

C.S.

STOCK	o
No.	303/22

1922

Governor

SUBJECT.

192 2

6th April

Previous Paper.

Regarding investigations and bacteriological examinations of Stock on the West Falkland.

MINUTES.

*PA 4/4/22*  
*PA 22/4/22*  
*PA 5/8/22*

*Minute from the Governor d 6<sup>th</sup> April 1922 — Encl (1)*  
*Letter from Mr. Blake d 5<sup>th</sup> February 1922 — Encl (1a)*

*Chief Inspector of Stock*

*Referred*

*[Signature]* 7/4/22

Hon Col Secretary

In answer to the letter from His Exelency the Govern<sup>r</sup> in reference to suspected abortion in sheep at Hill Cove and Port Howard, I beg to state that since the letter was written I have seen both Mr Miller and Mr Evans and they are of the opinion that this matter should be investigated, and they told me that they intended forwarding a request that this should be done.

It would be necessary for me to visit both Pprt Howard and Hill Cove early in August next and remain there until the lambing season was over.

I have everything necessary for bacteriological examinations but it would have to be done on the spot so would have

Subsequent Paper.

have to take the microscope with me, which could be easily arranged.

In answer to (4) there are no slides in this office nor were there any when I took over from Mr Atkins.

*B A Brown*  
Chief Inspector of Stock

24/4/22

*Y.B. Submitted*

*W.H. 20 April '22*

*H.P.S.*

Chief Inspector should arrange to visit West Falkland in August & carry out investigations as he proposes.

*W.H.*

*\* May 1922*

*C.I.S.*

*For note and return*

*W.H. 4/5/22*

*H.P.S.*

*Noted & returned*

*B A Brown*  
Chief Inspector of Stock

5/5/22

*Letter from Mr. S. Miller d 29<sup>th</sup> Apr 1922 — Encl (2)*

*Letter to Mr. S. Miller, 12. 5. 22, Encl. (8)*

*Letter from Mr. R. C. P. Evans 10. 5. 22 — Encl. (4)*

*Check and say  
what is being done  
W.H.*

Letter from R.C. Pole Evans of 10<sup>th</sup> May 1922 — Encl. (4)

Letter to Mr. R.C. P. Evans of 12<sup>th</sup> June 1922 Encl. (5)

Minute by Governor of 13<sup>th</sup> June 1922 — Encl. (6)

Ch. Insp. Stock

Will you please put up  
a programme accordingly

ttttt 18/6/22

Hon Col Secretary

I propose to visit Port Howard and Hill Cove stations for the purpose of investigating supposed Abortion in Sheep, in order to properly carry this out it will be necessary to be on the spot from July till the end of November as Ewes abort from about two months after being tupped up till the normal time of lambing.

I propose to spend most of the time equally between these two stations, leaving the microscope, stains etc at the most convenient place, and carrying the slides pippets etc on horseback, these can then be examined at leisure and if traces are found of Epizootic Abortion they can be forwarded to Sir Stewart Stockman.

I also propose to visit Charters and Port Howard (Packs) with a view of seeing if they are free from Lice. These inspections can be carried out when riding from Port Howard to Hill Cove.

*BA Brown*

Chief Inspector of Stock  
19/6/22

*Y.S.*  
*Submitted*

The time spent on each station is perhaps necessarily dependant on what is found but weekly or fortnightly papers reports should be submitted or a diary kept?

ttttt 19 June

H.O.S.

The Chief Inspector of Stock should furnish weekly reports on progress of his investigation.

2. The reports should be posted, each week, if this is possible, irrespective of whether he has any knowledge where there is likely to be any mail communication.

3. In the absence of any mail communication for four successive weeks the Chief Inspector of Stock should send a brief summary of his reports by wireless: it is intended that these summaries should be as brief as possible but they should contain information as to stations visited & periods spent on each.

H.O.

20 Jan 1922

Ch. Insp. Stock

Accd

11/11 21 Jan

Hon. Col Secretary

Noted

BC/Brown

22/6/22

Report from Chief Inspector of Stock

of 25<sup>th</sup> July 1922

Encl (1)

J.P.  
 Progress Report submitted  
 H.H.H. 2/10/22  
 2 August 1917  
 Letter from Chief Inspector of Stock of 22nd Sept 1917. Encl (8)

J.P.  
 H.H.H. 26/11/22

H.A.S.  
 Will you please ask Chief Inspector  
 of Stock to furnish a report on his  
 investigations up to the time of his  
 leaving West Hillland in the end of October  
 & his conclusion.

copies of the final report of C.I.S. should  
 go to Mr. Evans, Mr. Griffin & Mr. Harding.

C.I.S.  
 For report accordingly please  
 H.H.H. 31/10/22

Hon Colonial Secretary

I beg to state that I thoroughly examined the ewe flocks at Hill Cove and Port Howard during the lambing season, the stud flocks at both stations and three selected flocks at Hill Cove, all these flocks were kept under close personal observation, and all the shepherds on both stations were warned to report at once any signs of abortion among the outside flocks.

Only two cases of abortion were noted at Port Howard these being due to injuries and not Epizootic Abortion

Slides were made of both cases but no signs of the Vibrio of Epizootic Abortion were found .

There were no cases at Hill Cove.

I am quite convinced that this disease dose not exist on these stations

*B. A. Brown*

Chief Inspector of Stock

3.1.23

*M.S.*

*Submitted*

*W. H. H. 5/1/23*

TELEGRAMS: MERRIOTT.  
STATION: CREWKERNE, L.S.W.R.

MARK'S BARN,  
CREWKERNE.

Sunday. Feb 5<sup>th</sup> 1922.

My dear Harding.

These Reports from the Board of Agriculture only reached me last night and after glancing through them I feel convinced the New Island sheep are suffering from a severe attack of enzootic abortion, which must not be confounded with Epizootic. About 1902 I saw something of a bad outbreak of abortion in sheep in England and followed the matter closely. I think it must be 8 or 10 years since I thought the Heildon flocks were infected but could never get confirming evidence from Miller, and I have not been in the Falklands for a Lambing season for 24 years. I believe Evans has given the confirming proofs to my suspicions, but he has done it without knowing, I mean he puts the cause to reasons other than abortion.

I want you to pass these reports to His Excellency if you have an opportunity, if he would interest himself in the matter and send the Stock Inspector to the West about three months before Lambing begins he will be able to observe what happens scientifically. Ewes may begin aborting at two months, or the Lamb may be carried the full time, I have seen both <sup>happen</sup> cases, in the flock I mentioned. I have sent copies of this Report to both Miller and Evans and shall tell them both a copy has gone to you.

THE NATIONAL ARCHIVES  
COLLEGE PARK, MARYLAND  
SERIALS ACQUISITION  
1015 N. E. STREET  
ANNAPOLIS, MARYLAND 21403  
TEL: 410-293-7100

I shall tell one or both of them to ask the Governor to allow  
the Stock Inspector to go to the herd.

I do not think the Company's flocks can be infected  
because the lambing is as good as it was 30 years ago.

Yours sincerely,

Robert Blake.

Dated Feb 25<sup>th</sup>

—Yeabridge,  
—South Pertherton.—

pounds in weight in the preceding with thinking and worrying over the steamer.

By the last mail I sent you some Board of Agriculture Reports on abortion in sheep and asked you to pass these on to the Governor and have a talk with him if you get the chance. I think the trouble in the West is that the sheep are suffering from enzootic abortion, and I asked you to get the Governor to allow the Inspector to spend two or three months in the West and investigate the question. XX

If my surmise is correct it is quite sufficient to account for the bad Lambings. How far Matthews agrees with my conclusions I am not sure, I have sent him sheets of writing but he is a cautious man, and rightly so. There must be a cause for an increase only giving a 55% increase, and I cannot see how the rams can be blamed, they have always done their duty when taken from the West to the East, and in enzootic abortion infection is not carried by the male, while in Epizootic abortion it may be, and often is. Of course I may be wrong in my deductions and reasoning, but the presence of enzootic abortion would amply account for what no man in the Islands can give any reason for.

Of course I have written very fully to Miller about certain changes I want him to make, to see if these will improve the lambing percentages, if these fail we must take more drastic methods. I would give some thing for the purpose of being able to ride again, if I had that I should be out in the Islands again immediately.

By chance my brother had a very bad outbreak of abortion some twenty

+ The Inspector should be in the West before the end of June, and stay there till lambing starts, for ought to be in control during the aborting period.

years ago and I had nothing particular to do then so followed the matter up closely, and corresponded with various authorities about it. As you know was in England lamb in the grass fields probably one is set aside for this purpose, abortion may take place when the ~~part~~ foetus is only two months old, or the lamb may be aborted later, that is how the land gets tainted, but all my investigations go to show that it is after the ewe is in lamb that the abortion bacillus is taken into the system, or at any rate begins to do harm. When the ewes at Bridge were bad my brother was told not to put sheep for a whole year where they had been running during the aborting time, and his ewes have not aborted since. So in the faldlands it appears to me we can sometimes stamp out the disease if drastic remedies are taken, but it may mean we may have to discontinue breeding for a year or two, if no aborted foetus or aborted lamb drops on the land for a year or two the disease may be stamped out.

I am inclined to think if a so called infected flock were taken from the ground where they have been running for years and, put on an absolutely new ground which had not carried sheep before, that the lambing would immediately go up to 70% or over. I have explained all my conclusions to Miller and only hope he may be able to follow my reasoning. It is quite likely the F.I. stock inspector has never come across *Corynebacterium abortus* it may be on only one farm in a County & that farm may not see it again for many years, but when it does appear time does not appear to be much difficulty in stamping it out if the right procedure is followed. On the West it has a bad hold, but from what the Report says and

from what I personally have seen I believe the trouble can be banished.

Yours sincerely,  
Astford Blake.

From Governor to Colonial Secretary.

DATE.....



W. W. A. Harding has given me the two letters, which are attached, and which Mr. R. Blake has written to him on the subject of abortion in sheep on Hills Cove + Port Howard. From the letters it appears that a formal request will be made by Mr. Miller + Mr. Swan that the matter should be investigated by the Chief Inspector of Stock.

2. In anticipation of such a request being made the Chief Inspector of Stock should make all necessary arrangements to visit Hill Island for the purpose of investigating to what extent abortion occurs + whether it is contagious or of a sporadic + accidental character.

3. In the meantime the Chief Inspector of Stock should state whether he has everything which he requires to enable him to carry out bacteriological examinations in connection with <sup>such</sup> investigations.

4. A microscope was obtained some years ago for the C.I.S. and also a collection of slides of diseases of animals. I have seen the microscope but I should be glad to know whether the collection of slides is complete.

(over)

From Governor to Colonial Secretary

5. copies of reports of departmental Committee on  
Epizootic abatement, (Part III. abatement in sheep ca 7156 +  
+ appendix ca 7154) which Mr Harding has given me  
as attached.

A.

6 April 1922



April 29 1922

Sir,

I have the honour to beg, that you will lay before His Excellency the Governor, the following request.

Mr. Blake one of the Directors of this Company, sent me last mail some Board of Agriculture Reports on abortion, owing to the bad lambings, we have had on this Station for several years past.

It is a disease that requires a qualified man to follow up. May I ask, if you will request His Excellency to allow Captain Brown, the Stock Inspector, to visit this Station, about July next, to help me investigate the matter, during the time the ewes are in lamb.

I have written to the Stock Inspector informing him when the rams are put to the ewes, so that he will know, if July is the right time to come

I am, Sir,

Yours obediently,

Sydney Miller J. P.

Manager.

The Hon.

The Colonial Secretary,

Stanley.

3

303/22.

12th May, 22.

Sir,

I am directed by the Governor to acknowledge the receipt of your letter of the 29th of April, 1922, on the subject of abortion in sheep at Hill Cove, and to inform you that His Excellency has arranged for the Chief Inspector of Stock to visit your station early in August for the purpose of carrying out investigations in the matter.

I am,

Sir,

Your obedient servant,

H. Henniker-Heaton,

Colonial Secretary.

S. Miller, Esq., J.P.,

Hill Cove,

WEST FALKLAND.



4

Port Howard  
West Falklands  
May 10<sup>th</sup> 1922.

Sir,

Abortion in Ewes. There is an idea that this may be prevailing in the breeding flocks of this station. I shall be glad to know if the Chief Inspector of stock can be allowed to visit here during the Winter and Spring to look into the matter.

I am,

Sir,

Your obedient servant,  
R. B. Pole Evans.

The Colonial Secretary  
Stanley.

5

303/22.

12th June,

22.

Sir,

I am directed by the Governor to acknowledge the receipt of your letter of the 10th of May, 1922, on the subject of abortion in Ewes and to inform you that the Chief Inspector of Stock has been instructed to visit your station early in August for the purpose of investigating the matter.

I am,

Sir,

Your obedient servant,

H. Henniker-Heaton,

Colonial Secretary.

R. C. Pole-Evans, Esq., J.P.,

Port Howard,

WEST FALKLAND.

From Governor to Colonial Secretary.

DATE... 13<sup>th</sup> Jun 1922



on 4<sup>th</sup> Jun the Chief Inspector  
informed me that in connection with investigations  
regarding abortion in EWS + prevalence of scab  
he proposed to proceed to West Falkland  
early in July + that he would require to  
remain there until towards the end of  
December.

2. I shall be glad if the Chief Inspector of  
Stock will prepare for me a detailed  
programme of the work which he proposes  
to undertake in West Falkland for the  
period from July to December. He should  
give particulars of the stations which he  
proposes to visit, the work to be carried  
out at each station + the period which  
such work will occupy at each station  
which is to be visited.

Sd.

13 Jun 1922



7

Hill Cove

25th July 1922

Sir

I beg to report that on arrival at Hill Cove and after the departure of the S.S. Falkland I visited and inspected two ewe flocks at this station, and at present have not found any signs of abortion.

I propose to visit the five remaining ewe flocks here during the next two weeks. The seven flocks will laterly be split up into eight for lambing, and if any abortion is noticed there will a nother subdivision be ordered to have the aborting flock under close observation.

At the end of two weeks it is my intention to proceed to Port Howard and arrange with Mr Evans to inspect his ewes, unless there are any signs of abortion there before. Probably it will be necessary to remain there two or three weeks as I am anxious to inspect all his sheep in order to see if there are any signs of lice, also at the same time it would be advisable to see the sheep at racks Port Howard for the same reason.

I have sent out the forms relating to the loss of lambs and the weights of wool, specimens of which are enclosed. You will notice that I have asked for the lambing return from 1915 to 1922 which should give a good average to work from.

The wool return only goes back as far as 1900 as there seems to be some doubt if accurate figures can be obtained earlier, however I have asked the different farmers to let me have any earlier data if possible.

