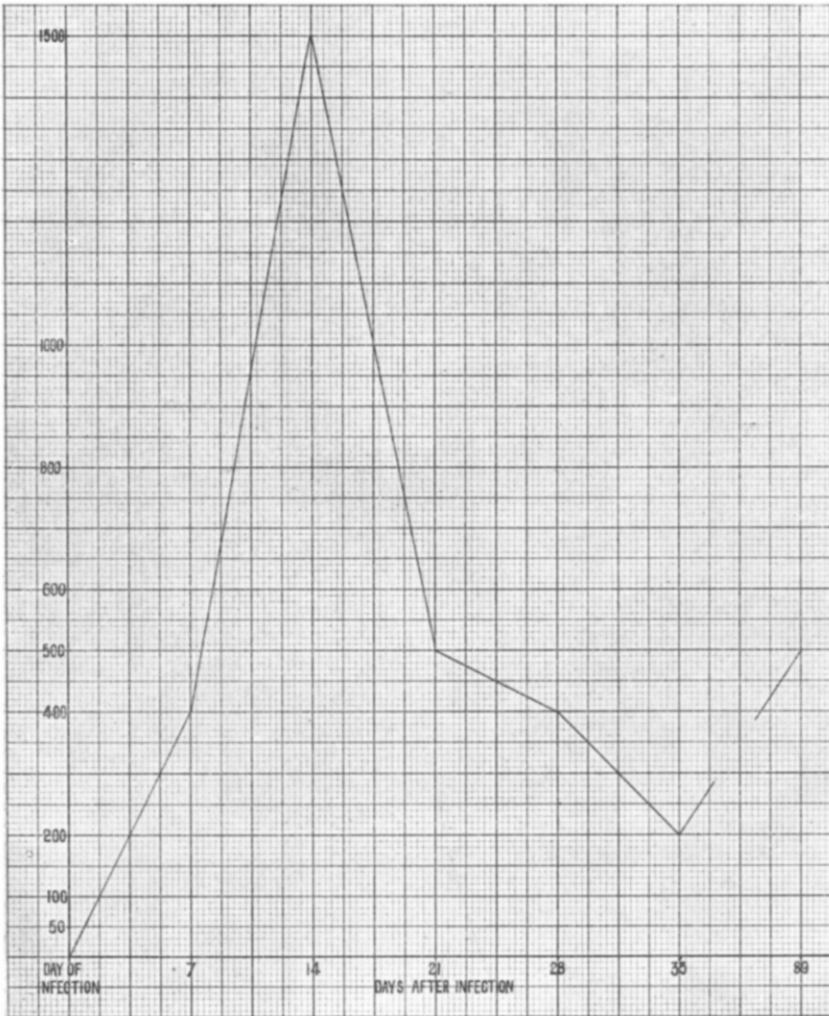


CHART I. Yearling No. 3, infected intravenously with one tube of culture.

On the 16th March an emulsion or mixture of abortion bacilli was prepared from surface growths on sloped serum agar. The tubes measured $\frac{5}{8}$ th inch in diameter, and the slopes were about 3 inches long. The cultures had been incubated at 38° C. for three days. The emulsion was made with normal saline solution in

the proportion of 1 cc. to each tube, and the mixed product from all the tubes was used to infect the ten yearlings as shown below.

<i>No. of Animal.</i>	<i>Sex.</i>	<i>Tubes of Culture.</i>	<i>Method of Infection.</i>
2	Male	1	Subcutaneous
3	"	1	Intravenous
13	"	2	Subcutaneous
21	"	2	Intravenous
24	"	4	Subcutaneous.
26	"	6	"
27	"	8	"
28	Female	2	Mouth
29	Male	2	"
1	Female	2	Vagina

In infecting No. 1 the syringe, without any needle, was introduced for about 4 inches into the genital passage, but it was estimated that about half the liquid escaped when the syringe was withdrawn.

In the case of Nos. 28 and 29 the jaws were separated with

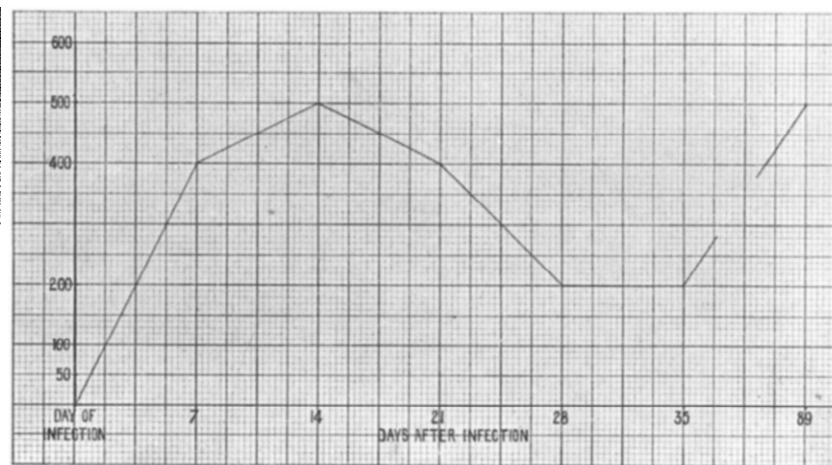


CHART II. Yearling No. 21, infected intravenously with two tubes of culture.

the long axis of the head in a horizontal position, and the emulsion was injected into the mouth by means of the syringe.

After infection blood was taken from each of the animals at intervals of seven days until the end of the fifth week, and submitted to the agglutination and complement tests. After a further interval of fifty-four days the blood was again submitted to the agglutination test, viz., on the eighty-ninth day from the date of infection. The results of all these tests are shown in the following tables.

[TABLES.

TABLE I.

Tests, 23rd April, Seven Days after Infection.

No.	Method of Infection.	Dose of Bacilli.	Agglutination.					Complement.*		
			20	40	100	200	400	2	3	5
2	Subcutaneous	1 tube	comp.	n. comp.	dist.	neg.	neg.	recog.	neg.	neg.
3	Intravenous	1 "	comp.	comp.	comp.	comp.	n. comp.	comp.	n. comp.	recog.
13	Subcutaneous	2 tubes	comp.	comp.	comp.	n. comp.	recog.	neg.	neg.	neg.
21	Intravenous	2 "	comp.	comp.	comp.	comp.	n. comp.	comp.	comp.	comp.
24	Subcutaneous	4 "	comp.	comp.	comp.	n. comp.	n. comp.	comp.	comp.	comp.
26	Do.	6 "	comp.	comp.	adv.	neg.	neg.	n. comp.	neg.	dist.
27	Do.	8 "	adv.	n. comp.	comp.	comp.	n. comp.	comp.	comp.	comp.
28	Mouth	2 "	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.
29	Do.	2 "	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.
1	Vagina	2 "	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.
100	Control cow which had aborted	—	comp.	comp.	comp.	recog.	neg.	comp.	comp.	comp.

* The columns under this heading show the extent to which complement was absorbed in the different tubes. For explanation of the contractions, see p. 143.

TABLE II.
Tests, 30th April, Fourteen Days after Infection.