If you could obtain similar returns from the West for the same periods it would be very useful. The returns from the West will not be received in time for this mail but should be in for the next one

I have the honour to be .. Sir

Your obedient servant

The Hon Colonial Secretary

Stanley

B A Brown

Chief Inspector of Stock

*Mr. J. Wilson  
Will the sheep  
only with us or  
Packy Parkman  
Excused  
to  
5/11/22  
5/12/22*



1574

8

Port Howard

September 22nd 1922

Sir

Please note for the information of His Excellency the Governor that upon arrival at Fox Bay in the "Alterglow" I proceeded with Mr Wilson to Dunrose Head where I examined all the flocks on that station, as some of the ewes had been obtained from ~~XXXXXXXXXXXX~~ Port Howard two years ago, there were no signs of lice. From there returned to Fox Bay and then went to Charters where I examined the sheep that were on the Charters-Port Howard boundary and failed to find any signs of lice. From there I proceeded to Port Howard and dipped two flocks that were slightly infected, one flock of about 400 cast ewes that were badly infected was destroyed by Mr Evans as he did not wish to drive them over clean ground to the dip. This was the only flock that showed severe infection since the dipping of last February and I consider Mr Evans most thorough in his endeavours to stamp out this pest. I then proceeded to Hill Cove as the lambing season was coming on. Up to the present I have failed to find any signs of abortion either in the ewes on this station or at Port Howard. I am returning to Port Howard in a day or <sup>50</sup> so as Mr Evans is trying the experiment of herding about 1000 ewes by day and yarding them at night and having two shepherds to watch them whilst in the yard, this will enable a check to be kept on the lambing as regard age, number of barren ewes etc and should be very instructive. After the lambing is over I shall be going to Port Stephens and Spring Point and Roy Cove if possible before returning to the East.

Excerpted to 123/22 Encl 2

Hon Col Secretary  
Stanley

I am, Sir.

Your Obedient Servant

*B A Brown*

Chief Inspector of Stock

**R E P O R T**  
OF THE  
**DEPARTMENTAL COMMITTEE**  
APPOINTED BY THE  
**BOARD OF AGRICULTURE AND FISHERIES**  
TO INQUIRE INTO  
**EPIZOOTIC ABORTION.**

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**Part III.**  
**ABORTION IN SHEEP.**

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*Presented to both Houses of Parliament by Command of His Majesty.*

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### Copy of Minute of Appointment and Reference to the Committee.

---

I hereby appoint a Committee to inquire, by means of experimental investigation and otherwise, into the pathology and etiology of Epizootic Abortion, and to consider whether any, and if so what, preventive and remedial measures may with advantage be adopted with respect to that disease.

The Committee will be constituted as follows :—

Sir EDWARD STRACHEY, Bart., M.P.

The Very Rev. Dr. JOHN GILLESPIE.

Professor JOHN McFADYEAN, M.R.C.V.S., M.B., B.Sc., Principal of the Royal Veterinary College.

Mr. WILLIAM HUNTING, F.R.C.V.S.

Dr. GEORGE H. FALKNER NUTTALL, F.R.S.

Mr. STEWART STOCKMAN, M.R.C.V.S., Chief Veterinary Officer of the Board of Agriculture and Fisheries.

And I hereby appoint Professor JOHN McFADYEAN to be Chairman and Mr. JAMES RALPH JACKSON, M.R.C.V.S., of the Board of Agriculture and Fisheries, to be Secretary.

4, Whitehall Place,  
London, S.W.,  
19th April 1905.

(Signed) AILWYN E. FELLOWES,  
President of the Board of Agriculture  
and Fisheries.

### Copy of Minute extending the Reference to the Committee.

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The reference to the Committee appointed by the Board's Minute of the 19th April 1905, "to inquire, by means of experimental investigation and otherwise, into the pathology and etiology of Epizootic Abortion, and to consider whether any, and if so what, preventive and remedial measures may with advantage be adopted with respect to that disease" is hereby extended, so as to include an inquiry as to the administrative measures which in view of the results of the investigation made by the Committee should now be taken to deal with cases of the disease and to prevent the spread of infection.

The following gentlemen are hereby appointed to be additional members of the said Committee :—

His Grace the DUKE OF DEVONSHIRE, P.C. ;

Mr. A. W. ANSTRUTHER, C.B., one of the Assistant Secretaries to the Board of Agriculture and Fisheries ;

Captain C. G. ALISON ; and

Mr. GEORGE LANE FOX, M.P.

(Signed) CARRINGTON,  
President of the Board of Agriculture  
and Fisheries.

May 24th, 1909.

R E P O R T.

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PART III.—ABORTION IN SHEEP.

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**Final Report of the Departmental Committee  
appointed by the Board of Agriculture and Fisheries  
to inquire into Epizootic Abortion.**

---

ABORTION IN SHEEP.

TO THE PRESIDENT OF THE BOARD OF AGRICULTURE AND FISHERIES.

SIR,

WE have the honour to submit the following Report and Appendix embodying the principal results of our investigations concerning abortion as it has been found to occur in sheep. The Appendix, which gives a detailed account of the experiments and observations on which the Report is based, is by Sir John McFadyean and Sir Stewart Stockman, by whom the work has been carried out.

The inquiry has been surrounded by many unavoidable difficulties necessitating a considerable amount of organisation in addition to investigation, and although some of the most important results were arrived at as far back as 1906, it was thought inadvisable to report sooner, for reasons which will be apparent on perusal of the Report.

Although the present Report deals specially with abortion in sheep, we have during the last three years paid attention to certain points to which reference was made in our last Report in connection with cattle abortion, and especially to the comparative merits of the agglutination test and other methods of arriving at a correct diagnosis in suspected cases of contagious abortion and the value in actual practice of protective inoculation as a means of dealing with outbreaks of the disease.

With regard to the first of these, we now feel warranted in stating that by means of one or other of the methods of diagnosis described in a previous Report (Part I., Appendix) it is now possible to distinguish with practical certainty between cases of contagious abortion in cows and those of a sporadic or accidental character. Moreover, the agglutination test by itself appears to be likely to render great service in dealing with outbreaks in the future, since it can be employed to detect the disease while it is still in the incubative stage, *i.e.*, before the act of abortion has occurred.

Our experiments and observation have not led us so far with regard to the question of immunising cattle against contagious abortion. We are satisfied that a high degree of immunity against the disease can be conferred on healthy animals, and to that extent the question has been carried beyond what may be called the laboratory stage of investigation. Furthermore, protective inoculation has already been employed in a considerable number of infected herds, but some time must elapse before it will be possible to judge the results in these cases or to estimate the general value of this method of dealing with outbreaks.

The work in connection with immunisation is being carried out at the laboratory of the Board of Agriculture and Fisheries, and we suggest that it might be left to the Board of Agriculture to complete this work, and report upon the result when they are satisfied that a sufficient number of observations have been made to justify a special report.

*History.*

Abortion in ewes does not seem to have engaged the attention of scientific investigators in the past, and in consequence there is practically nothing to relate regarding the history of previous investigations into the pathology of ovine abortion. In Report, Part I., it was shown that pregnant ewes could be experimentally infected with the virus of cattle abortion, and bearing this in mind, together with the fact that cattle and sheep are not infrequently pastured on the same land, it did not seem improbable that the disease in these two species arose from the same cause.

There appeared to be, however, epizootiological reasons for thinking that the diseases might be different, viz., that unlike the disease of cattle, abortion in sheep assumes enzootic characters, that is to say, it seems to confine its operations to a comparatively small proportion of farms without showing much tendency to become widespread. On the other hand, it may be pointed out that a disease does not owe its epizootiological character to its contagiousness alone, but also to the opportunities given it for dissemination. The opportunities which operate in relation to the disease in cattle and not in sheep are as follows:—(a) Breeding goes on all the year round in the case of cattle, whereas with sheep it is almost entirely confined to a season, and a considerable interval of rest intervenes. (b) Cows are frequently sent to the bull on other premises, where the disease may prevail, but this does not apply to ewes to any extent. (c) There is a very considerable interchange of in-calf and newly-calved cows through markets, while ewes are practically always tugged on the owners' premises, and remain there throughout pregnancy. In fact, the distinction between an epizootic and an enzootic is somewhat artificial, and bearing the above considerations in mind it will be understood how the same disease in sheep and cattle might present very different characteristics according to the species of animal affected.

In a previous Report (Part I., Abortion in Cattle, page 15) it was stated that we had never found the microbe of cattle abortion in connection with outbreaks of abortion among sheep, and that a totally different microbe—a vibrio—had been repeatedly isolated from outbreaks of abortion in ewes, and that it had been successfully used at the laboratory to experimentally infect pregnant ewes with abortion.

It is almost entirely with this vibrionic disease of sheep that the present Report is concerned.

#### *Method of obtaining Material for Investigation.*

It was found to be a much more difficult matter to obtain suitable material for the investigation of ovine abortion than was the case in the disease of cattle, and it was still more difficult to keep the disease going at the laboratory after material had been obtained. These difficulties did not arise from the disease not being prevalent, although in certain years the absence of reports from the field was a material hindrance to progress. They were for the most part unavoidably connected with the investigation of a seasonal disease and the peculiar pathology of the disease in question. The difficulties will be more readily realised after perusal of Appendix III., but it will be useful to state some of them as shortly as possible here for the purpose of providing a preliminary idea of the disease and the organisation which was necessary for its investigation, and in explanation of why it was considered advisable not to publish results, some of which were acquired several years back, until they had been repeatedly confirmed.

(a) Abortion in sheep is from existing circumstances a seasonal disease. It only becomes alarming enough to be reported about six weeks before the end of the lambing season, and in consequence the period during which suitable material can be obtained from the field is limited to the short space of six weeks in the year.

(b) Under ordinary conditions of infection (that is when only comparatively small amounts of virus have been introduced) the foetus often dies some time before it is aborted. The uterus is open, however, and becomes rapidly invaded along the external genital passages by the germs of putrefaction, with the result that in most cases when indications of approaching abortion present themselves, and an examination is made, the uterine contents consist of a putrefying mass, which is useless for bacteriological or experimental examination.

(c) One of the essential proofs of a microbe being the cause of abortion in the field is to find it in pure culture in an aborting uterus of an animal belonging to the flock in which abortion is taking place. Having regard to the facts stated in (b) and that ewes practically never show signs of being infected until the uterus has been open for some time, the obvious method of killing an animal in the infected herd for examination before the uterus becomes open did not offer much chance of success. Nevertheless, by adopting this method we were fortunate enough on several occasions to obtain material from uteri in which the vibrio prevailed to such an extent over the other microbes present that the latter were clearly contaminations, and on two occasions the ewes selected from infected flocks for slaughter and examination showed typical lesions of the disease in the uterus, which was still closed, and contained enormous numbers of the vibrio in absolutely pure culture (see Appendix III., and particularly Flocks 2, 13, and 15). The investigation was aided by the fact that the vibrio was found to invade the tissues of the foetus, and could be obtained in pure culture in its

intestinal fluids for a short time after the uterus had become contaminated from without, but this, of course, could not be accepted as proof that the microbe was the cause of the disease.

(d) Since the disease is only reported towards the end of the lambing season it is a difficult matter to obtain a large supply of ewes in the earlier stages of pregnancy for experiment at the laboratory with material obtained from outbreaks in the field. To meet this difficulty maiden ewes were obtained, and put to the tup each month starting from October, so that they would lamb late in the season. It was found to be no easy matter, however, to ensure an adequate supply of late lambing ewes without employing an unreasonable number to start with, and it was found extremely difficult to get the ewes to take the ram later than February and become pregnant. In fact, it was found impracticable to keep in hand a sufficient supply of pregnant ewes, even by using Dorsets, to keep up a supply of natural virus to continue the investigation through the interval between the ordinary lambing and tupping seasons.

(e) It was also found that the vibrio soon lost its virulence under conditions of artificial cultivation, so that the experimental work with pure cultures could only be carried on for a limited period after the supply of natural virus gave out.

Owing to the above-mentioned circumstances the experimental work on this disease was limited to a period of about two months in the year.

A circular letter was issued to the Board's Honorary Agricultural Correspondents and Inspectors asking them to make it known to farmers that the Committee were carrying on investigations in relation to abortion in sheep. We desire to take this opportunity of thanking those gentlemen who complied so sympathetically with our request, and to state that by means of their assistance we were put in touch with a great deal of material which might otherwise have been lost. When a report was received that abortion was occurring amongst ewes, the practice was to send one of the Board's Veterinary Inspectors to obtain full particulars of the outbreak, and to forward what material the investigators at the laboratory required. This consisted mainly of smears from cotyledons from aborting ewes, aborted lambs, and, in some cases, the gravid uteri of ewes suspected of being infected. The last-mentioned material was collected in the following way. The ewes were selected with the assistance of the shepherd and slaughtered. The abdominal cavity was immediately opened, the neck of the uterus ligatured, and the organ was excised. It was then packed in a waterproof bag which was placed in a padded box, and despatched to the laboratory by passenger train, a telegram of advice being sent. The instructions were to make these post-mortem examinations in the early morning so that the material might reach the laboratory in time to be dealt with on the day or evening of arrival. The pregnant ewes, for experiment, were kept for the most part in isolated pastures some distance from the laboratory, and were only brought on the premises to be put under experiment. After infection they were isolated in pens or loose-boxes under strictly experimental conditions.

A list of questions bearing on the prevalence and distribution of abortion in sheep was also sent out to farmers who had been good enough to promise to tabulate their experience; a summary of the information obtained in this way will be found under other headings.

#### *Post-mortem Appearances of Ewes affected with Vibrionic Abortion.*

By consulting Appendix III. B., in which the post-mortem description of ewes obtained from infected herds in the field and those experimentally infected at the laboratory are given in detail, it will be seen that the appearances in both cases are identical. The method adopted was to open the abdomen after slaughter, ligature the neck of the uterus, cut out the organ, and remove it to the laboratory for minute examination. To obtain material for bacteriological examination the surface of the uterus was sterilised with a red-hot iron, an incision was made with a heated scalpel, and scrapings of exudate were removed with a sterile platinum scoop.

Lesions were not found in any organ other than the uterus, but in one case hæmorrhage had taken place into the pelvic tissue (Appendix III. B., Experiment 40, Ewe 226). Externally, the uterus may show no alteration, but in a number of cases it is considerably thickened and œdematous in the posterior third. On cutting into the œdematous part the tissue has a gelatinous appearance, and a yellowish fluid exudes from the cut surface (Appendix III. B., Ewe 101, and Flock 5, Ewe E.). In some cases livid patches on the mucous membrane could be seen through the uterine wall (Appendix III. B., Experiment 21, Ewe 103).

The mucous membrane of the uterus may be normal in appearance in the early stages. When the disease is more advanced the mucous membrane is reddened, and it occasionally shows patches of intense congestion in the sub-mucous tissue. In the early stages there is only a small amount of grey exudate of a mucoid character; it looks as if the normal uterine secretion had been slightly increased owing to catarrh (Appendix III. B., Flocks 2 and 5). In the later stages there is a very considerable amount of exudate between the chorion and the uterus. This exudate is of a dark red colour, indicating the presence of blood colouring matter. The consistence of the more solid parts is that of thick mucus, of which it seems to be mainly composed. Sometimes a large amount of red watery fluid is also present, in which the mucus appears as floccules. The cotyledons are firmly attached in the early stages, but on separating them a milky juice is seen on the surface. At a later stage the cotyledons are deeply congested, soft, and pulpy, and some are found separated. The exudate is odourless in uncomplicated cases, but it may be, and frequently is, very putrid, owing to the uterus being invaded by putrefactive germs. Sometimes the separated cotyledons show yellow anæmic looking areas, such as are found in connection with the bovine disease (Appendix III. B., Flocks 4 and 5).