No.	Method of Infection.	Dose of Bacilli.	Agglutination.										Complement.			
			20	40	50	100	200	400	500	600	800	1000	1500	3	5	7
2	Subcutaneous . .	1 tube	—	—	comp.	comp.	comp.	comp.	comp.	comp.	n. comp.	n. comp.	recog.	neg.	recog.	recog.
3	Intravenous . .	1 "	—	—	comp.	comp.	comp.	comp.	n. comp.	n. comp.	n. comp.	n. comp.	neg.	neg.	neg.	neg.
13	Subcutaneous . .	2 tubes	—	—	comp.	comp.	comp.	comp.	comp.	n. comp.	n. comp.	dist.	comp.	comp.	comp.	comp.
21	Intravenous . .	2 "	—	—	adv.	adv.	n. comp.	n. comp.	n. comp.	adv.	adv.	n. comp.	recog.	comp.	comp.	recog.
24	Subcutaneous . .	4 "	—	—	dist.	n. comp.	n. comp.	n. comp.	comp.	comp.	comp.	comp.	recog.	neg.	neg.	recog.
26	Do. . .	6 "	—	—	comp.	comp.	n. comp.	adv.	adv.	adv.	neg.	neg.	comp.	neg.	neg.	neg.
27	Do. . .	8 "	—	—	comp.	comp.	comp.	comp.	n. comp.	n. comp.	adv.	recog.	comp.	dist.	neg.	neg.
28	Mouth . .	2 "	neg.	neg.	—	neg.	neg.	neg.	—	—	—	—	neg.	neg.	neg.	neg.
29	Do. . .	2 "	comp.	comp.	—	adv.	recog.	neg.	—	—	—	—	neg.	neg.	neg.	neg.
1	Vagina . .	2 "	n. comp.	adv.	—	recog.	neg.	neg.	—	—	—	—	neg.	neg.	neg.	neg.
100	Control aborted cow .	—	—	—	comp.	comp.	adv.	recog.	neg.	neg.	neg.	neg.	comp.	neg.	neg.	neg.

Note.—A tube with two units of complement was also included in this series, but the results in that tube have not been included in the table, because at the time when the tubes were examined (one hour after adding the sensitised corpuscles) hæmolysis was incomplete in tube 1 of the "A" series of controls (*see* p. 145).

TABLE III.
Tests, 7th May, Twenty-one Days after Infection.

No.	Method of Infection.	Dose of Bacilli.	Agglutination.										Complement.			
			50	100	200	400	500	600	800	1000	1500	2000	3	5	7	10
2	Subcutaneous . .	1 tube	comp.	comp.	comp.	comp.	comp.	comp.	n. comp.	adv.	adv.	recog.	recog.	neg.	neg.	neg.
3	Intravenous . .	1 "	comp.	comp.	comp.	n. comp.	n. comp.	adv.	adv.	dist.	dist.	recog.	comp.	comp.	dist.	neg.
13	Subcutaneous . .	2 tubes	comp.	comp.	comp.	n. comp.	n. comp.	n. comp.	adv.	adv.	recog.	neg.	neg.	neg.	neg.	neg.
21	Intravenous . .	2 "	comp.	comp.	n. comp.	n. comp.	adv.	adv.	recog.	recog.	recog.	neg.	comp.	comp.	n. comp.	dist.
24	Subcutaneous . .	4 "	comp.	comp.	comp.	comp.	comp.	comp.	n. comp.	n. comp.	n. comp.	adv.	comp.	trace	neg.	neg.
26	Do. . .	6 "	comp.	comp.	n. comp.	recog.	recog.	neg.	neg.	neg.	neg.	neg.	comp.	comp.	comp.	trace
27	Do. . .	8 "	comp.	comp.	comp.	comp.	n. comp.	adv.	recog.	recog.	neg.	neg.	recog.	recog.	neg.	neg.
28	Mouth . .	2 "	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	trace	neg.	neg.	neg.
29	Do. . .	2 "	comp.	comp.	n. comp.	adv.	recog.	neg.	neg.	neg.	neg.	neg.	trace	neg.	neg.	neg.
1	Vagina . .	2 "	n. comp.	recog.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	trace	neg.	neg.	neg.

Note.—A tube with two units of complement was also included in this test, but the results in that were ignored for the reason mentioned in the note to the previous test.

TABLE IV.
Test, 14th May, Twenty-eight Days after Infection.

No.	Method of Infection.	Dose of Bacilli.	Agglutination.								Complement.					
			50	100	200	400	500	600	800	1000	1500	2000	3	5	7	10
2	Subcutaneous .	1 tube	comp.	comp.	comp.	n. comp.	n. comp.	n. comp.	adv.	adv.	recog.	recog.	dist.	neg.	neg.	neg.
3	Intravenous .	1 "	comp.	comp.	comp.	n. comp.	adv.	adv.	recog.	recog.	neg.	neg.	comp.	comp.	neg.	neg.
13	Subcutaneous .	2 tubes	comp.	comp.	comp.	n. comp.	adv.	adv.	recog.	neg.	neg.	neg.	comp.	neg.	neg.	neg.
21	Intravenous .	2 "	comp.	comp.	comp.	adv.	adv.	adv.	recog.	neg.	neg.	neg.	comp.	comp.	comp.	comp.
24	Subcutaneous .	4 "	comp.	comp.	comp.	comp.	comp.	n. comp.	n. comp.	adv.	dist.	dist.	dist.	neg.	neg.	neg.
26	Do.	6 "	comp.	comp.	n. comp.	adv.	dist.	recog.	neg.	neg.	neg.	neg.	comp.	n. comp.	neg.	neg.
27	Do.	8 "	comp.	comp.	comp.	n. comp.	adv.	adv.	dist.	recog.	neg.	neg.	dist.	neg.	neg.	neg.
28	Mouth .	2 "	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.
29	Do.	2 "	comp.	comp.	adv.	recog.	neg.	neg.	neg.	neg.	neg.	neg.	trace	neg	neg.	neg.
1	Vagina .	2 "	comp.	comp.	comp.	adv.	adv.	adv.	recog.	neg.	neg.	neg.	comp.	neg.	neg.	neg.
100	Control aborted cow.	—	—	—	—	—	—	—	—	—	—	—	comp.	neg.	neg.	neg.

TABLE V.
Test 21st May, Thirty-five Days after Infection.

No.	Method of Infection.	Dose of Bacilli.	Agglutination.								Complement.	
			50	100	200	400	500	600	800	1000	3	5
2	Subcutaneous	1 tube	comp.	comp.	comp.	n. comp.	n. comp.	adv.	adv.	dist. ¹	n. comp.	trace
3	Intravenous	1 "	comp.	comp.	n. comp.	dist.	recog.	neg.	neg.	neg.	comp.	n. comp.
13	Subcutaneous	2 tubes	comp.	comp.	comp.	comp.	n. comp.	adv.	recog.	neg.	n. comp.	trace
21	Intravenous	2 "	comp.	comp.	n. comp.	recog.	neg.	neg.	neg.	neg.	comp.	n. comp.
24	Subcutaneous	4 "	comp.	comp.	comp.	comp.	n. comp.	adv.	dist.	recog.	comp.	n. comp.
26	Do.	6 "	comp.	comp.	n. comp.	dist.	recog.	neg.	neg.	neg.	comp.	comp.
27	Do.	8 "	comp.	comp.	comp.	adv.	recog.	neg.	neg.	neg.	dist.	tr
28	Mouth	2 "	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.
29	Do.	2 "	comp.	comp.	adv.	neg.	neg.	neg.	neg.	neg.	dist.	trace
1	Vagina	2 "	recog.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	comp.	dist.
100	Control aborted cow	—	—	—	—	—	—	—	—	—	n. comp.	dist.

¹ Agglutination was also recognisable in tubes 1500 and 2000 with this animal's serum.

TABLE VI.
Test 15th July, Eighty-nine Days after Infection.

No.	Method of Infection.	Dose of Bacilli.	Agglutination.				
			50	100	200	400	500
2	Subcutaneous . .	1 tube	comp.	n. comp.	adv.	adv.	recog.
3	Intravenous . .	1 „	comp.	comp.	comp.	comp.	comp.
13	Subcutaneous . .	2 tubes	comp.	n. comp.	adv.	recog.	neg.
21	Intravenous . .	2 „	comp.	comp.	comp.	comp.	comp.
24	Subcutaneous . .	4 „	n. comp.	adv.	neg.	neg.	neg.
26	Do. . .	6 „	comp.	dist.	neg.	neg.	neg.
27	Do. . .	8 „	comp.	n. comp.	adv.	neg.	neg.
28	Mouth . . .	2 „	neg.	neg.	neg.	neg.	neg.
29	Do. . . .	2 „	adv.	neg.	neg.	neg.	neg.
1	Vagina . . .	2 „	neg.	neg.	neg.	neg.	neg.

No further observations were made on any of the animals of this series after eighty-nine days except in the case of No. 21 (infected intravenously with two tubes of cultures). Blood was taken from this animal on the 2nd December and the serum was tested on the following day, with the result that agglutination was complete in 1 in 50 and 1 in 100, and advanced in 1 in 200, which proved to be the limit.

Consideration of the Results.

It was, of course, inevitable that the serum of all the animals inoculated subcutaneously or intravenously would after an interval react to the agglutination and complement tests, but it was not certain that the attempt to infect by the vagina or mouth would succeed. There seems to be no room for doubt that the attempt failed in No. 28, which received two tubes of culture by the mouth, and succeeded in the fellow animal (No. 29) and in No. 1, which received two tubes of culture *per vaginam*.

The escape of No. 28 is indicated by the fact that its serum never caused agglutination, and that, except on one occasion (twenty-first day), the result of the complement test with its serum was also entirely negative. On that one occasion there was only a trace of absorption in the tube containing the smallest amount of complement.

The effects, as indicated by the results of the agglutination tests, in the case of each of the nine infected animals may next be considered, taking in the first place the two (Nos. 3 and 21) that were inoculated intravenously. The accompanying charts¹ show that

¹ In constructing the charts no account has been taken of any lesser effect than "nearly complete" agglutination, but it must be remembered that beyond this in many cases there was manifest agglutination described by the terms "advanced" or "distinct."

No. 3, inoculated with only one tube of culture, developed a high power of agglutination (*see* Chart I., p. 147), which reached its maximum on the fourteenth day and then fell steadily during the next three weeks. At the end of three months from the date of infection the agglutinin content of the blood had again risen con-

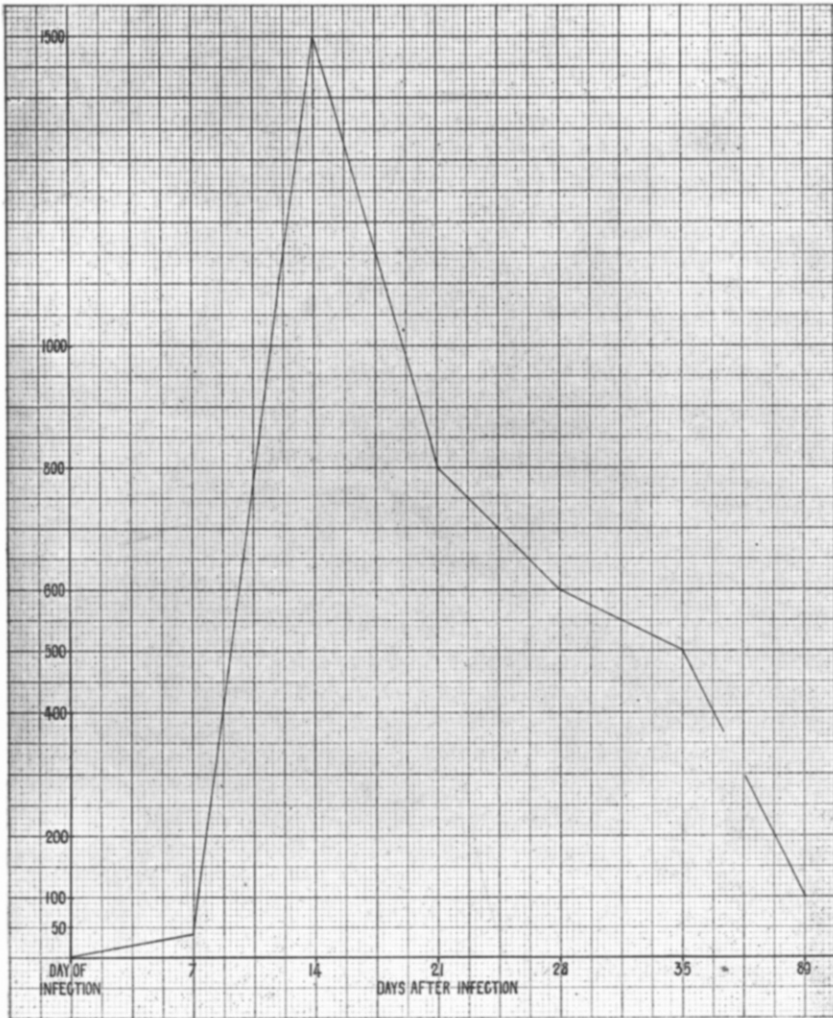


CHART III. Yearling No. 2, infected subcutaneously with one tube of culture.

siderably, and then stood higher than it usually does in naturally infected cows immediately after the act of abortion.

No. 21, which received intravenously double the dose given to No. 3, did not develop nearly so high a power of agglutination, but the maximum (1 in 500) was reached on the same day (fourteenth), and afterwards fell steadily during the next three weeks (*see*

Chart II., p. 148). A point of similarity with the other animal inoculated intravenously is that three months after infection the power of agglutination had again risen and then stood at 1 in 500.