It may be stated here that no particular difference is discernible between the advanced lesions in which the vibrio is found in pure culture and in those in which the contents of the uterus are putrid, and no vibrios can be found. It will be observed from the above description that the appearances are very different from those seen in aborting cattle.

It would appear from the experimentally produced cases that the condition of the foetus depends to some extent on the period of pregnancy at which infection took place, that is to say, a foetus may die shortly after infection but a considerable time before it is aborted. Thus a foetus which is aborted at a late period of pregnancy may be very little bigger than a rat. In such cases the foetus is dark brown in colour, macerated, and sometimes pulped up with the membranes; it is then difficult to find the vibrios. In other cases the foetus may be well developed for the period of pregnancy and show no lesions, but it is not uncommon to find blood-tinged fluid in the abdominal muscles and peritoneal cavity.

We may add as the result of inquiry in the field, that in the great majority of cases the foetuses and membranes are expelled in a putrefying condition.

#### *The Microbe of Ovine Abortion.*

The best preparations are obtained from the exudate, contents of the foetal stomach, and the surfaces of the separated cotyledons. In early cases the microbes can be found in the latter situation when no exudate is present, but, given an uncontaminated exudate, it is the best material for examination.

If smears be made from the exudate, and suitably stained, large numbers of "S" shaped microbes are seen in the preparation. There is also present a considerable number of spirillar forms, which give the appearance of being made up of a variable number of the curved elements joined end to end. No limiting membrane is specially evident. The individual organisms vary in length from 1.5 to 3 microns, and the breadth is from  $\frac{1}{4}$  to  $\frac{1}{3}$  of a micron at the middle part, which is broader than the extremities. The spirillar forms vary in length, according to the number of elements composing them, and it is not uncommon to find six or even more spirals. The appearance is very like that of a spirochæte, indeed it was at first thought to be a spirochæte, until its behaviour under conditions of artificial cultivation put this classification in doubt.

Awaiting a more definite classification in relation to spirillar micro-organisms, which include those which have been referred to by various authors as spirillum, vibrio, and spirochæte, the organism under description is referred to as a vibrio.

Some of the single elements show a deeply staining area at one end like the head of a comma. Some of the vibrios have a granular appearance, and they apparently break up into small round granules, which can be seen free in the exudate as well as in cultures. In hanging drop preparations the "S" and spiral forms are actively motile; the granules can also be seen. Examination by the method of dark ground illumination gives very good results.

*Staining.*—The various forms, with the exception, perhaps, of the free granules, stain well with preparations of methylene blue or diluted carbol-fuchsin. They do not retain the stain when treated by Gram's method. The granular forms and granules stain well with toluidin blue.

*Physical requirements in Culture.*—The vibrio grows best at the incubative temperature—30°–37° C. It grows well, however, in the substance of solid media at room temperature, but its growth is slower. In the incubator growth is apparent in from 3 to 4 days, but it is 10 days or more at room temperature before it becomes visible. It does not grow under strictly anærobic conditions, but a rarefied atmosphere seems to suit it much better than a free supply of oxygen. The peculiar requirements of the vibrio in relation to media and subcultivation are specially referred to below. The medium should be changed about every 10 days to keep the strain active in the vegetative sense. The vibrio appears soon to lose its pathogenic power under conditions of artificial cultivation, and so far our efforts have failed to devise a method of keeping this property active apart from the natural habitat.

With regard to the thermal death point of the vibrio, subcultures cannot be obtained from material which has been heated for 10 minutes at between 55° and 56·5° C. Subcultures were obtained, however, from material which had been exposed to the same temperature for only five minutes.

*Cultural Characters.*—Full details in relation to the cultural characters of the vibrio are given in Appendix III. A.

*Agar-gelatin-serum* was made use of in the first instance, as it was in that material that the bacillus of cattle abortion was first obtained in artificial culture. The medium was varied at times by the introduction of ascitic fluid from the foetus in place of the serum. This medium has nothing special to recommend it, and its preparation and manipulation are more complicated than some of the others in which the vibrio grows equally well. The employment of the medium, however, brought out the rather remarkable fact that the vibrio, when it was inoculated into tubes which had been liquefied and quickly solidified by plunging into water, gave a growth which was identical in appearance to that given by the bacillus of cattle abortion, and preparations had to be examined by the microscope before it was found to consist of a totally different microbe. After three days incubation at 35°–37° C. a faint grey cloudy ring appears about  $\frac{1}{4}$  inch below the surface of the medium. The grey ring is composed of innumerable colonies of vibrios, and distinct macroscopic colonies can be made out after about 10 days.

If plain *agar* tubes are liquefied, cooled to about 45° C., sown with material containing the vibrio, and solidified by plunging into cold water a growth similar to that on agar-gelatin-serum appears. Frequently there is also an abundant grey growth just under the surface of the medium, such as is found in agar-gelatin-serum cultures of the bacillus of cattle abortion, when a large amount of material is used for sowing. In cultures about a month old the sub-surface colonies vary in size from a pin point to a pin head, and the larger ones show a reddish-brown centre. In agar cultures of this age, one or two large isolated round colonies also frequently appear deep down in the medium, practically in the anærobic areas. There may even be a large number of small colonies in the deep layers of the medium. At first sight these large isolated colonies were thought to be contaminations, but repeated examinations showed them to consist of pure cultures of the vibrio. The explanation of their presence seems to be that the vibrio grows best in a rarefied atmosphere. When impure material containing vibrios and other organisms is sown in liquefied agar, it is often possible to identify the vibrios by their mode of growth. On the surface of agar slopes the vibrio grows very poorly; most frequently there is no growth at all. In a few tubes in which growths appeared they were usually in the form of a very thin grey film of the most meagre description.

On *gelatin* good cultures can be obtained by liquefying the medium before sowing, and solidifying afterwards. In gelatin tubes, which are kept at room temperature to prevent liquefaction, the vibrio grows more slowly. There may be no visible growth for 10 days or even longer, according to the temperature. The first appearance of growth is in the form of a delicate grey cloudy ring about half an inch below the surface of the medium, and no growth takes place in the deeper layers or on the surface. The colonies never attain the same size as those in agar. No liquefaction of the gelatin takes place.

On solid *potato* no growth of the vibrio has been obtained, but it was found that small blocks of potato added to broth favoured its development.

In *peptone broth* good growths are obtained in from 24 to 48 hours after sowing with natural material, such as exudate, scrapings from the cotyledons, and fluid from the foetal stomach. If the tubes be shaken up after a period of incubation a greyish vibrating cloudiness is observed.

five days before full time, but no vibrios could be found. Ewe 150 aborted 40 days after contact began and 43 days before full time. Vibrios were found in the discharges, the foetal membranes, and in the stomach fluid of the foetus from Ewe 150.

We have considered whether any part in the spread of ovine abortion is attributable to the ram. It seems most improbable that a ram could spread infection mechanically from ewe to ewe in a flock merely from the fact of having been contaminated by an aborting ewe, because in the great majority of cases both rams and ewes, when the breeding season begins, have been excluded from sexual intercourse for nearly a year, and the ewes must have been free from maternal duties, other than suckling, for about six months. There seems only to be the possibility that a ram from infected premises may be a carrier of infection owing to the upkeep in some way of virus in its body, but of this being actually the case there is no evidence of any consequence.

#### *Symptoms.*

No particular symptoms in connection with abortion seem to have been observed in the field except just before the act, when, if the period of pregnancy is advanced, and it usually is, the ligaments are relaxed, the mammae and the vulva are swollen and a sanious, mucoid discharge is seen around the latter. The symptoms immediately following infection in some of the experimental cases, such as elevation of temperature and diarrhoea, can hardly be considered to be of practical interest, as they almost certainly arose from the virus having been introduced by unnatural channels, or from the material having been somewhat putrid.

We would, however, draw attention to the fact that some of the experimentally infected animals showed the sanious, mucoid discharge at the vulva some days, and even weeks, before abortion took place, and sometimes even a few days after infection. Vibrios could in some cases be demonstrated with the microscope in this discharge, and we would point out that a pregnant ewe, which before her time shows soiling of the fleece by sanious material, should be regarded with great suspicion, and the discharge should be examined. The evidence from the field is that many of the foetuses are putrefying when expelled. We would point out, however, that this seems to apply more particularly to those which have died in utero at a comparatively early stage of pregnancy.

It would appear from an examination of the cases experimentally produced by introducing virus by way of the natural orifices, that the interval between infection and abortion varied between 13 and 113 days, and in this connection we would again refer to the two cases of infection from the pasture (Experiment 66) in which one ewe aborted 46 days and another 40 days from the beginning of contact. In the 15 outbreaks which it was possible to investigate particularly, the percentage of abortions varied between 5 per cent. and 50 per cent., average 23·2 per cent.

It is not uncommon to meet with inflammation of the womb, which may end fatally, as the result of abortion in ewes, especially when the contents of the uterus are putrid.

#### *Distribution and Prevalence of the Disease.*

In the absence of an Order requiring notification of the disease we can only report upon what has come to our knowledge through our inquiries, and the sympathetic co-operation of farmers, honorary correspondents of the Board, and veterinary practitioners. Of the 15 outbreaks which were particularly inquired into, three were in Hampshire, one in Devonshire, three in Lincolnshire, one in Somerset, one in Berkshire, two in Suffolk, two in Wiltshire, one in Sussex, and one in Cambridgeshire. Vibrionic abortion was also found in material from aborting cows in Ireland and Wales. We are not prepared to say that the vibrio is responsible for all serious outbreaks of abortion amongst ewes; we can only report that we have not as yet met with any other specific form of the disease.

By means of the circular letter of inquiry containing a series of specific questions we have been able to collate from farmers a considerable amount of presumptive evidence in relation to the prevalence and distribution of abortion in sheep. Replies on this point were received from 476 sheepowners, running approximately 252,738 breeding ewes year in and year out. The recipients of circulars were asked to eliminate barren ewes as far as possible from consideration, and to enter the latter in a separate column, so that an opportunity might be obtained of judging whether the proportion of barren ewes was sufficiently high in any case to lead to the belief that foetuses had

been cast unobserved in the very early stages of pregnancy. It would appear from replies, that abortion commonly occurs amongst ewes to the extent of 1 per cent. to 3 per cent., or even more, without any very serious outbreak ever arising.

In the 15 outbreaks specially investigated (*see* previous section), the average number of abortions was 23·2 per cent., but in only three cases was it below 10 per cent., viz., 9 per cent., 8·3 per cent., and 5 per cent. We have, therefore, for the purposes of this section of the Report fixed 10 per cent. and over as the indication of something definite having operated on the ewes, though we are aware that the percentage is very considerably above the ordinary.

On 70 of the 476 farms (14·7 per cent.), representing 34,135 breeding ewes, 10 per cent. of abortions and over had occurred in one or more years of the five to which inquiry related. On 17 farms the proportion was 20 per cent. and over, and on seven it was 30 per cent. or over.

The flocks in which 10 per cent. of abortions and over had occurred were located in the following counties:—

Berkshire.	Leicestershire.	Carnarvonshire.
Buckinghamshire.	Lincolnshire.	Denbighshire.
Cambridgeshire.	Norfolk.	Flintshire.
Cumberland.	Northamptonshire.	Radnorshire.
Derbyshire.	Northumberland.	Lanarkshire.
Devonshire.	Oxfordshire.	Orkney.
Durham.	Somerset.	Peeblesshire.
Essex.	Suffolk.	Perthshire.
Hampshire.	Sussex.	Roxburghshire.
Isle of Wight.	Wiltshire.	Stirlingshire.
Huntingdonshire.	Worcestershire.	
Kent.	Yorkshire.	

In 26 flocks, representing 16,850 sheep, the proportion of barren ewes was 10 per cent. or over.

#### *Species of Animals which are susceptible.*

In the section of Report, Part I., dealing with the same subject in relation to bovine abortion it was mentioned that this vibrio had been isolated from aborting ewes, and it was stated that it had not been found possible to infect cows with vibrionic abortion. Since the publication of our first Report the work of investigation in connection with sheep abortion has been extended, and it has been found that cows can be experimentally infected with vibrionic abortion. By consulting Appendix III. D. (Experiments 57a-64), it will be seen that infective material was administered to seven pregnant bovine animals by the veins, natural orifices, or by both channels, and that only one cow (Cow 46a, Experiment 63) became infected. It is of great importance to note, however, that two natural outbreaks of vibrionic abortion, one in Ireland and one in Wales, were met with in cows (Appendix III. D.), therefore it must be admitted that the disease enters into the pathology of cattle, and that cattle may be a factor in the epizootiology of the disease.

A goat aborted (Experiment 59) four days after being inoculated with virulent material, but no vibrios were found in the cotyledons, discharges, or uterus. The period, however, between inoculation and abortion was too short to admit of a plentiful culture having taken place.

The small animals of the laboratory—guinea-pigs and rabbits—do not suffer any illness when inoculated with vibrios, but, in one of the few cases where pregnant guinea-pigs were submitted to experiment, abortion followed inoculation in 12 days (Experiment 8, Guinea-pig 451). We desire to point out, however, that the experiments carried out on pregnant animals other than sheep were necessarily comparatively few in number, for reasons which have already been mentioned.

We might add here that from information collected from the replies to our circular, nine sheepowners stated having abortion in their cattle and none in their sheep, and 21 admitted having had over 10 per cent. of abortion amongst their ewes, and at the same time abortion amongst their cows.

#### *Diagnosis.*

When one or two ewes in a flock abort or bring live lambs prematurely, it is of the utmost importance in relation to preventive measures to know whether or not

the trouble is contagious. The symptoms and the proportion of abortions mentioned in the two previous sections will to a certain extent act as a guide to diagnosis. The discharges before the act, the expelled membranes (especially the cotyledons), and the contents of the foetal stomach should be examined microscopically for the specific micro-organism. Its presence is sufficient to establish the diagnosis, but failure to find it does not justify the conclusion that the case is not one of vibrionic abortion (consult Appendix III. B., Flocks 6, 7 and 8); especially when the material examined is putrid.

It is known that as the result of infection with a micro-organism, the serum of the blood may acquire certain specific properties in relation to it, one of which is that it causes the micro-organisms to become agglutinated into clumps when added to an emulsion of an artificially grown culture. This general method of diagnosis was particularly referred to in Report, Part I., and Appendix. The applicability of the agglutination test in relation to the diagnosis of vibrionic abortion was studied at the laboratory (Appendix III. E), and it would appear that, in so far as the observations have gone, the test promises to be of practical value. Further observations on the agglutination test and the fixation of the complement test (Report, Part I., and Appendix) are being carried out, and we would here observe that differential tests acquire all the more importance from the fact now established that bovine animals may also become naturally infected with the vibrionic disease, and abort.

#### *Natural and Acquired Immunity.*

We have found no evidence to justify the opinion that any particular breed of sheep possesses natural immunity, though it seems not improbable that some individual animals are more resistant to infection than others. This is suggested by the fact that the same virus introduced experimentally in the same way to a number of ewes at the same time did not cause abortion in every case, added to the field observations in which only a proportion of the ewes aborted although the flock had been kept under conditions which were calculated to expose all the animals to infection (crowding and penning on crops). There remains the question of whether the ewes of a flock which have suffered from abortion, acquire a degree of resistance which is maintained over one or two subsequent breeding seasons. It appears to be the case that abortion in sheep does not usually repeat itself in the same flock in two successive seasons. The replies from 205 owners, owning about 131,023 ewes, show that outbreak had recurred in successive seasons in the same flock in only 45 cases. On the other hand, we do not feel justified in attributing, without reserve, the absence of successive outbreaks to the original animals of the flock having acquired immunity, because in the case of 158 owners the disease was not observed to recur in the new breeding ewes introduced to the old flock in the following season. Only 86 owners replied that the new ewes suffered from abortion in the following season.