The five animals infected by subcutaneous inoculation were: No. 2, one tube; No. 13, two tubes; No. 24, four tubes; No. 26, six tubes; and No. 27, eight tubes; and the accompanying charts show in a graphic manner the very different way in which they "reacted" to the infection, as indicated by the agglutination test. Not the least interesting fact is that No. 2, which received the smallest dose, developed a power of agglutination which was equalled by only two other animals, viz., No. 3, which received one tube of culture into

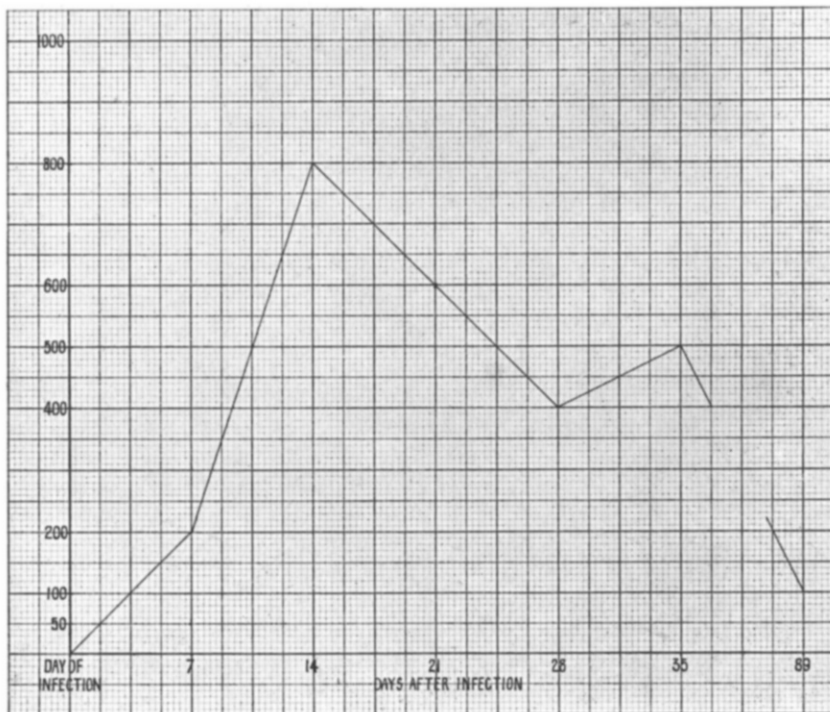


CHART IV. Yearling No. 13, infected subcutaneously with two tubes of culture.

the jugular vein, and No. 24, which was inoculated subcutaneously with four tubes of culture.

No. 24, as a result of subcutaneous inoculation with four times the dose given to No. 2, developed the same agglutinating power, and the only material difference between the charts of these two animals is the more rapid fall in the case of No. 2.

A point difficult of explanation is that No. 13, which in respect of the dose used for infection occupied an intermediate position between No. 2 and No. 24, developed a much lower power of agglutination than either of these.

Among the animals infected subcutaneously the one which developed least agglutinin in its blood was No. 26, which was

inoculated with the six tubes of culture. Its highest mark for "nearly complete" agglutination was 1 in 200, which was also the highest point touched by No. 1, infected *per vaginam* with one tube, and No. 29, infected *per os* with two tubes.

It is of special interest to compare the charts of the two animals

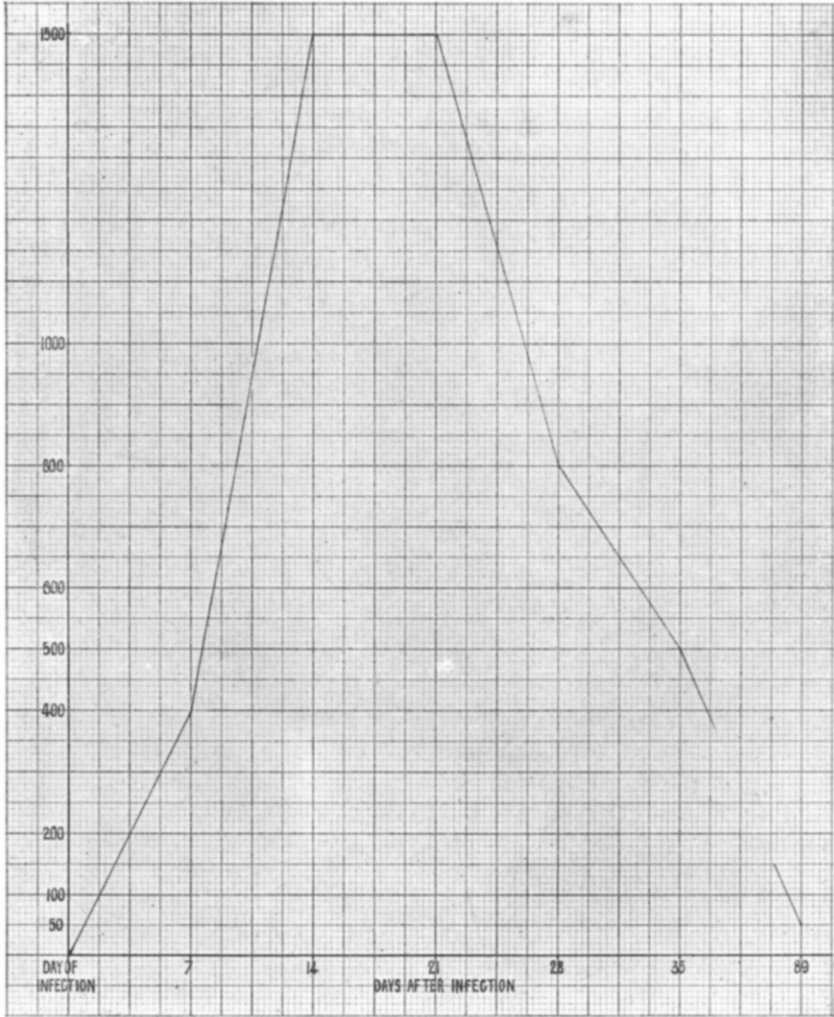


CHART V. Yearling No. 24, infected subcutaneously with four tubes of culture.

inoculated into the jugular vein with those of the two which were inoculated subcutaneously with the same doses.

No. 2 and No. 3 each received one tube, the former subcutaneously and the latter into the vein. Their charts are almost identical, except that a higher point had been reached at the end of the first week in the case of the animal infected intravenously, and that there

was a decline between the thirty-fifth and eighty-ninth days in the one inoculated subcutaneously.

A comparison of the chart of No. 13, which received two tubes subcutaneously, with that of No. 21, which was given the same dose into the jugular, shows that the higher point was reached by the former, but that the latter had the advantage of a rise between the thirty-fifth and eighty-ninth days.

Turning next to the results of the complement tests, it may be said that these were upon the whole concordant with the results of the agglutination test. It has not been thought necessary to give here the results of the complement tests of each animal's serum in the form of a chart, partly because we do not believe that successive complement tests can be absolutely trusted to measure variations in the amount of sensitiser unless these are very considerable. We have, however, for our own information constructed such charts; and these justify the statement just made, viz., that the results of the agglutination and complement tests were broadly concordant. Indeed,



CHART VI. Yearling No. 26, infected subcutaneously with six tubes of culture.

in most of the cases this concordance was striking, the agreement including the height of the reaction to the two tests and the time at which the highest point was touched. In only two cases were the results very divergent, viz., in Nos. 3 and 13. In No. 3 there was no absorption of complement on the fourteenth day, although the agglutinin content of the same serum was very high ("nearly complete" agglutination in 1 in 1500). During the following week the power of agglutination fell very considerably, whereas the amount of sensitiser increased, and it remained high during the two succeeding weeks, although the agglutinin content continued to fall.

In No. 13 the result of the complement test, although positive on the fourteenth day (coinciding with the highest point of the agglutinin curve), was negative on the twenty-first day, at which time the agglutinating power of the serum was still high.