#### *Immunisation.*

Experiments were conducted for the purpose of inquiring whether it would be possible to immunise ewes by subcutaneous injection of living cultures of the vibrio about two months before putting them to the ram (Experiment 65, Appendix III. F.). It seemed likely, from the experience acquired in connection with bovine abortion, that good results, if obtainable, would only be acquired by using large doses. This presented some difficulty, because the vibrio did not grow luxuriantly on artificial media. The difficulty was got over to a certain extent by adding solid portions of potato to the peptone broth (Appendix III. A.), but it could not be said that the cultures were very luxuriant.

It does not appear from the results of these few experiments that the immunising dose had a preventive effect of any value. We think, however, that these observations might be continued, and extended, by the Board of Agriculture and Fisheries. Sero-therapy will be dealt with in the next section.

#### *Curative Measures, including Sero-therapy.*

For the production of an anti-serum a heifer was hyper-immunised by subcutaneously injecting artificially prepared cultures of the vibrio. The difficulty of obtaining luxuriant cultures arose as in the case of immunisation, but this was got over

by injecting enormous doses. We understand, however, that there is hope of being able to cultivate the vibrio freely on the surface of specially prepared solid media, which will greatly lessen the difficulties in the way of obtaining material for immunisation, serum production, and the diagnosis tests.

The design of Experiment 67 (Appendix III. H.) was to infect six pregnant ewes (Ewes 140 to 146) with vibrio, and afterwards treat them by injections of anti-serum to see if abortion could be prevented by such treatment after infection.

The experiment was undertaken because it had been learned that abortions began to occur seriously towards the beginning of the lambing season, and that, given a heavy infection, abortion might take place in less than a week. It was therefore thought possible: (a) That owing to heavy infection of the pastures from recent abortions, it might be that a large number of ewes aborted at the end of pregnancy from quite recent infection with an enormous dose of virus. (b) That once it was established by examination of the first cases that the trouble was micro-organismal in character, it might be possible, by injecting a potent serum, to endow the remaining ewes with immunity of sufficient duration to carry them on to full time.

It will be observed that, in Experiment 67, one ewe (240) aborted 48 days after infection and only the day after treatment began, that is before the serum could have exercised any effect. The appearance in the uterus was typical of the disease, but no vibrios could be found. All the other ewes carried their lambs to full time. It is to be noted, however, that the material used for infection consisted of pure cultures, which had been for some time under conditions of artificial cultivation, and, having regard to the irregularity of results with such virus, it is possible that it failed to establish a footing in the uterus owing to its nature alone, and not owing to the action of the serum.

No experiments with drug treatment have as yet been undertaken, but, bearing in mind the nature of the micro-organism and the specific effect which certain drugs have recently been shown to exercise on similar micro-organisms in the body, it does not appear impractical to think that in the early stages of infection at least, when the parasites are confined to the cotyledons, certain drugs may be found useful. Further investigations are proceeding in relation to the possibilities of the serum and drug treatment.

#### *Preventive Measures.*

It has been already explained that serious outbreaks of ovine abortion do not usually occur on the same farm year after year. There may be, and often is, little or nothing to warn an owner that infection has entered his flock until the disease has fairly well established itself and a number of ewes have aborted.

Except in those cases where the disease repeatedly recurs, it is improbable that many farmers would go to the trouble and expense of regularly carrying out preventive treatment in anticipation, even if a reliable method were available. Bearing this in mind, together with what has been said in the section dealing with immunisation, it would appear that, for the time being at least, preventive measures are only likely to be applied in the sense of trying to prevent further spread once the disease has shown itself.

*Destruction of Virulent Material and Disinfection of Everything contaminated by it.*—This is obviously important. The membranes, fœtus, the discharge, and any litter soiled by the material should be removed at once and destroyed. It is even preferable to destroy their virulence on the spot by mixing the material freely with caustic lime and slaking, or enveloping it in straw soaked in paraffin and burning it. Any patches of the pen or pasture which are known to have been soiled should be disinfected with a strong disinfectant. The boots, clothing, and hands of attendants should be disinfected.

*Isolation of Aborting Animals and those which have Aborted.*—When a ewe before her time shows a discharge from her genital organs, or should she abort, it is essential that she should be removed from contact with her fellows. The same applies to ewes which have given birth to live lambs prematurely, in which case both the ewes and lambs may be excreting infective material. It would be preferable, of course, to remove the still pregnant ewes when this is possible.

*Penning or Folding of Ewes before Lambing.*—In some cases it is customary to pen ewes at night towards the end of pregnancy, and it is also not unusual to fold them by hurdles to feed on certain crops. As far as abortion is concerned, these

practices may do no harm, so long as there are no infected ewes in the flock; but given one or two cases of abortion occurring in the narrow area of a pen or fold, it will be obvious how great will be the opportunity for other ewes to become infected, and we would again point out here that a ewe may abort from infection acquired only a short time before her normal time to lamb. The replies to our inquiry by circular bring out that, of the 70 owners of 34,135 breeding ewes who had experienced 10 per cent. or more of abortion, 46 owners were in the habit of penning or folding their ewes some time before they were due to lamb.

It is advisable, then, not to adopt these practices if they can be avoided, but if they are adopted the greatest vigilance should be exercised in watching for cases of abortion. Should such occur, two courses are open, either to destroy all evacuated material, disinfect the ground, and remove the ewe, or to immediately remove the other ewes to clean ground. The second course is the more recommendable, if it can be carried out.

*Disinfection of the Genital Passages of Ewes which have Aborted.*—This may be done by gently injecting an antiseptic wash (3 per cent. pure carbolic acid in water, or corrosive sublimate 1-2,000) by way of the external genital organs. It is advisable to carry out this treatment, because it may have the effect of destroying a certain amount of virulent material which has passed from the uterus into the vagina, and it may also help to prevent further invasion of the passages and womb by hurtful microbes from without. Ewes which have aborted should be put in improvised pens, and treated in the same way until the discharge ceases.

#### *Legislative Measures of Prevention.*

Having regard to the conditions under which sheep breeding is carried on, which have already been referred to, and the facts so far brought out by investigation, it does not appear to us that legislative measures of prevention are applicable to this disease in sheep.

We wish to place on record our appreciation of the manner in which the duties of Secretary have been carried out by Mr. J. R. Jackson. Besides conducting the purely secretarial work of the Committee he has lent valuable assistance in connection with the experimental part of the inquiry.

We have the honour to be,  
Sir,  
Your obedient servants,

(Signed) J. McFADYEAN (*Chairman*).  
DEVONSHIRE.  
STRACHIE.  
STEWART STOCKMAN.  
A. W. ANSTRUTHER.  
CHARLES ALISON.  
C. R. LANE FOX.  
WILLIAM HUNTING.  
GEORGE H. F. NUTTALL.

JAMES R. JACKSON,  
*Secretary.*

Oct. 20th, 1913.

REPORT  
OF THE  
DEPARTMENTAL COMMITTEE  
APPOINTED BY THE  
BOARD OF AGRICULTURE AND FISHERIES  
TO INQUIRE INTO  
EPIZOOTIC ABORTION.

APPENDIX TO PART III.—ABORTION IN SHEEP.

BY

SIR J. McFADYEAN, M.R.C.V.S., M.B., C.M., B.Sc., LL.D.,

AND

SIR STEWART STOCKMAN, M.R.C.V.S.,  
CHIEF VETERINARY OFFICER OF THE BOARD OF AGRICULTURE AND FISHERIES.

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## INTRODUCTION.

The experiments described in this Appendix were carried out at an Experimental Station of the Epizootic Abortion Committee, at the Laboratory of the Board of Agriculture and Fisheries at Alperton, and at the Royal Veterinary College, on behalf of the Departmental Committee on Epizootic Abortion.

**Report of the Departmental Committee  
appointed by the Board of Agriculture and Fisheries  
to inquire into Epizootic Abortion.**

ABORTION IN SHEEP.

A.

BACTERIOLOGY.

From our studies in connection with epizootic abortion in cattle we learned that pregnant ewes could be easily infected experimentally with this disease (see Report, Part I., and Appendix) in the same way and by the same channels as cattle. Taking this into account, in addition to the highly contagious character of the disease, its great prevalence amongst cattle, and the fact that cattle and sheep are often in more or less direct association at pasture, it was not unnatural to suppose that, if sheep suffered extensively from abortion, there was at least a strong possibility that it arose from the same cause as epizootic abortion in cattle. From the first, however, it did not seem probable that abortion would exist amongst sheep in an epizootic form for the reasons which follow. The epizootic character does not depend entirely on a disease being highly contagious, but also on the opportunities for dissemination which present themselves. As is well known, the great majority of sheep enjoy an annual period of rest from reproduction of about seven months, and during this period the genital organs, if they have been infected, have a greater chance of becoming cleansed than in the case of cows. Ewes are not as a rule sent to the ram on other premises, as a cow is sent to the bull, nor is there an extensive interchange of newly-lambled or pregnant ewes, as in the case of milch cows. What usually happens is that a sheep farmer, if he buys in at all, buys gimmers which have never been pregnant; the rams are turned in with the ewes in the autumn or late summer, and the ewes remain on the premises throughout the period of pregnancy. The opportunities for dissemination would not, then, appear to be great, but, of course, the chance of such a disease spreading from ewe to ewe on the same pastures is obvious.

Some time after the inquiry into abortion in cattle had commenced, we became aware that abortion prevailed from time to time amongst sheep on certain farms, in a form so serious that there was a probability of contagion being the cause. The disease, however, was enzootic in character, and although this did not eliminate epizootic abortion of cattle as the cause, having regard to the conditions under which sheep-breeding is conducted, the bacillus of cattle abortion was never found in connection with a natural outbreak in sheep. At the same time we were greatly surprised to find that in certain cases uncontaminated material from the uteri of aborting sheep, and the stomach fluid of aborted lambs, gave growths on upright tubes of agar-gelatin-serum which corresponded macroscopically to the appearance which was at the time supposed to be typical of the abortion bacillus of cattle (see Flock 2, Appendix B). As will be seen, however, the investigation of the microscopical, biological and pathological characters of the microbe found in sheep left no possible doubt that it was entirely distinct from the bacillus of cattle abortion.

At the time the micro-organism about to be described was isolated, somewhat arbitrary rules for the classification of such organisms had been formulated by systematists. Under these rules a spiral organism was not to be classed as a spirochæte, unless it divided longitudinally, and could not be cultivated on artificial media. With this organism isolated from sheep, although its requirements are peculiar, we were fortunate enough almost from the start to make use of methods and media which enabled artificial cultures to be obtained, and kept up indefinitely, and we were unable to

see anything resembling multiplication by longitudinal division. We therefore classed it with bacteria. We would mention, however, that of late longitudinal division as a method of multiplication in spirochætes has been much disputed, that some protozoologists do not admit spirochætes among the protozoa, preferring to class them with the bacteria or in a class by themselves, and that some of the spirochætes, particularly *S. gallinarum*, which have been generally accepted as belonging to the spirochaudinna, have now been cultivated in artificial media, using methods and media similar to those by which we were enabled to obtain and maintain cultures of the organisms of ovine abortion in 1906. Awaiting a final classification of spirillar micro-organisms, we have thought it advisable to adopt the term vibrio, associating the organism with the bacteria, with which it appears to have the greater affinities.

The bacteriological studies which we have made during this part of the inquiry have been brought together for description in this section. Most of the observations on the culture of this special micro-organism were made during 1906 and 1907, but they were supplemented, and confirmed, all through the investigation by studying different strains of the organism from the various outbreaks in which it was met at a later date.

By consulting the observations in section B on the different flocks it will be seen that other micro-organisms besides the one specially referred to were found in connection with the uterine contents of aborting ewes. These, however, were mainly bacteria of the putrefactive type. None, with the exception of that described fully in this appendix, gave evidence of having any specifically pathogenic characters, and for that reason we thought it useless to engage in an elaborate study of them.

*Cultivation of the Vibrio of Sheep Abortion in Artificial Media.*

*Agar-gelatin-serum.*—This is the medium in which Bang first obtained a culture of the bacillus of cattle abortion. It has nothing to recommend it specially for the cultivation of the vibrio, but, as we began the inquiry into abortion in sheep with the probability before us that it might be due to the same cause as the disease in cattle, we naturally employed the recognised media in the first instance. It was on this medium that a growth of the vibrio was first obtained from the uterine contents of Ewe B. (Flock 2), and from the stomach fluid of Lamb B. 19, by sowing in the liquid state and allowing the medium to solidify quickly. The growths to the naked eye appeared identical with those of the bacillus of cattle abortion, and it was only when the bacteria came to be examined by the microscope that it was discovered that they differed totally from that microbe. After two or three days' incubation at 35° to 37° C. a faint grey, cloudy ring appears at about  $\frac{1}{4}$  to  $\frac{1}{2}$  of an inch below the surface of the medium. The grey ring is composed of innumerable small colonies of vibrio, and distinct macroscopic colonies cannot be distinguished until after about ten days incubation.

*Agar.*—If agar tubes be liquefied, cooled to about 45° C., sown with material containing the vibrio, and solidified quickly by placing the tubes in cold water, a growth similar to that on agar-gelatin-serum appears. Very frequently, however, there is also an abundant growth just under the surface of the medium. The surface growth is in the form of a grey film, and extends to the depth of about  $\frac{1}{4}$  of an inch. In cultures about

a month old the sub-surface colonies vary in size from a pin-point to a pin-head. The larger ones show a reddish brown centre. Frequently in agar cultures of this age one or two large, isolated, brown colonies appear deep down in the medium, practically in the anaerobic areas. There may even arise a multitude of small colonies in the deep layers of the medium. At first sight these large, isolated colonies were taken for contaminations, but we have repeatedly assured ourselves by microscopical examinations that they consist of pure cultures of the vibrio. The explanation of their presence seems to be that the vibrio can grow in a rarefied atmosphere. When impure material containing vibrios and other organisms is sown in liquefied agar, it is often possible to identify the former by their mode of growth.

On the surface of agar slopes the vibrio grows very poorly; most frequently there is no growth at all. In the few tubes in which growths appeared, they were usually in the form of small, round, grey colonies which were seldom larger than a pin-head, and remained isolated. It may also develop in the form of a thin, grey film which never becomes heaped up on the surface, and close examination shows that extension takes place only into the sub-surface layers of the agar. Subcultures have not been obtained from agar growths of a greater age than nine weeks. Subcultures, however, should be made every ten days to keep the growth active.

*Gelatin*.—When tubes of liquid gelatine are sown and solidified, and kept at room temperature, the vibrio grows more slowly. There may be no appearance of growth for ten days or even longer according to the temperature. The first sign of growth is in the form of a delicate, grey, cloudy ring. In gelatin this invariably appears about half-an-inch below the surface of the medium, and no growth takes place in the deeper layers or on the surface. The colonies never attain the same size as those in agar. No liquefaction of the gelatin takes place.

*Potato*.—No growth of the vibrio has been obtained on solid potato, but, as will be seen later, potato added to broth favours its development.

*Peptone Broth*.—When ordinary peptone broth is sown with natural material containing vibrios, and incubated at 37° C., a growth is usually evident in from 24 to 48 hours. If the tubes be shaken up a greyish, vibrating cloudiness is observed. In hanging-drop preparations one sees with the microscope single elements shaped like the letter S, and long spiral forms which appear to be made up of a variable number of the S-shaped bodies joined end to end. The longer spirals are only seen in older cultures inside and outside of the body. Both forms are actively motile. They seem to move rapidly by a snake-like wriggling of the body. Fixed preparations are easily stained with Loeffler's blue or dilute carbol-fuchsin. They are decolorised by Gram's method. In the stained preparations the S-shaped and spiral forms are, of course, more distinctly outlined. Some of the single elements show a deeply stained area like the head of a comma at one end, when stained with methylene blue. In preparations made from old cultures the substance of the vibrio has a granular appearance, and many of the elements seem to have broken up into small granules of a circular shape, which take up the stain feebly. These granular forms stain well with toluidin blue. Preparations from cultures and tissue fluid show many free granules.

Multiplication seems to take place by transverse division. In hanging-drop preparations the last segments of the spiral can sometimes be seen to shoot off, and become a single element. Flagella have not been demonstrated by staining methods.

It has invariably been found impossible to obtain subcultures in peptone broth from cultures in peptone broth, but if liquefied agar or gelatin tubes be sown with a broth culture growth readily occurs in the former media, and if portions of the solid cultures be transferred to peptone broth, growth occurs in the latter.

An excellent liquid medium for the cultivation of the vibrio can be prepared by adding portions of potato to the flasks or tubes of peptone broth and

sterilising them in the autoclave. Rich cultures of the organism can be obtained in this medium. Moreover, subcultures can be carried on from tube to tube, contrary to what obtains in the case of plain peptone broth.

*Peptone Solutions*.—Practically no growth takes place in the various peptone solutions, which are stated to be excellent media for cultivating vibrios.

*Physical Requirements*.—Attempts to cultivate the vibrio under anaerobic conditions have proved negative, but the organism is capable of active growth in a rarefied atmosphere. Its cultural activity is greatest at the incubation temperature, 35°–37° C., but it grows well, though more slowly, at room temperature. The fact that it can grow at ordinary temperatures and the apparent suitability of media containing vegetable material for its growth suggest that it may perhaps lead a saprophytic existence on the pastures. It also seems certain that culture under artificial conditions soon impairs its pathogenic power, as the virulence of a pure culture of the vibrio obtained directly from the uterus is strikingly greater before than after incubation in artificial media (compare Experiments 32 and 35, see also Sections C. and F.).

*Thermal Death Point*.—Subcultures cannot be obtained from material which has been subjected to a temperature of 56.5°–57° C. for 10 minutes, but exposure to the same temperature for only five minutes does not destroy the vibrio.

*Filtrability*.—If an active culture of the vibrio, containing all forms, be passed through a Berkefeldt V filter, no growth appears on any medium sown with the filtrate.

## B.

### PARTICULARS OF OUTBREAKS OF ABORTION IN THE VARIOUS FLOCKS INQUIRED INTO, AND EXPERIMENTAL INVESTIGATIONS WITH MATERIAL OBTAINED FROM EACH FLOCK.

#### LAMBING SEASON 1906.

##### Flock 1.

Abortion was reported in a flock in Hampshire. The Secretary to the Committee paid a visit to this flock on the 13th January, and obtained the following particulars. The flock consisted of 300 well-bred Hampshire ewes which had been tupped during September, 1905, and were, therefore, due to lamb in February 1906.

During their whole period of pregnancy they had been folded, and fed on thousandhead, white turnips, swedes, hay, and a little corn. They had been in good health all through the winter, and had not been exposed to continued bad weather. An outbreak of abortion had occurred amongst the ewes on this farm three years before, but no particulars of this outbreak could be obtained. In the lambing season of 1905 (the previous season) only two ewes aborted. At the date of the Secretary's visit 25 ewes (8.3 per cent. of the flock) had aborted, or had lambed prematurely about a fortnight before time, and the information was afterwards obtained that the flock finished lambing on the 7th February, and no further cases occurred. Some of the aborted lambs were still-born, others lived only for an hour or two, but there were several weakly lambs in the flock which were not considered to have been born prematurely although it is possible that they were.

Six dead lambs were examined, but no lesions were observed. According to the shepherd, the ewes sometimes showed indefinite symptoms a short time before aborting. At least, they sometimes gave him the impression that something was wrong. By request he selected a ewe which in his opinion would probably abort. This animal (Ewe A) was slaughtered, the neck of the uterus was ligatured, the organ was excised, and despatched to the laboratory, where it arrived in the evening of the same day. At the laboratory the uterus was immediately cut into after the surface had been seared with a hot iron.

*Lesions*.—Between the chorion and the uterine mucous membrane there was a considerable amount of white mucus, that is to say, a greater quantity than is usually found in a healthy pregnant uterus, but there was nothing otherwise abnormal.

*Microscopical and Cultural Examination.*—The mucous material contained a variety of bacteria, but nothing resembling the bacillus of cattle abortion was found. Broth and agar-gelatin-serum tubes were sown with the mucus. These, however, gave mixed cultures in which none of the bacteria exhibited the characters of the bacillus of cattle abortion. It is, of course, questionable whether this ewe would have aborted had she lived. The proportion of abortion was 8.3 per cent. No experiments were carried out in connection with this flock.

#### Flock 2.

At the end of January 1906 abortion was reported to be occurring in this flock, which was in Devonshire. The Secretary to the Committee visited the farm on the 1st February and obtained the following particulars. It consisted of 150 well-bred Devon Long-wooled ewes. The first ewe was due to lamb on the 8th February. The flock was folded on white turnips and swedes. The old ewes had been tupped by a ram which had been used for three seasons, and a new yearling ram had been used for the progeny of the former. There was nothing which could be held to account for the introduction of any contagion into this flock, and no previous history of a serious outbreak of abortion could be obtained.

Early in January, that is about two or three weeks before time, a number of ewes aborted fetuses the size of rats, and abortions continued to take place at intervals, the fetuses being larger at the later periods. At the date of the Secretary's visit (1st February), 50 ewes (33.3 per cent. of the flock) had aborted. It was noticed 24 to 30 hours before abortion that the udders became larger, the vulva pendulous and flaccid, and a dark reddish-brown discharge came from the genital passage. No fetal membranes were available for examination, but the shepherd described them as being dark yellow in colour and rotten. The ewes showed no distinct signs of illness either before or after abortion. On the 2nd February a ewe (Ewe B), due to lamb on the 24th of that month, was selected for slaughter, as she was showing slight enlargement of the udder. The uterus was excised after the neck had been ligatured, and the organ was dispatched to the laboratory, where it arrived on the evening of the same day; it was examined nine hours after excision.

*Lesions.*—The uterus was normal externally. After the surface had been seared, excisions were made through the wall of the organ, and material was removed for microscopical and cultural examination. Between the chorion and the uterine mucous membrane there was an abnormal amount of white mucus as in Ewe A, but otherwise the appearances were normal. The fetus (Lamb B 19) was well developed. It showed no macroscopic lesions.

*Microscopical and Cultural Examination.*—Smears made from the uterine mucus and stained with Loeffler's blue showed large numbers of a peculiar organism, which was apparently present in pure culture. Most of the elements were comma shaped, but long spirillar forms, apparently consisting of several commas joined end to end, were also present. In hanging drops these organisms were actively motile. At first sight it was thought the organism might be a spirochete, but subsequently the growths obtained in culture-media put this view of its classification in doubt.

The stomach fluid of Lamb B 19 also contained large numbers of this vibrio in pure culture. The uterine mucus and fluid from the fetal stomach were sown in oxygenated broth and in liquefied agar-gelatin-serum, which was immediately solidified. The broth tubes showed an abundant and pure culture of the vibrio after about four days' incubation, but it was found impossible to obtain subcultures in broth from these. This was afterwards found to be characteristic of cultures of the vibrio in broth. The agar-gelatin-serum tubes after incubation for a week showed a dense grey ring of colonies about  $\frac{1}{4}$  to  $\frac{1}{2}$  inch under the surface of the medium. At first it was thought that cultures of the bacillus of cattle abortion had been obtained, as the solid tubes showed a picture which Bang described as characteristic of that organism. The microscope, however,

showed that the growth consisted of vibrio-like organisms in pure culture. Subcultures in broth were readily obtained by sowing with the solid agar growths, but attempts to continue these growths by subculturing from broth tube to broth tube failed.

At this stage of the Committee's work attention was being directed mainly to abortion in cattle, and there was *prima facie* evidence that the disease in all animals might be due to the same cause. We had not a supply of pregnant ewes at the laboratory, and the season was too far advanced to obtain them. Moreover, it was thought advisable to employ for experiments only animals whose previous history was known, and which had been tupped at the laboratory on ascertained dates.

Although up to this time there was little evidence to show that this vibrio-like organism caused abortion in sheep, it was decided to study the organism for the following reasons:—(1) A vibrio-like organism was then looked upon as a relatively uncommon microbe, and on this account its presence in connection with an outbreak of abortion lent some support to the possibility that it might be casually connected with the disease. (2) There seemed to be no doubt that this vibrio was capable under natural conditions of invading the normal pregnant uterus and its fetus, and propagating therein, a property with which only a very small minority of microbes are endowed. (3) This vibrio grew on agar-gelatin-serum after the manner of the bacillus of cattle abortion, and this form of growth was believed at the time, though erroneously, to arise owing to the uterine microbes requiring certain physical conditions which were suggested also to exist in the pregnant uterus.

It was thought advisable in the first place to ascertain whether the vibrio was pathogenic to non-pregnant animals by inoculation, and for this purpose cultures had to be employed, as the supply of pure natural material was very limited.

#### Experiment 1, Lamb 23.

This lamb, which was about six weeks old and healthy, received on the 17th February 5 c.c. of a glycerin-broth-serum culture of the vibrio into the aurial vein, and 2 c.c. of the same culture under the skin of the thigh. The culture, which had been incubated for about a week, was made from the uterine exudate of Ewe B, and it was very rich in vibrios. Two hours after inoculation the lamb was dizzy, and the temperature had risen 1° F. On the following day the lamb was rather dull, but the temperature was normal. After the second day this lamb showed no signs of disturbance. Blood smears stained by Giemsa and other stains were made and examined every day for a week, but no vibrios could be found.

#### Experiment 2, Heifers 6 and 17.

On the 19th February these two heifers, which were not pregnant, each received into the jugular vein 2 c.c. of an actively growing broth culture made from the uterine exudate of Ewe B. On the third day after inoculation the temperature of Heifer 6 rose to 104° F., but fell to normal on the day following. Heifer 17 showed no rise of temperature. Neither of these heifers exhibited any signs of illness. Blood smears were examined at intervals for two weeks, but no vibrios could be found.

#### Experiment 3, Rabbits 173 and 175.

These rabbits received respectively by subcutaneous inoculation 1 c.c. and 2 c.c. of an actively growing culture of vibrios from the uterine exudate of Ewe B. In both cases the result was negative.

#### Experiment 4, Guinea-pigs 174, 176, and 177.

These animals received subcutaneously 2 c.c. of an actively growing culture of vibrios from the uterus of Ewe B. Number 174 died six days after inoculation, but no vibrios were found in the blood or organs. The other two remained healthy.

#### Experiment 5, Rabbit 247.

On 23rd June 1906 this rabbit received into the aurial vein 2 c.c. of an actively growing broth-serum

culture of vibrios made from the stomach fluid of Lamb B 19 (*see* Flock 2, Ewe B). The result was negative.

#### Experiment 6, Guineapigs 246 and 248.

The culture used in this experiment was the same as that in Experiment 5. Guineapig 246 received 2 c.c. of culture into the peritoneal cavity. Guinea pig 248 received 2 c.c. of culture subcutaneously. In both cases the results were negative.

#### Experiment 7, Rabbits (Does) 449 and 450.

On the 8th September 1906 these does, which were believed to be in the early stage of pregnancy, were inoculated into the aural vein with 5 c.c. of a broth subculture of the vibrio. The original culture was obtained from the stomach contents of Lamb B 19 (*see* Flock 2, Ewe B). Both of these does, however, were found to be non-pregnant. They exhibited no signs of illness after inoculation.

#### Experiment 8, Guineapigs 451 and 452.

On the 8th September 1906 these guineapigs, which were pregnant, received by the mouth, 2 c.c. of the same culture as that used in Experiment 7. On September 15th (7 days after infection), Guineapig 452 was killed and examined, but no vibrios could be found in the uterus. Guineapig 451 aborted on the 20th September (12 days after receiving the culture), but as she ate most of the fetus and its membranes, no examination could be made.

#### Experiment 9, Ewes 34 and 28 (Dorsets).

Ewe 34 was tupped on October 7th, and Ewe 28 on October 19th, 1906. On November 20th the following mixture of cultures of vibrio was prepared:—One agar-gelatin-serum culture (two months old), 45 c.c. of a broth culture, which had been incubated for 14 days, and 10 c.c. of sterile broth. These were subcultures from the stomach fluid of Lamb B 19 (*see* Flock 2, Ewe B), and it will be observed from the date that the cultures had been kept up in the laboratory by subculturing for some months. Subsequent observations seemed to show that the vibrio may soon lose its virulence under artificial cultivation.

On November 20th, that is 44 days after being tupped in the case of Ewe 34 and 32 days in the case of Ewe 28, each received 5 c.c. of the above mixture into the aural vein and 20 c.c. into the vagina. On the 2nd January 1907 each again received by the mouth and vagina an emulsion in broth containing the growths from three cultures of vibrio on agar-gelatin-serum. These were also subcultures obtained from the strain started from Lamb B 19. No temperature reaction followed any of the injections.

Ewe 28 was killed for examination on February 19th, 1907, that is about a month before full time. She was not pregnant. No bacteria could be obtained either by microscopical or cultural examination of scrapings from the uterine mucous membrane.

Ewe 34 gave birth to one live and one dead lamb on March 7th, 1907, that is at full time. The lambs were fully developed. No vibrios or other bacteria were found in the fluid from the stomach of the dead lamb. The membranes were not recovered for examination.

It was thought, owing to the negative results obtained with actively growing cultures of the vibrio, that it probably had nothing to do with abortion in sheep. Nevertheless, the study of the biological characters of the microbe was continued on account of its peculiar features.

#### Summary of Observations in connection with Flock 2.

Percentage of abortions in flock, 33.3 per cent. Vibrios were demonstrated. One pregnant ewe had old cultures introduced into the vagina with negative results. One pregnant guineapig was infected by mouth, using old cultures; the guineapig aborted.