Leaving out of account No. 28, which did not become infected, the tables show that as early as the seventh day all the animals infected by intravenous or subcutaneous inoculation reacted distinctly to the agglutination test, whereas the first reaction in the case of No. 29, infected by the mouth, was obtained on the fourteenth day,

at which time there was also an appreciable reaction with the serum of No. 1, infected *per vaginam*.

A reaction to the complement test was obtained on the seventh day in all the animals infected subcutaneously or intravenously, with the exception of No. 13, which had received two tubes of culture subcutaneously. No. 29, infected by the mouth, did not react to the complement test till the twenty-first day, although the agglutination had been positive (1 in 100) a week previously. In No. 1 also, infected *per vaginam*, the complement test was not positive before the twenty-first day, although the agglutination test had given evidence of infection on the fourteenth day.

We defer for later consideration the question whether the results of this series of experiments justify any conclusion regarding natural

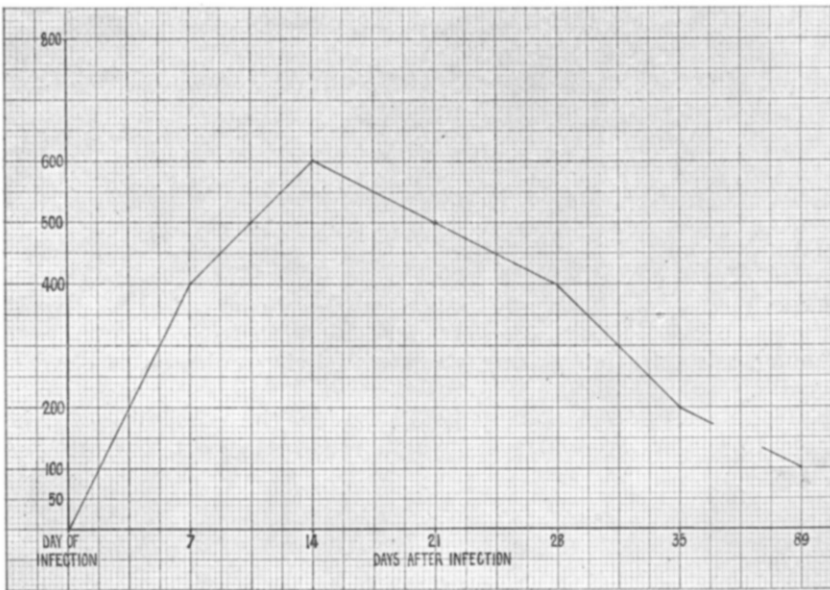


CHART VII. Yearling No. 27, infected subcutaneously with eight tubes of culture.

infection with contagious abortion, and the only other point requiring notice touches the question of immunity.

There is an obvious difficulty in the way of measuring the degree of an animal's immunity against contagious abortion, arising from the fact that the bacillus which is the cause of it is scarcely pathogenic except in the pregnant female. The test which would justify the most confident conclusion cannot be carried out in male subjects or in females that are not pregnant.

If, however, one accepts the agglutinating power of the serum as a measure of immunity, the experiments show that a high degree of immunity can be produced by experimental inoculation.

Experience appears to warrant the opinion that of cows that become naturally infected with the bacillus of contagious abortion a decided majority make a complete recovery soon after the expulsion

of the foetus, and acquire a degree of immunity which is sufficient to prevent their reinfection during the immediately succeeding pregnancy. Such appears to be the rule, although it is undeniable that it is subject to a considerable number of exceptions. There is also already sufficient experience to show that the agglutinin content of the blood immediately after the act of abortion in cows

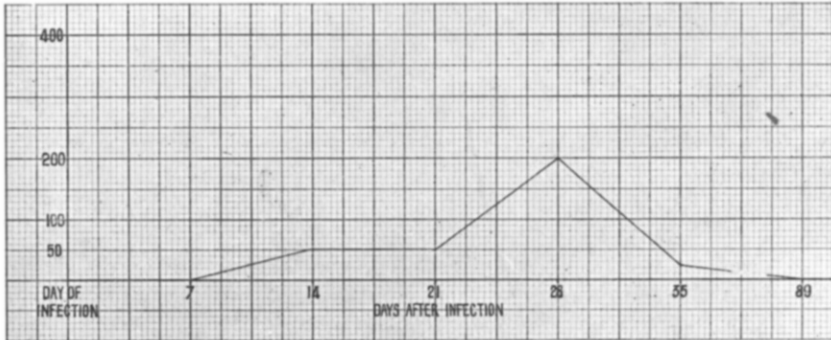


CHART VIII. Yearling No. 29, infected *per os* with two tubes of culture.

naturally infected is generally not higher than it was in some of the cases of experimental infection dealt with in this article. The experiments may therefore be said to be encouraging as regards the prospects of being able to confer a useful degree of immunity on young cattle by inoculating them with living cultures of abortion bacilli.

There are, of course, many points besides the possibility of being able to confer immunity which will require to be taken into con-

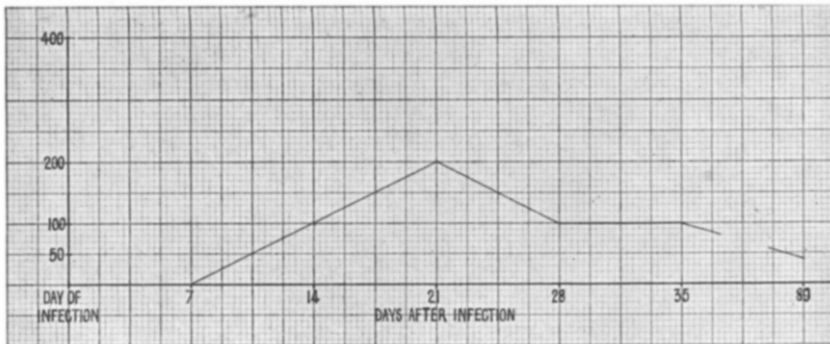


CHART IX. Yearling No. 1, infected *per vaginam* with two tubes of culture.

sideration before deciding to recommend the inoculation of healthy cattle with the living bacilli of contagious abortion.

The before-described experiments are perhaps not sufficiently numerous to warrant hard and fast conclusions regarding the best method of inoculating healthy cattle with a view to protecting them against the risks of natural infection, but, assuming again that the

agglutinin content of the blood affords any index to an animal's immunity, it would appear that it is not necessary, and not even advantageous, to inoculate with large doses of bacilli.

In this connection it is interesting to compare again the charts of the animals inoculated with the same dose of bacilli, but by different methods.

Nos. 2 and 3 were each inoculated with one tube of culture, the former subcutaneously and the latter into the jugular vein. The charts are very similar, but it will be observed that in the case of No. 3 the agglutinating power had risen considerably between the thirty-fifth and eighty-ninth days, whereas in the other animal it had fallen. A similar difference is observable between the charts of No. 13, inoculated subcutaneously with two tubes of culture, and that of No. 21, inoculated intravenously with the same dose of bacilli. Our observations afford no information concerning the fate of the inoculated bacilli or the period for which they persist in any part of the body, but the form of the curve in the two animals inoculated intravenously rather suggests that in them the bacilli had remained for a longer time active in the system than in the case of those infected by subcutaneous inoculation.

SERIES II.

On the 6th May 1912 an attempt was made to infect the following seven animals:—

<i>No. of Animal.</i>	<i>Sex.</i>	<i>Tubes of Culture.</i>	<i>Method of Infection.</i>
11	Bull	1	Prepuce
25	"	1	"
19	"	1	Mouth
6	Heifer	1	"
54	"	1	Vagina
Ayrshire	Cow	1	Mouth
Welsh	"	1	"

Nos. 11, 25, 19, 6, and 54 were yearlings. The Ayrshire cow had aborted on the 3rd February 1911, and had not since been put to the bull. The Welsh cow was an old animal which had been at the College for a year and was non-pregnant.

The material used for infection was the mixed product of seven tubes of culture (*see* p. 147) which had been incubated at 38° C. for three days. The growth was washed off with normal saline solution, 1 cc. to each tube, and each animal thus received 1 cc. of the mixed emulsion. In the case of Nos. 11, 25, and 54 the dose was diluted with 3 cc. of normal saline solution before it was injected into the prepuce or vagina.

In infecting Nos. 11 and 25 a piece of flexible rubber tubing was attached to the syringe, and introduced for about 5 inches into the prepuce. While the liquid was being injected the orifice of the prepuce was closed with the fingers, and after the rubber tube had been withdrawn the prepuce was massaged gently in the backward direction in order to distribute the emulsion throughout the preputial cavity. The animals were cast for the operation, and

little or none of the emulsion escaped from the prepuce when they were allowed to get up.

Exactly the same procedure was adopted in the case of No. 54, the emulsion being injected about 5 inches into the vagina, but about half the liquid escaped when the lips of the vulva were released.

In the other animals the mouth was held open and the dose of emulsion was deposited on the dorsum of the tongue.

Before the attempt to infect these animals was made blood had been taken (on the 6th May) from each of them, and the serum was submitted to the complement and agglutination tests. The results were negative in all the animals with the exception of the Ayrshire cow, in which both tests were positive. Agglutination was complete in 1 in 100, and advanced in 1 in 200, but not recognisable in 1 in 400. A week previously (30th April) this cow's serum had given a similar result, except that agglutination was recognisable in 1 in 400. Attention may be called to the fact that this was fifteen

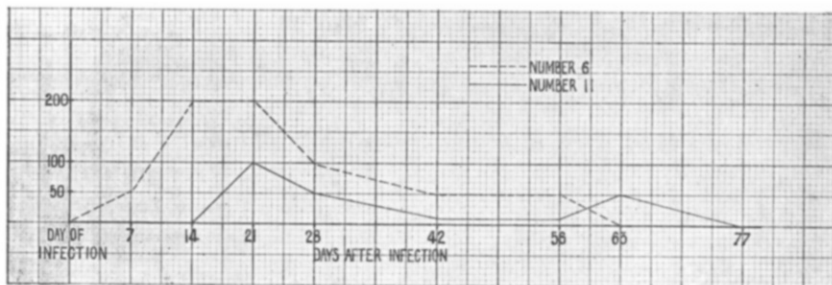


CHART X. Yearling No. 6, infected by the mouth, and yearling No. 11, infected by the prepuce.

months after the cow had aborted. This cow was included in the experiment in order to see whether one could cause a re-infection and recognise it by an increased agglutinating power of the serum.

Blood was taken from these animals on the seventh, fourteenth, twenty-first, twenty-eighth, forty-second, fifty-sixth, sixty-third, and seventy-seventh days after the attempt to infect them was made, and the serum was submitted to the agglutination test, with the results shown in the following tables (*see pp. 163, 164*).

Consideration of the Results.

The agglutination tests which were carried out from the seventh to the seventy-seventh day after the attempt to infect had uniformly negative results in the case of the yearling heifer No. 54, which received the emulsion of abortion bacilli *per vaginam*, and in the aged Welsh cow, to which it was given by the mouth. It may therefore be concluded that the attempt to infect these two animals failed.

Similarly, it may probably be inferred that the Ayrshire cow which had aborted fifteen months previously was not re-infected by

No.	Method of Infection.	13th May 1912.				20th May 1912.							
		50	100	200	400	50	100	200	400	500	600	800	1000
11	Prepuce	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.
25	Do.	neg.	neg.	neg.	neg.	comp.	n. comp.	recog.	neg.	neg.	neg.	neg.	neg.
54	Vagina	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.
19	Mouth	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.
6	Do.	n. comp.	recog.	neg.	neg.	comp.	comp.	n. comp.	adv.	adv.	dist.	recog.	neg.
Ayrshire cow	Do.	comp.	comp.	n. comp.	adv.	comp.	n. comp.	recog.	neg.	neg.	neg.	neg.	neg.
Welsh cow	Do.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.

No.	Method of Infection.	27th May 1912.					3rd June 1912.				
		50	100	200	400	500	50	100	200	400	500
11	Prepuce	comp.	comp.	adv.	neg.	neg.	n. comp.	adv.	recog.	neg.	neg.
25	Do.	comp.	comp.	n. comp.	recog.	neg.	comp.	comp.	comp.	recog.	neg.
54	Vagina	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.
19	Mouth	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.
6	Do.	comp.	comp.	n. comp.	neg.	neg.	comp.	comp.	adv.	recog.	neg.
Ayrshire cow	Do.	comp.	n. comp.	adv.	neg.	neg.	comp.	n. comp.	adv.	neg.	neg.
Welsh cow	Do.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.

No.	Method of Infection.	17th June 1912.						1st July 1912.					
		50	100	200	400	500	600	50	100	200	400	500	600
11	Prepuce . . .	adv.	recog.	neg.	neg.	neg.	neg.	dist.	neg.	neg.	neg.	neg.	neg.
25	Do. . . .	comp.	comp.	n. comp.	recog.	recog.	neg.	comp.	dist.	neg.	neg.	neg.	neg.
54	Vagina . . .	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.
19	Mouth . . .	neg.	neg.	neg.	neg.	neg.	neg.	comp.	dist.	neg.	neg.	neg.	neg.
6	Do. . . .	comp.	recog.	neg.	neg.	neg.	neg.	comp.	recog.	neg.	neg.	neg.	neg.
Ayrshire cow	Do. . . .	comp.	comp.	n. comp.	recog.	neg.	neg.	comp.	comp.	n. comp.	adv.	recog.	neg.
Welsh cow	Do. . . .	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.

No.	Method of Infection.	8th July 1912.					22nd July 1912.		
		50	100	200	400	500	50	100	200
11	Prepuce	n. comp.	adv.	neg.	neg.	neg.	adv.	recog.	neg.
25	Do.	dist.	recog.	neg.	neg.	neg.	n. comp.	neg.	neg.
54	Vagina	neg.	neg.	neg.	neg.	neg.	Not tested.		
19	Mouth	comp.	recog.	neg.	neg.	neg.	n. comp.	recog.	neg.
6	Do.	recog.	neg.	neg.	neg.	neg.	adv.	neg.	neg.
Ayrshire cow	Do.	comp.	comp.	adv.	dist.	neg.	Not tested.		
Welsh cow	Do.	neg.	neg.	neg.	neg.	neg.	Not tested.		

the dose of culture given by the mouth. Immediately before the experiment was made this cow's serum still caused complete agglutination with a dilution of 1 in 100, and afterwards the same effect was never produced by any weaker dilution. It is true that on some occasions partial agglutination was caused in more dilute mixtures, but the difference was not greater than one may find with any infected animal's serum in two successive tests with a short interval.