#### LAMBING SEASON 1907.

The lesson learned in 1906 was that abortion in sheep usually occurred towards the end of the lambing season, and that in order to utilise material from the

field for the study of outbreaks by experimental methods it would be necessary to arrange to have at the laboratory a supply of pregnant ewes due to lamb at late periods. A supply of young ewes was obtained, and a certain number were put with the ram each month, starting from October 1906. It was found difficult, however, to get ewes to take the ram later than the month of April. In fact, we found it impossible to keep in hand a sufficient supply of pregnant ewes, even by using Dorsets, which would carry us through the interval which occurs between the ordinary lambing and tupping seasons. This turned out to be a serious obstacle to the progress of the investigation, as it was afterwards found that the vibrio under the conditions of artificial cultivation lost much of its original virulence, and it was not possible to keep this up from season to season by passing it through a continuous series of pregnant sheep. In fact, we always were left practically without active material to work upon during the greater part of the interval between the lambing seasons.

#### Flock 3.

Early in 1907 abortion was reported to be occurring in this flock, which was in Lincolnshire. It consisted of 100 Lincoln ewes, 40 of which (40 per cent.) aborted. The abortions began from a month to three weeks before the date at which the ewes were expected to lamb.

On February 9th two lambs (C 1 and C 2), which had been aborted, were sent to the laboratory. An aborted calf and its membranes were received from the same place on February 12th. In the membranes and fluid from the stomach of this calf (C 3) a fair number of the bacilli of cattle abortion were found. The owner of the farm, knowing that his cattle were infected with cattle abortion, was inclined to think that they had infected his ewes, as the cows and sheep had been grazed on the same pastures. The lambs sent were nearly up to time in development. No macroscopic lesions could be found, but microscopical examination revealed a small number of vibrios in the fluid taken from the stomachs. The vibrios were in pure culture, and nothing resembling the bacillus of cattle abortion could be found either by cultural or microscopical examination.

The finding of this peculiar vibrio in connection with outbreaks of abortion in sheep in two such widely separated counties as Devonshire and Lincolnshire lent fresh support to the impression that the organism was causally connected with the outbreaks. The following experiment was carried out with the stomach fluid from the above-mentioned lambs.

#### Experiment 10, Ewes 72 and 73.

Ewe 72 (Cheviot) was tupped on January 9th, 1907. She was, therefore, due to lamb early in June. Ewe 73 (Dorset) was tupped on January 11th, 1907, and was also due to lamb early in June. A mixture of two parts of the thick fluid from the stomachs of the above lambs was diluted with one part of broth, and, on February 9th each of these ewes received 2 c.c. of the mixture into the jugular vein and 5 c.c. into the vagina. No temperature re-action followed upon inoculation. Ewe 72 gave birth to a live lamb on June 6th, 1907, that is about full time. Neither vibrios nor bacilli of cattle abortion could be found in smears made from the fetal cotyledons. Material was removed by swabs from the genital passage shortly after the lamb was born, but no special microbe was found in smears made from the swabs. On the same day (June 6th) it was thought advisable to kill her fellow, Ewe 73, and examine her uterus before it became open.

*Lesions.*—After slaughter the neck of the the uterus was tied, the organ excised, and taken to the laboratory. No external lesions were present. On opening into the organ no exudate could be discovered. The cotyledons looked healthy, and the uterine mucous membrane appeared normal. The fetus (Lamb 53) was well developed and up to time.

*Microscopical and Cultural Examination.*—No bacteria could be found by microscopical or cultural examination of material taken from the uterine mucous membrane.

*Summary of Observations in connection with Flock 3.*

Percentage of abortion in flock, 40 per cent. Vibrios were demonstrated. Two pregnant ewes were infected with natural material by the veins; neither aborted.

*Flock 4.*

This flock was located in Somerset. On the 4th February 1907 abortions were reported to have occurred, and the following particulars were obtained. The flock consisted of 241 ewes which had been tupped at different periods to lamb down from the last week of December to the beginning of March. About the first week in December a number of ewes commenced to abort, and from that time onwards abortions continued to take place. As many as four or five ewes would abort on the same day. Most of the fœtuses were putrefying when expelled. At the earlier dates the fœtuses were devoid of wool, and were apparently not nearly up to time. In the month of January, however, most of the fœtuses were covered with wool. Only a few were alive when expelled, and none of these lived for more than an hour. Up to the 4th February 70 ewes had aborted, and before the end of the lambing season abortions had occurred in slightly over 30 per cent. of the flock. A lamb (D 1) which had been aborted with its membranes on the morning of the 11th February was forwarded to the laboratory, where it arrived the same evening. The lamb was nearly at full time, and showed no macroscopic lesions. The fetal membranes, which were fresh, showed very little alteration, except that certain of the cotyledons were yellow and anæmic-looking, like some of those taken from cows which have aborted (*see* Report, Part I., Epizootic Abortion in Cattle, p. 5). The vibrio was found in large numbers in smears made from the fetal cotyledons, but, of course, the cotyledons had become contaminated by extraneous material. The fluid from the stomach of the lamb (D 1) also contained vibrios, and pure cultures of the organism were obtained from it in fluid and solid media. The following experiments were carried out with the material obtained from this flock:—

*Experiment 11, Ewes 89 and 90.*

Ewe 89 (Cheviot) was tupped on October 10th, 1906, and was due to lamb early in March. Ewe 90 (Cheviot) was tupped on February 5th, 1907, and was due to lamb early in July. On February 12th an emulsion was made by washing the cotyledons from Lamb D 1 (sent up from Flock 4) in sterile water. The cotyledons had been preserved on ice since the time of arrival (February 11th). On this day, *i.e.*, February 12th, each of the above ewes received 80 c.c. of the mixture by the mouth and 50 c.c. by the vagina. As the object at this stage of the inquiry was to find out whether or not material from aborting ewes would produce abortion in others, it was thought advisable to introduce some of it by the veins as well as by the natural orifices, and on February 15th each ewe received into the jugular vein 3 c.c. of fluid from the stomach of Lamb D 1 diluted by one half with sterile broth. No temperature reaction followed inoculation.

Ewe 89 aborted a dead fœtus (Lamb 40) on March 12th, that is 153 days from the commencement of pregnancy (practically at full time), and 25 days after inoculation into the vein. The lamb was well developed, but the lungs had never been inflated. The fluid from its stomach contained a small number of cocci and longish rods, but the culture media sown with the material remained sterile. It is probable that these were chance organisms, which had invaded the fœtus after expulsion, as it had lain in the pen for some time before it was discovered. The ewe was immediately slaughtered, the neck of the uterus ligatured, and the organ cut out for examination in the laboratory.

*Lesions.*—On opening into the organ many of the fetal and maternal cotyledons were found to be still attached. The surfaces of those separated were bathed in what looked like a milky juice. The uterine mucous membrane was covered at parts by a reddish-brown exudate resembling thick mucus in consistence. In other respects the membrane appeared normal.

*Microscopical and Cultural Examination.*—The exudate contained a considerable number of vibrios, but, as was expected, seeing that the uterus had been open, they were not present in pure culture. Enormous numbers of vibrios in pure culture, however, were found in preparations made from the fetal and maternal cotyledons after separation by traction. These preparations also showed a large number of leucocytes. Nothing resembling the bacillus of cattle abortion could be found either by microscopical or cultural examination. Pure cultures of the vibrio were obtained in tubes of glycerine-broth-serum sown with scrapings from the freshly-separated cotyledons.

Ewe 90 was slaughtered on the 6th June, that is about a month before the time when she was expected to lamb. The uterus, however, was empty and had no appearance of ever having been pregnant. No bacteria could be found in scrapings from the uterine mucous membrane of Ewe 90, either by microscopical or cultural examination.

*Experiment 12, Ewe 74.*

Ewe 74 (Cheviot) was tupped on January 15th, 1907. On February 15th, that is a month after taking the tup, she received into the jugular vein 3 c.c. of a dilution to a half with broth of the fluid from the stomach of Lamb D 1 received from Flock 4. The fluid had been collected in sterile pipettes, and had been preserved on ice since the 11th February, the date on which the lamb arrived at the laboratory; it had given pure cultures of vibrio. For two days after inoculation the temperature of this ewe varied from 104.6 to 105, but on the third day it fell to normal and the ewe showed no disturbance of health. On May 25th, *i.e.*, 145 days after taking the tup (nearly at full time), and 99 days after inoculation, Ewe 74 aborted two lambs. One of these was born alive, but only lived for about an hour. One set of membranes came away with the lamb. It was covered by a thick reddish-brown exudate, some of which had become almost solid. Microscopical preparations made from this exudate showed enormous numbers of vibrios. The lambs (51 and 52) were practically up to time, and vibrios were found in the fluid from the stomachs of both of them. In the case of Lamb 51, which was born alive, other organisms than the vibrio were also present, but in the stomach fluid of Lamb 52 the vibrio was the only organism which could be found by microscopical examination, and pure cultures were obtained in solid and fluid media. Nothing resembling the bacillus of cattle abortion could be found in the material taken from these lambs. The ewe was killed about two hours after she had aborted; the neck of the uterus was ligatured; the organ was cut out and taken to the laboratory for examination.

*Lesions.*—The uterus was partially contracted, but its wall was œdematous and much thicker than normal. On cutting into the organ the uterine mucous membrane was normal in appearance, but was covered at different parts by a yellowish and reddish-brown exudate. The one set of membranes was still attached to the uterus. On pulling apart the fetal and maternal cotyledons a white milky juice was found in the separated surfaces.

*Microscopical and Cultural Examination.*—Preparations made from the uterine exudate and from the cotyledons showed large numbers of vibrios under the microscope. Cultures of the vibrio were obtained from the uterine exudate and cotyledons, but, as was not unexpected, seeing that the uterus had been open, these cultures contained other bacteria.

*Experiment 13, Ewes 101, 104, and 106.*

Ewe 101 (Cheviot) was tupped on February 13th, 1907. Ewe 104 (Cheviot) was tupped on February 16th, 1907. Ewe 106 (Cheviot) was tupped on February 17th, 1907. All three ewes, therefore, were due to lamb early in July. On the 17th April, that is 63 days after being tupped in the case of Ewe 101, 66 days in the case of Ewe 104, and 67 days in the case of Ewe 106, each of these three ewes was infected with an emulsion of pure cultures of the vibrio obtained on artificial media. The original culture had been obtained from the cotyledons of Ewe 89 (*see* Experiment 11).

Three agar-gelatin-serum tubes, which were first subcultures from Ewe 89 and which had been incubated for 24 days, and one glycerine-broth-serum culture (6 c.c.), which was also a first subculture and had been incubated for the same time, were triturated in a sterilised mortar with 36 c.c. of sterile broth. Ewes 101 and 104 each received 5 c.c. of the above mixture into the jugular vein and 5 c.c. into the vagina. Ewe 106 received 5 c.c. into the jugular vein. No temperature reaction followed inoculation in any of these ewes. On May 11th each of the three received intravenously 5 c.c. of a glycerine-broth-serum subculture from the stomach fluid of Lamb D 1 sent up from this Flock (4) on February 11th. The culture was diluted with 5 c.c. of sterile water before injection. On June 4th each of the ewes was again injected into the jugular vein with 3 c.c. of a mixture prepared from two agar-gelatin-serum subcultures of the stomach fluid of Lamb 52 (see Experiment 12, Ewe 74) by triturating the solid cultures with 10 c.c. of a glycerine-broth-serum subculture which had the same origin. In addition each received by the vagina on this date 10 c.c. of a mixture prepared by triturating with 30 c.c. of sterile water an agar-gelatin-serum subculture which had originally been obtained from the uterine exudate of Ewe 74 (see this Flock, Experiment 12). The reason for introducing the cultures from Ewe 74 (which was the same strain of virus) into this experiment was that they had been about six weeks less than those from Ewe 89 under conditions of artificial cultivation, and it was thought that those from Ewe 89 might possibly have lost their virulence at the date they were inoculated, as they showed evidence of less active growth in the incubator.

Ewe 101 showed what were believed to be signs of approaching parturition on the morning of June 11th, that is 118 days from the date of service, and 55 days after the first infection. This ewe was immediately slaughtered, and her uterus examined. The appearance of the uterus on *post mortem* did not indicate that abortion was about to take place. The membranes were firmly attached, the fœtus was well developed for the period of pregnancy, and no exudate was present on the uterine mucous membrane. On separating the maternal and fetal cotyledons by traction, however, the surfaces of some of them showed a very small amount of white milky juice, but no vibrios could be found by microscopical examination, and cultures were not attempted.

Ewe 106 showed signs of uneasiness on the morning of June 7th, *i.e.*, 114 days from the date of service and about five weeks from full time. A thick brown discharge was observed around the genital organs.

As the symptoms continued, this ewe was slaughtered on the afternoon of the same day; the neck of the uterus was ligatured, and the organ was cut out and taken to the laboratory for examination.

*Lesions.*—The wall of the organ was œdematous and thickened. After searing the surface with a hot iron incisions were made into the uterine cavity. A reddish-brown exudate of the consistence of thick mucus was found on the mucous membrane and on the chorion, particularly in the neighbourhood of the cotyledons. The fetal membranes were œdematous. The fœtus (Lamb 54), although fairly well developed, had evidently been dead for some little time, as a blood-tinged fluid was present in the peritoneal cavity. The maternal and fetal cotyledons, which could be easily pulled apart, showed a considerable amount of white milky juice on the separated surfaces.

*Microscopical and Cultural Examination.*—Smears made from the exudate and cotyledons showed a considerable number of vibrios which were apparently present in pure culture. Pure cultures of the vibrio were obtained on fluid and solid media from the uterine exudate and from scrapings of the cotyledons. No vibrios could be found in the fluid from the fetal stomach by microscopical examination, but pure cultures of vibrio were obtained from it on solid media.

Ewe 104 went over her time, and she was slaughtered in the belief that she was not pregnant, which turned out to be correct. She had never shown any signs of having aborted, and the uterus on examination gave no evidence of having been pregnant. No bacteria

could be found in smears made from the uterine mucous membrane.

#### Experiment 14, Ewes 105 and 107.

Ewe 105 (Cheviot) was tupped on February 17th, 1907. Ewe 107 (Cheviot) was tupped on the 20th February. Both ewes, therefore, were due to lamb about the middle of July. On May 27th, *i.e.*, 101 days after service in the case of Ewe 105, and 98 days after service in the case of Ewe 107, each ewe received by the mouth 10 ounces of an emulsion which was prepared by bruising the fetal membranes of Ewe 74 (see Experiment 12) in sterile water. Ewe 74 had aborted on the 25th May, and during the interval between that date and the 27th the membranes had been kept on ice.

Ewe 105 gave birth to a live lamb on July 25th, *i.e.*, at full time. The lamb was healthy and well developed. Smears were made from swabs which had been inserted into the vagina immediately after parturition, but no vibrios or bacilli of cattle abortion could be found. This ewe afterwards developed metritis, which probably was only a coincidence, and had nothing to do with the experiment.

Ewe 107 was slaughtered after she had gone about a fortnight over time, as it was decided that she was not pregnant. The uterus on examination was quite normal, and apparently had never been pregnant. No bacteria could be found in smears or cultures made from scrapings from the uterine mucous membrane.

#### Experiment 15, Ewes 102 and 29.

Ewe 102 (Cheviot) was tupped on February 15th, 1907. Ewe 29 (Dorset) was tupped on February 6th, 1907. The former, then, was due to lamb about the middle of July and the latter about the first week in July. On May 27th, *i.e.*, 101 days after service in the case of Ewe 102 and 108 days in the case of Ewe 29, each ewe received into the vagina 50 c.c. of an emulsion made by bruising the cotyledons of Ewe 74 with sterile water. The material had been kept on ice since the 28th May (12 days), when Ewe 74 aborted. In addition each received into the jugular vein on the same day 3 c.c. of a broth culture from the cotyledons of Ewe 74 (see Experiment 12). The culture had been incubated for 48 hours. No temperature reaction followed inoculation.