In the remaining four animals the results of the agglutination tests indicate infection, as may be seen more clearly from the accompanying charts (Nos. X. and XI.).

The result was most decisive in the case of bull No. 25, which was infected by the prepuce, and heifer No. 6, which was infected by the mouth. In each of these animals the serum was found to cause complete or nearly complete agglutination in 1 in 200, this point being reached on the fourteenth day in No. 6 and on the twenty-first day in No. 25. If one applies the term "incubative period" to the interval which elapses before the test shows agglutinin to be present

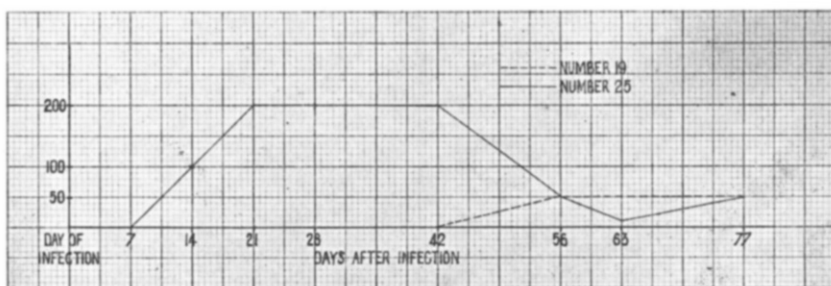


CHART XI. Yearling No. 19, infected by the mouth, and yearling No. 25, infected by the prepuce.

in amount above the normal, then in both these animals it was not more than a week.

It must be concluded that the other bull (No. 11) which received the culture into the prepuce also became infected, although complete agglutination did not occur in any weaker dilution than 1 in 100, and the period of incubation was something between fourteen and twenty-one days.

In the case of bull No. 19, which was given culture by the mouth, we think that the results of the agglutination tests point with some probability to an actual infection, although complete agglutination occurred only in 1 in 50. Assuming this view to be correct, it is remarkable that there was no evidence of infection until after the sixth week.

The yearlings Nos. 11, 25, 19, and 6 were throughout the experiment kept together in the same loose-box, and with them there was another yearling (steer No. 59) which was not included in the experiment. The late agglutination in No. 19 suggested that infection might have occurred naturally from one of the experimentally infected animals, and the serum of No. 59 was therefore tested on the 22nd July (after eleven weeks of contact), but with entirely negative result. It need hardly be said that we do not regard this result as proof that No. 19 did not become infected through contact.

Unquestionably the chief interest in this experiment attaches to the two bulls that became infected in consequence of the injection of the abortion bacilli into the prepuce. The view that contagious abortion is frequently spread by the agency of the bull in the act of copulation is very widely held, but it has generally been supposed that in such cases the transmission is of a mechanical character, the bacilli being simply transferred from the vagina of a diseased cow to that of a healthy one by means of the penis.

As soon as it was found that animals of either sex could be infected *per os* and young non-pregnant females *per vaginam* it was natural to suppose that infection by the prepuce might also be possible, and that must now be regarded as proved.

It would be easy to draw from these experiments far-reaching conclusions regarding the rôle of the bull in the dissemination of infection. In reality, however, they do not justify any conclusion regarding the frequency of infection through the bull as compared with infection by other means, but they indicate the propriety of testing the blood of the bulls, as well as of the cows and heifers, in dealing with outbreaks.

In conclusion, it ought to be said that the injection of the abortion bacilli into the prepuce did not occasion any recognisable catarrh of the lining membrane.

SERIES III.

On the 11th June 1912 the growth of abortion bacilli on twelve tubes of sloped serum-agar was made into an emulsion with normal saline solution and used as shown below.

No.	Sex.	Method of Infection.	Dose of Emulsion.
22	Heifer	Vagina	2½ cc.
39	"	"	1 cc.
30	Steer	Prepuce	"
10	"	"	"
37	"	"	"
42	"	"	"
44	"	"	"
23	"	Mouth	"
47	"	"	"
50	"	"	"

All these animals were about a year and a half old. The emulsion was administered as described in connection with the preceding series. The whole of the liquid injected into the prepuce appeared to be retained, but about half the dose escaped from the vagina in Nos. 22 and 39.

On the previous day blood had been taken from each of the animals, and in each case the complement and agglutination tests when applied to the serum had negative results.

The animals were bled seven, fourteen, twenty-one, and twenty-eight days after the attempt to infect, and the serum was tested with the results shown in the following table. The Ayrshire cow is the same animal as was included under that description in the previous series, and it was here used only as a control to the agglutination tests.

No.	Method of Infection.	18th June 1912.			25th June 1912.			2nd July 1912.			9th July 1912.		
		50	100	200	50	100	200	50	100	200	50	100	200
22	Vagina . . .	neg.	neg.	neg.	n. comp.	recog.	neg.	dist.	recog.	neg.	n. comp.	adv.	recog.
39	Do. . . .	neg.	neg.	neg.	comp.	n. comp.	neg.	comp.	adv.	recog.	comp.	comp.	adv.
30	Sheath . . .	neg.	neg.	neg.	neg.	neg.	neg.	recog.	neg.	neg.	adv.	neg.	neg.
10	Do. . . .	neg.	neg.	neg.	n. comp.	recog.	neg.	neg.	neg.	neg.	recog.	neg.	neg.
37	Do. . . .	neg.	neg.	neg.	comp.	adv.	recog.	comp.	neg.	neg.	comp.	adv.	recog.
42	Do. . . .	neg.	neg.	neg.	neg.	neg.	neg.	n. comp.	recog.	neg.	dist.	neg.	neg.
44	Do. . . .	neg.	neg.	neg.	n. comp.	recog.	neg.	n. comp.	recog.	neg.	n. comp.	recog.	neg.
23	Mouth . . .	adv.	neg.	neg.	neg.	neg.	neg.	recog.	neg.	neg.	n. comp.	recog.	neg.
47	Do. . . .	neg.	neg.	neg.	neg.	neg.	neg.	dist.	recog.	neg.	neg.	neg.	neg.
50	Do. . . .	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.
Ayrshire cow	Do. . . .	comp.	comp.	n. comp.	Not tested.			comp.	comp.	n. comp.	n. comp.	n. comp.	adv.

Consideration of Results.

Apparently the attempt to infect *per vaginam* succeeded in both the cases (Nos. 22 and 39), and there was also evidence of infection in at least four of the five animals which received the bacilli into the prepuce. Of the three animals which were given the emulsion of bacilli by the mouth, one (No. 50) failed to develop any evidence of infection; in one (No. 47) the result was doubtful; and in the third (No. 23) the agglutinating power of the serum reached a height which probably warrants the conclusion that the animal had become infected.

This interpretation of the results is based upon the experience of McFadyean and Stockman,¹ who tested the serum of 535 presumably healthy cattle (steers, bulls, heifers, and calves) and found that in only five of these animals was "complete" or "nearly complete" agglutination caused by a dilution of 1 in 50. And it must be remembered that the possibility that these five animals were actually infected was not excluded. When that is kept in mind it appears to be impossible to account for the numerous instances in which "complete" or "nearly complete" occurs in the preceding table, except by assuming that, at least in the great majority of the cases, the result was in no sense accidental or normal, but attributable to actual infection.

SERIES IV.

This series includes only two experiments, but both of them are of special interest.

Experiment I.

On the 28th July 1912 heifer No. 22 was served by bull No. 11. The first of these was one of the animals included in Series III., and it had been infected *per vaginam* on the 11th June, or forty-seven days before service. The bull was one of the animals included in Series II., and it had been infected on the 6th May by injecting culture into the prepuce.

On the 2nd January 1913 the blood of heifer No. 22 was submitted to the agglutination test, but the result was negative even in the 1 in 50 tube.

It was resolved to hyper-immunise the heifer, and with that object it was given by subcutaneous inoculation repeated and increasing doses of artificial culture of abortion bacilli. The periods at which these injections were given and their effects are shown in Chart XII., and the quantity of culture injected on each of the occasions was as follows:—

- | | | | | | | | |
|-------------------|---------|-----------|-------|---------|-----------|-----------|--------------|
| 1. Jan. 10, 1913. | 5 cc. | glycerine | broth | culture | incubated | at 38° C. | for 3 weeks. |
| 2. Jan. 21, 1913. | 50 cc. | " | " | " | " | " | " |
| 3. Feb. 4, 1913. | 400 cc. | " | " | " | " | " | " |
| 4. Feb. 18, 1913. | 600 cc. | " | " | " | " | " | " |
| 5. Mar. 5, 1913. | 600 cc. | " | " | " | " | " | " |
| 6. Mar. 17, 1913. | 800 cc. | " | " | " | " | " | " |
| 7. Apr. 11, 1913. | 900 cc. | " | " | " | " | " | " |

¹ "Journal of Comparative Pathology and Therapeutics," Vol. XXV., p. 28.

On the 16th May 1913 the animal gave birth to a healthy-looking, well-developed calf. The parturition occurred early on the morning, and the foetal membranes came away naturally during the same day. The whole of the cotyledons and the membranes generally appeared perfectly normal, and no abortion bacilli could be found in these or in material obtained by swabbing the vagina.

Blood was taken from the calf on the 19th May, and the serum when tested was found to cause complete agglutination in 1 in 50.

It must be considered remarkable that this heifer produced a full-term, healthy calf in the circumstances stated, but as the case appears to be absolutely unique, inasmuch as both parents had been infected.

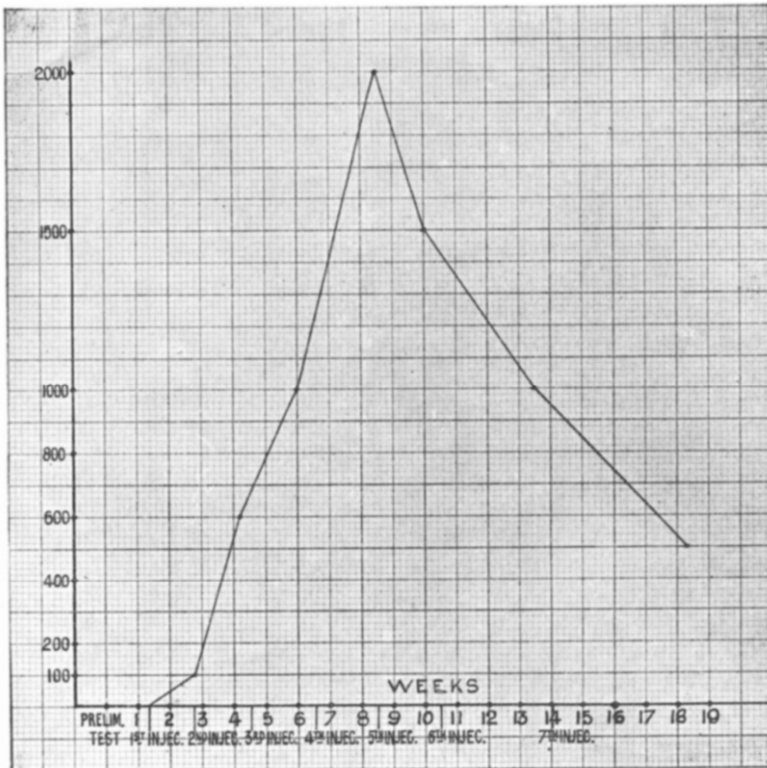


CHART XII. Heifer 22.

not long before the act of copulation, it would be hazardous to draw any general conclusion from it. It appears, however, to be not unlikely that as a result of the intra-vaginal injection on the 11th June, and the consequent infection, the heifer had acquired immunity before the date of service, forty-seven days later. However that may be, it is very curious that a re-infection was not caused by the very large doses of culture administered to the animal during the course of the pregnancy.

It may be added that the heifer's serum still (20th June) possesses high agglutinating power, although the last injection of bacilli was given on the 11th April.

Experiment II.

A cow which had calved normally on the 18th April 1912, and which was suckling her calf, was given one tube of culture of abortion bacilli intravenously on the 23rd May 1912. The object of the experiment was to see whether the calf would become infected by means of the cow's milk or otherwise from contact.

The following table shows the results of agglutination tests of the cow's serum at various dates after infection.

<i>Date.</i>	$\frac{1}{2}$	1	2	4	5
30.5.12	comp.	neg.	neg.	neg.	neg.
6.6.12	comp.	recog.	neg.	neg.	neg.
13.6.12	comp.	comp.	recog.	neg.	neg.
20.6.12	comp.	comp.	n. comp.	n. comp.	adv.
27.6.12	comp.	comp.	comp.	n. comp.	n. comp.
4.7.12	comp.	comp.	comp.	n. comp.	adv.
11.7.12	comp.	comp.	n. comp.	n. comp.	adv.
18.7.12	comp.	comp.	comp.	n. comp.	adv.
12.8.12	comp.	adv.	recog.	neg.	neg.

On all the dates shown in the table serum from the calf was also tested, and with uniformly negative results, there being no recognisable agglutination even in the 1 in 50 tube.

On many occasions since the cow and calf were separated the calf's serum has been tested with similar negative results. The cow's serum was tested on the 20th June of this year, and agglutination was then advanced in 1 in 50, recognisable in 1 in 100, and negative in 1 in 200.

During the course of the experiment no attempt was made to cultivate abortion bacilli from the cow's milk, but, whether they were present or not, the agglutination tests indicate that the calf did not become infected.

CONCLUSIONS.

1. Cattle of any age and either sex may be infected by natural channels with the bacillus of epizootic abortion.
2. Male animals (bulls or steers) can be infected by way of the prepuce.
3. The agglutination and complement tests when applied to the serum of animals experimentally infected yield results that are broadly concordant.
4. Either of these tests may give positive results within from seven to twenty-one days after infection.
5. In animals in which the result of the agglutination test is negative three weeks after the attempt to infect, it generally remains negative, and indicates that the animal has not become infected.