Ewe 29 aborted one lamb at noon on the 14th June, *i.e.*, 128 days after service, about three weeks before full time, and 18 days after infection. The lamb (Lamb 56) was born alive, but it only lived for half an hour. A brown coloured discharge was present around the genital organs, and this contained an immense number of vibrios, though not, of course, in pure culture. The ewe was slaughtered immediately after the death of the lamb, the neck of the uterus was ligatured, the organ cut out, and taken to the laboratory for examination.

*Lesions.*—No lesions were found on the external surface of the uterus. On cutting into the organ, however, congested areas were found on the mucous membrane. A fairly plentiful amount of exudate resembling blood-stained mucus was found in the uterine cavity. The fetal membranes were still attached, but could be easily separated by traction. On separation the surfaces appeared to be covered with an abundant milky-looking juice.

*Microscopical and Cultural Examination.*—The uterine exudate and the juice from some of the cotyledons contained large numbers of vibrios, but the exudate, as was not unexpected, seeing that the uterus had been open, show a few other organisms. Pure cultures of the vibrio were obtained in fluid and solid media by sowing with the juice of the cotyledons which had been freshly separated. Tubes of culture media inoculated from some of the cotyledons, however, remained sterile. No vibrios could be found by microscopical examination in the fluid from the fetal stomach, but cultures were obtained by sowing media with this material. They were, however, impure, owing probably to the lamb having lived for some little time after birth. Nothing resembling the bacillus of cattle abortion could be found in the uterus or fœtus of this ewe.

Ewe 102 was slaughtered on August 13th, that is about two months over full-time. The uterus had apparently never been pregnant.

*Summary of Observations in connection with Flock 4.*

Percentage of abortions, 30 per cent.; vibrios were demonstrated in material. Ten ewes were put under experiment, four of which were not pregnant. Four pregnant ewes aborted and showed vibrios. One of the latter was infected by the veins, vagina and mouth, with natural material; two received natural material by the veins; one received natural material by the vagina and culture by the veins. Two pregnant ewes did not become infected. One of these received culture by the veins and vagina, and one received natural material by the mouth.

*Flock 5 (Hampshire Downes).*

During the second week of February 1907 abortion was reported to be occurring in this flock, which was in Berkshire. Mr. Brand, one of the Board's Veterinary Inspectors, was instructed to visit the farm and obtain all particulars, and to send material for investigation to the laboratory. The flock consisted of 300 ewes, of which over 80 (27 per cent.) aborted. Most of the ewes aborted about the sixteenth week of pregnancy, that is about four or five weeks before full-time, but the first abortion took place in a ewe during the twelfth week of pregnancy. A ram and about 20 shearling ewes had been brought on to the farm in the autumn. It was noticed by the shepherd that several of the ewes served by this ram returned for service after about six weeks, and then became pregnant. Some of these ewes along with others aborted, but it was not possible to connect the introduction of the disease with the imported lot. On the 14th February two aborted lambs (E 1 and E 2), both within three weeks of full time, and a mummified fœtus which had been aborted at the same date, were sent to the laboratory. These were dealt with the same evening. Nothing could be found in the mummified fœtus, but the fluid from the stomachs of the other two contained vibrios in considerable numbers, and actively growing pure cultures of the vibrio were obtained in fluid and solid media. On the following day, February 15th, a ewe (Ewe E), which was thought to be showing signs of approaching parturition three or four weeks before full time, was killed. The neck of the uterus was ligatured, the organ excised, and despatched at 2 p.m. to the laboratory, where it arrived the same evening at 6.30 p.m. This material was dealt with on arrival.

*Lesions.*—The wall of the uterus near the neck was œdematous and much thickened. The surface of the organ was seared with a hot iron, incisions were made with a heated scalpel, and material was removed for cultural and microscopical examination. On opening the uterus a considerable amount of mucus-like material was found on the mucous membrane and the chorion. In some places this exudate was grey in colour; it was, in fact, like the normal uterine mucus, except that its amount was greatly increased. In other places the exudate was reddish-brown in colour, as if at some period it had been mixed with blood. Its consistency varied from that of fluid mucus to that of dough. Many of the cotyledons were softened and broken down. Some had evidently separated some little time before the post mortem examination was made, and it is possible that this premature separation may have led to a certain amount of blood becoming mixed with the exudate. The fetal membranes were very œdematous. Two fœtuses (Lambs E 3 and E 4) were contained in the uterus. Both were perfectly fresh, and neither showed macroscopic lesions of any kind.

*Microscopical and Cultural Examination.*—Smears made from the uterine exudate showed vibrios in large numbers, but a few foreign organisms were also present. Cultures of the vibrio were obtained in fluid and solid media, but a few other micro-organisms also grew in the tubes. The fluid from the fetal stomachs also showed vibrios to the microscope, and pure cultures of the organism were obtained from this material.

*Experiment 16. Ewes 94, 95, and 96.*

Ewe 94 (Cheviot) was tupped on February 7th, 1907, and was due to lamb about the first week in July. Ewe 95 (Cheviot) was tupped on February 11th, 1907, and was due to lamb about the same time as the former. Ewe 96 (Cheviot) was tupped on the same day as Ewe 95. On February 16th, *i.e.*, nine days after service in the case of Ewe 94 and five days in the case of Ewes 95 and 96, each ewe received into the jugular vein 5 c.c. of a dilution of the fluid from the fetal stomach of Lamb E3 from Flock 5, containing two parts of stomach fluid and one part of sterile broth. At the same time each ewe received into the vagina 20 c.c. of a dense emulsion in broth of the uterine exudate from Ewe E.

In the case of Ewe 94 the temperature rose to 106 the day after inoculation, but on the following day it fell to normal. On February 20th, that is four days after the first inoculation, it was thought advisable also to inoculate into the veins with the uterine exudate from Ewe E, and each of the three ewes received into the jugular vein 3 c.c. of a dense emulsion in broth of this material.

The temperature of Ewe 94 rose to 107 the day after inoculation, but returned to the normal on the following day. The two other ewes did not appear to be disturbed systemically.

Ewe 94 was killed on May 4th, that is 86 days after service (64 days before full time), and 77 days after the first inoculation. The ewe was not showing any symptoms of approaching abortion, but it was thought that by killing her we might possibly be in a position to study the lesions of abortion in the early stage of the disease. The neck of the uterus was ligatured, the organ was excised, and taken to the laboratory for examination.

*Lesions.*—Externally the uterus showed no macroscopic lesions. Incisions were made through the wall of the organ after it had been seared, but nothing abnormal was found. On opening the uterus it was found to contain a fœtus (Lamb 48), which was well developed for the period of pregnancy. The uterine mucous membrane and the fetal envelopes were normal in appearance. No bacteria of any kind were found either by microscopical or cultural examination of material taken from the mucous membrane and the cotyledons. The contents of the fetal stomach contained no bacteria.

Ewe 95 was slaughtered on the 15th June, that is 124 days after service (nearly four weeks before full time), and 119 days after the first inoculation. This ewe was not showing signs of approaching parturition at the time she was slaughtered, but it was thought that as most of the animals observed in the field aborted about a month before full time, she ought at this date to show lesions, if she had become infected. The neck of the uterus was ligatured, the organ was excised and taken to the laboratory for examination.

*Lesions.*—Externally the uterus appeared normal. Its surface was seared in the usual way, and incisions were made with a heated knife. At the site of these incisions an abnormal though not very excessive amount of white-coloured mucus was present on the uterine mucous membrane and chorion, and it was possible to obtain some of this for cultural and microscopical examination. On opening the organ most of the cotyledons were firmly attached, but on separating them by traction they appeared markedly congested, and a considerable amount of milky juice was found on the separated surfaces. Some of the cotyledons also appeared to be softer than normal. The uterus contained one fœtus, which was well developed for the period of pregnancy, and showed no macroscopic lesions.

*Microscopical and Cultural Examination.*—Only a few vibrios were found in smears prepared from the exudate, but they were fairly plentiful in preparations made from the separated cotyledons. Pure cultures of vibrio were obtained in fluid and solid media by sowing with scrapings from the cotyledons. Apparently, however, the vibrio was not present in every cotyledon, as they could not always be found with the microscope, and some of the tubes sown remained sterile. The

contents of the fetal stomach showed no microbes under the microscope, and tubes of culture media sown with this material remained sterile. Ewe 96 apparently did not become pregnant. She was never observed to abort, and when the uterus was examined after slaughter on the 13th August it had no appearance of ever having been pregnant. No bacteria could be found either by microscopical or cultural examination of material scraped from the uterine mucous membrane.

*Summary of Observations in connection with Flock 5.*

Proportion of abortion, 27 per cent.; vibrios were demonstrated. Three ewes were put under experiment, one of which was not pregnant. The two pregnant ewes received natural material by the veins and vagina; one became infected, and showed vibrios.

*Flock 6.*

About the middle of February 1907 abortion was reported to be occurring in this flock, which was in Suffolk. The flock consisted of 196 ewes, which were on a lowland farm, and before the end of the lambing season 50 per cent. had aborted. The trouble began about a month before the ewes were due to lamb. The owner was asked to slaughter a ewe which he considered was showing premonitory symptoms of abortion, and to forward the uterus. This ewe (Ewe F) was slaughtered on the 21st February, and the uterus arrived at the laboratory on the evening of the same day. The neck of the organ had been ligatured according to instructions, and it was packed in a large rubber bag which had been sent from the laboratory to the farm for the purpose. In spite of the careful packing, however, the organ had become ruptured in transit. On arrival it was found that a considerable amount of light yellow liquid containing mucus-like flakes had been pressed out through the rupture into the water-proof cover. Smears from the uterine exudate were examined, and several varieties of organisms were found. The exudate, however, was not putrid. Some of the organisms had a certain amount of resemblance to the bacillus of cattle abortion. It was found, however, by cultural examination and by inoculation to a pregnant heifer that the bacteria present had no relation to the bacillus of cattle abortion. The culture tubes, in fact, showed that the bacteria under discussion were micrococci. No vibrios could be found in the smears made from the exudate, nor could they be observed in the mixed cultures obtained on artificial media. The fetus which was contained in this uterus was well developed, and showed no macroscopic lesions. No organisms were found by microscopical or cultural examination of the fluid from the fetal stomach. As the outbreak was reported at a very late stage of the lambing season, it was impossible to get further material from this flock.

*Experiment 17, Ewe 100.*

Ewe 100 (Cheviot) was tupped on February 13th, 1907, and was due to lamb early in July. On the morning of the 22nd February, *i.e.*, nine days after service, she received into the jugular vein 8 c.c. of an emulsion in broth of the uterine exudate of Ewe F (Flock 6). This material had been kept on ice since its arrival the previous evening, as it was then too dark to carry out an experiment. On the day after inoculation this ewe developed a very high temperature—106–107—and died of septic pneumonia on February 26th, four days after inoculation. The septic pneumonia was in all probability caused by the material inoculated, which contained various microbes. The uterus was pregnant, but no bacteria of any kind could be discovered by cultural or microscopical examination of material taken from the uterine mucous membrane and fetus.

*Experiment 18, Ewes 97 and 98.*

Both these ewes (Cheviots) were tupped on February 11th, 1907, and were, therefore, due to lamb early in July. On the 22nd February each received by the mouth 10 ounces of a dense emulsion in water of the uterine exudate of Ewe F. Starting from the day after inoculation, both ewes for a period of six days showed a temperature reaction varying from 103 to 106, and it is not improbable that this was due to the same microbes which had caused septic pneumonia in

Ewe 100, inoculated into the jugular vein with the same material.

Ewe 97 was slaughtered on June 15th, that is 124 days after service (about three weeks before full time) and 113 days after infection. At the time of slaughter she was not showing signs of approaching parturition, but it was thought advisable to kill her, in the hope that she might furnish an opportunity for studying the lesions in connection with abortion in sheep, and provide uncontaminated material for bacteriological examination. The neck of the uterus was ligatured; the organ was excised and taken to the laboratory for examination.

*Lesions.*—Externally no macroscopic lesions were present. One fetus (Lamb 59) was present in the uterus; it was well developed, and showed no macroscopic lesions. On cutting into the organ areas of marked congestion, which might have gone on to œdema had the animal lived, were found on the uterine mucous membrane. The cotyledons were fairly firmly attached, but on separation many of them showed a distinct amount of white milky juice on the separated surfaces.

*Microscopical and Cultural Examination.*—Vibrios in small numbers were found in smears made from some of the cotyledons. The uterus was left open some time before cultures were made, and although some of the culture-tubes showed vibrios after incubation other microbes also developed. No bacteria could be found either by microscopical or cultural examination of the contents of the stomach of the fetus.

It is not improbable, especially in the light of subsequent experience, that Ewe 97 would have aborted had she not been slaughtered.

It is to be noted that owing to the condition of the exudate from Ewe F, which was used for infection in this experiment, it was deemed advisable to administer it to this animal by the mouth only, and that, although no vibrios could be found in it by microscopical examination, the uterus of Ewe 97 contained vibrios 113 days after infection with the material administered by one of the natural orifices (the mouth). While not supplying absolutely conclusive evidence that the vibrio could cause abortion in sheep, this experiment certainly showed that the vibrio-like organism introduced by the mouth was able to invade the uterus and live therein.

Ewe 98 apparently did not become pregnant, as she did not bring forth a lamb and was never observed to abort. She was killed on the 13th August, and an examination of the uterus showed no signs of it ever having been pregnant. No bacteria could be obtained by culture or microscopical examination in scrapings from the uterine mucous membrane.

*Summary of Observations in connection with Flock 6.*

Proportion of abortions, 50 per cent.; vibrios were not demonstrated in material from the field.

Three ewes were experimentally infected, one of which died and another was found to be barren.

The remaining ewe, which received natural material by the mouth, became infected, and showed vibrios.

*Flock 7 (Hampshire Downs).*

Towards the end of February 1907 abortion was reported to be occurring in this flock, which was located in Wiltshire. Mr. Brand, one of the Board's Veterinary Inspectors, visited the flock on the 26th February, and obtained the following particulars. It consisted of 320 ewes, of which 50 (15.6 per cent.) had aborted by the end of the lambing season, which practically coincided with Mr. Brand's visit. Abortion also prevailed amongst the cattle on this farm, and it was thought by the owner that the ewes had probably been infected by the cattle. Abortion began amongst the ewes about a month before lambing time.

On the 27th February a ewe (Ewe G), believed to be showing signs of approaching abortion, was slaughtered. The neck of the uterus was ligatured, the organ was excised, and despatched to the laboratory, where it arrived on the same evening. The material was examined on arrival.

*Lesions.*—The uterus showed no external lesions. The surface of the organ was seared with a hot iron, and incisions were made with a heated scalpel. From these incisions it was possible to remove a considerable

amount of exudate for microscopical and cultural examination. On opening the organ it was found to contain two lambs. One was almost putrid, the other (Lamb G 1) was comparatively fresh. The uterine mucous membrane and the chorion were covered by a considerable amount of fluid exudate of a chocolate brown colour, but no other lesions were apparent.

*Microscopical and Cultural Examination.*—Smears made from the uterine exudate contained a variety of organisms, which was not unexpected seeing that one of the fœtuses had become putrid. Nothing resembling the vibrio or the bacillus of cattle abortion could be found in the smears. Culture tubes containing various media were sown with the uterine exudate, but these all developed a variety of bacteria, none of which were the vibrio or the bacillus of cattle abortion. The fluid from the fœtal stomach of Lamb G 1 also contained several different varieties of microbes; no vibrios or bacilli of cattle abortion were demonstrated in this material, either by microscopical or cultural examination.

A heifer, which eventually proved not to be pregnant, was inoculated with the uterine exudate.

#### Experiment 19, Ewes 99 and 33.

Ewe 99 (Cheviot) was tupped on February 11th, 1907. Ewe 33 (Dorset) was tupped on February 14th, 1907. They were both, therefore, due to lamb early in July.

On February 28th each ewe received by the mouth 10 ounces of a dense emulsion of the uterine exudate from Ewe G. This material had been kept on ice since the arrival of the uterus on the previous evening. Four days after receiving the exudate by the mouth, both these ewes showed signs of illness. Their temperatures fluctuated for nine days between 104 and 106, and the animals suffered from profuse diarrhœa. These symptoms probably arose from the exudate being putrid.

Ewe 99 was slaughtered on June 15th, *i.e.*, 124 days from the date of service, about three weeks before full time, and 107 days after infection. The reason for slaughtering this ewe was that she did not appear to be quite well, and it was thought that at this period after infection whatever lesions were going to develop would probably be in evidence, and it might be possible to obtain pure material for bacteriological examination from the uterus.

*Lesions.*—After slaughter the neck of the uterus was ligatured, the organ was excised, and taken to the laboratory for examination. The wall was slightly thickened by œdema. On opening into the organ it was found to contain one fœtus. A small amount of reddish-brown, mucoid exudate was found on certain parts of the uterine mucous membrane, and on the chorion. The uterine mucous membrane also showed large patches of hæmorrhagic congestion. Many of the cotyledons had separated. These were broken down, and had a pulpy appearance. They also appeared to be distinctly swollen. On separating others by traction the separated surfaces were covered by a milky juice, which in some cases was blood tinged.

The fœtus (Lamb 60) was well developed, and showed no macroscopic lesions.

*Microscopical and Cultural Examination.*—A considerable number of vibrios were found in pure culture in smears made from the surface of the cotyledons. Pure cultures of the vibrio were obtained by sowing culture media with the juice from the cotyledons. Some of the tubes, however, remained sterile, and one or two became foul.

Ewe 33 turned out not to be pregnant; she had never been observed to abort.

It is to be noted that in the case of Ewe 99 there was produced, subsequent to the administration of the uterine exudate from Ewe G by one of the natural orifices (the mouth), a condition which would almost certainly have ended in abortion, and that vibrios were found in pure culture in the uterine lesions of Ewe 99, although, owing probably to the multiple bacterial contaminations in the uterine exudate of Ewe G, which was used to infect the former, no vibrios could be demonstrated in it.

#### *Summary of Observations in connection with Flock 7.*

Proportion of abortions, 15 per cent.; vibrios were not demonstrated in material from the field.

Two ewes were infected, one of which was not pregnant. The remaining ewe, which received natural material by the mouth, became infected, and showed vibrios.

#### *Flock 8.*

Towards the end of February 1907 abortion was reported to be occurring in this flock, which was located in Lincolnshire. The flock consisted of 400 ewes, of which 100 (25 per cent.) had aborted by the end of the lambing season. The owner reported that abortion in ewes had been known on this farm for 20 years. It appeared at irregular intervals, however, and when an outbreak occurred, he generally reckoned that about 15 per cent. of his ewes would abort. Abortion usually began about a month before full time.

A lamb (Lamb H 1), which had been aborted with its membranes on the 28th February, was received at the laboratory on the evening of the same day. The chorion was covered at parts by a considerable quantity of slimy exudate which was soiled by admixture with extraneous material (earth, straw, hay, &c.) in which it had been packed. Vibrios were not found in this exudate.

#### Experiment 20, Ewe 92.

Ewe 92 (Cheviot) was tupped on February 5th, 1907. On February 28th, *i.e.*, 23 days after service, she received into the jugular vein 2 c.c. of the stomach fluid from Lamb H 1, and at the same time 10 ounces of dense emulsion in water of the slimy material from the membranes of the same lamb by the mouth. No temperature reaction or illness followed the administration of the materials. On July 16th, *i.e.*, at full time and 138 days after infection, Ewe 92 gave birth to a healthy living lamb. The fœtal membranes were examined, but neither vibrios nor bacilli of cattle abortion could be found.

#### Experiment 21, Ewe 103.

Ewe 103 (Cheviot) was tupped on the 15th February 1907, and was due to lamb about the middle of July. On February 28th, *i.e.*, 13 days after service, she received by the mouth 10 ounces of a dense emulsion in water of the slimy material from the membranes of Lamb H 1. This ewe showed no signs of ill-health after receiving the material, but the temperature remained a degree higher than normal for three days. On June 15th, *i.e.*, 120 days after service (about a month before full time), and 107 days after infection, Ewe 103 was slaughtered as she did not appear to be quite well. The neck of the uterus was ligatured, the organ was excised, and taken to the laboratory for examination.

*Lesions.*—No lesions were present on the external surface of the organ, but through the wall one could see a large livid patch of congestion on the mucous membrane. On cutting into this patch it showed hæmorrhagic congestion, which would probably have been followed by œdema had the animal lived. Some of the cotyledons were separating, but others were fairly firmly attached. They had an abnormally congested appearance, and showed a small amount of milky juice on the surface when separated. Covering the patch of congestion there was a considerable amount of white mucus-like exudate which was not blood stained. One fœtus (Lamb 61) was present. It was well developed and showed no macroscopic lesions.

*Microscopical and Cultural Examination.*—The mucoid exudate contained a considerable number of vibrios in pure culture, but no culture tubes were sown with it, as the uterus had been widely dissected before the material was observed. Vibrios were also found in scrapings from the cotyledons, but cultures were not obtained, probably because the actual cotyledons selected for obtaining seed material were not infected.

No bacteria could be found in the contents of the foetal stomach either by microscopical or cultural examination.

*Summary of Observations in connection with Flock 8.*

Proportion of abortions, 25 per cent.; vibrios were not found in material from the field.

Two ewes were put under experiment. One, which did not become infected, received natural material by the mouth and veins. The other, which became infected and showed vibrios, had received natural material by the mouth.

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*Flock 9.*

A few cases of abortion were reported to have taken place in this flock, which was located in Wiltshire. The flock consisted of 300 ewes, of which 30 (10 per cent.) aborted about 10 days before full time.

On 8th February 1908, two lambs (J 1 and J 2) and their membranes, which had been aborted by different ewes in this flock, were sent to the laboratory.

No vibrios or bacilli of cattle abortion could be found in the fluid of the stomach of the lambs or in material taken from the membranes.

Experiment 22, Ewes 157 and 158.

Ewe 157 (cross bred) was tupped on 7th January 1908. Ewe 158 (cross bred) was tupped on 9th January 1908. Both ewes were expected to lamb early in June.

The foetal membranes of Lambs J 1 and J 2 were washed with distilled water, and the fluid from the stomachs of the same lambs was added to the washings.

On February 8th, Ewes 157 and 158 received 15 ounces of the above mixture by the mouth and 10 c.c. by the vagina. Both ewes unfortunately turned out to be non-pregnant. They had never been noticed to abort.

*Flock 10.*

This flock was located in Lincolnshire. A considerable amount of abortion had occurred in different flocks in the district, but it was not reported to the Committee until the lambing season was practically at an end. This flock was the only one from which it was thought still possible to obtain material. It consisted of 254 ewes, of which 63 (24.8 per cent.) aborted; 15 of the ewes which had aborted had died. The farmers of the neighbourhood attributed the trouble to the exceptionally wet season, owing to which the pregnant ewes had to run on sodden and very heavy ground.

On March 12th Mr. Gooch, M.R.C.V.S., who was attending this flock, killed a ewe (Ewe K), which was at full time, but appeared to be unwell. The neck of the uterus was ligatured, the organ was excised, and brought to the laboratory, where it was examined the same day.

*Lesions.*—The wall of the organ was thickened and oedematous towards the neck. On opening into the uterine cavity it was found to contain a very large quantity of a reddish-coloured fluid. This fluid was much thinner than that usually present in cases of abortion in ewes; in fact, the appearances suggested that it might be something different from what had been previously met with. One foetus was present in the uterus. It had apparently been dead for some time, as the subcutaneous tissue was blood-stained, and the intestinal tissue was becoming pulpy. No vibrios or bacilli of cattle abortion could be found in the exudate or in the fluid from the foetal stomach. A variety of microbes, however, which suggested that the uterus had been open, were present.

Experiment 23, Ewes 159 and 160 (Cross-breds).

Both ewes were tupped on January 10th, 1908, and were expected to lamb early in June.

On March 13th, that is 61 days after service, each received 250 c.c. of the fluid exudate from the uterus of Ewe K., by the mouth and 10 c.c. of the same fluid were injected into the vagina. Both ewes became very ill as the result of the dose administered by the mouth.

This was probably due to the variety of putrefactive microbes contained in the exudate. The symptoms were those of enteritis, and diarrhoea was severe.

Ewe 159 succumbed on March 23rd, that is 10 days after receiving the uterine exudate of Ewe K. The intestinal mucous membrane was in a highly-congested condition, and patches of superficial necrosis were present. The carcass was rapidly becoming putrid. The uterus was excised in the usual way and taken to the laboratory for examination.

*Lesions.*—The uterus contained a foetus. The putrefactive process had extended, however, to the whole of the organ and its contents, rendering the material quite unsuitable for further investigation. Scrapings from the uterine mucous membrane contained a multitude of different microbes, but no vibrios or bacilli of cattle abortion could be found.

Ewe 160 showed a dark chocolate-coloured discharge from the vulva on March 30th, *i.e.*, 17 days after receiving the material from Ewe K. This discharge was examined microscopically, and was found to be very foul. No special microbes could be identified in the preparations.

*Lesions.*—Ewe 160 was immediately slaughtered. The neck of the uterus was ligatured, the organ was excised, and taken to the laboratory for examination. The uterus had recently been pregnant, but the foetus had been expelled, and on searching the litter where this ewe had been penned, a foetus, which had been trampled almost into a pulp, was found. From the shrunken appearance of the eyes, however, it was concluded that it had probably been dead some days before expulsion.

On cutting into the uterus it was found to contain a small amount of a thick creamy exudate. As was expected, this material contained a variety of microbes. No vibrios or bacilli of cattle abortion could be detected by the microscope, but this might have been due to the rapid putrefactive process going on.

Experiment 24, Ewes 161 and 162 (Cross-bred).

Ewe 161 was tupped on the 13th January 1908. Ewe 162 was tupped on the 15th January 1908, and both were expected to lamb early in June.

As Ewe 160 (Experiment 23) had aborted subsequent to the administration of material obtained from an outbreak of abortion in the field, and as none of the microbes which we were accustomed to associate with abortion had been found either in the material administered to Ewe 160 or in the uterine exudate of Ewe 160, it was thought advisable to inquire whether the trouble could be explained by the action of an ultra-microscopic virus. A dense emulsion of the uterine exudate of Ewe 160 was prepared by shaking up the material with a quantity of physiological salt solution. A portion of this emulsion was passed through a Chamberland filter (F.). Ewe 161 on March 31st, that is 79 days after service, received 70 c.c. of the filtrate by the mouth and 10 c.c. by the vagina, and on the same date Ewe 162 received into the jugular vein 10 c.c. of the unfiltered emulsion.

Ewe 161 gave birth to a live lamb on June 4th, that is at full time. The membranes were recovered immediately after parturition. They appeared to be perfectly normal, and nothing but apparently chance organisms could be found in smears made from the cotyledons.

Ewe 162 unfortunately turned out not to be pregnant. She had never been observed to abort.

*Summary of Observations in connexion with Flock 10.*

Proportion of abortions, 24.8 per cent.; no vibrios were demonstrated in material from the field. The material was very putrid.

Two ewes were put under experiments. One ewe died. The other aborted, but no vibrios were found.

*Control Experiments to the Series carried out during the Lambing Season of 1908 at the Abortion Committee's Laboratory.*

It was thought advisable to control the experiments done at the Committee's laboratory at Sudbury by observations made on other premises where abortion in sheep had never been introduced. These experiments were carried out at the Royal Veterinary College

Five ewes, believed to be pregnant, were sent to the College on January 18th, 1908, and on the 20th January pure cultures of the vibrio on agar and potato broth were sent from the Committee's laboratory to be administered to these animals. The only cultures available at the time were 36th subcultures from Ewe 29 (*see* Experiment 15). The tubes of culture sent had been incubated for four days, and before being administered to the animals they were kept in the College laboratory outside the incubator for five days. It is to be observed, then, that the material used had been kept up for a prolonged period under conditions of artificial cultivation. An emulsion was prepared which contained three agar cultures (36th subcultures from Ewe 29) triturated with about 15 c.c. of a liquid culture in potato broth from the same animal. The liquid culture was also a 36th subculture, and had been incubated for four days.

Experiment 25, Ewes 152, 156, 148, 155, and 151.

Ewe 152 (cross-bred) was tupped on October 9th, 1907, and was expected to lamb early in March 1908.

Ewe 156 (cross-bred) was tupped on January 7th, 1908, and was expected to lamb early in June.

Ewe 148 (cross-bred) was tupped on December 12th, 1907, and was expected to lamb early in May.

Ewe 155 was tupped on January 6th, 1908, and was expected to lamb early in June.

Ewe 151 was tupped on October 8th, 1907, and was expected to lamb early in March 1908.

Ewes 152 and 156 were not infected, but were kept as controls.

On the 25th January 1908 Ewe 148 received into the jugular vein 2 c.c. of the above-mentioned emulsion. Ewe 155 received 3 c.c. into the jugular vein, and Ewe 151 received an injection of 6 c.c. into the vagina.

Ewes 148 and 155 unfortunately turned out not to be pregnant. They had never been observed to abort.

Ewe 151 gave birth to a live lamb on March 11th, 1908, that is at full time. The membranes were normal in appearance, and no vibrios could be found in smears made from the cotyledons.

Control Ewe 152 gave birth to a live lamb at full time on March 6th, 1908.

Control Ewe 156 aborted on April 17th, 1908, that is about seven weeks before full time. The foetus, which was shrunken, appeared to have been dead for some time. Unfortunately, however, notice of the abortion was received too late for a proper examination to be conducted, and at the time it was examined it was in a state of advanced putrefaction. It is not improbable that the result in this control ewe may have been due to conveying her nine miles in a cart from the laboratory to the College. No definite conclusions could be drawn from these control experiments.

#### LAMBING SEASON 1909.

##### Flock 11.

This flock consisted of 1,000 Hampshire Down ewes, and was located in Hampshire. Information was received at the laboratory towards the middle of January 1909 that some of the ewes in this flock had aborted. Mr. Brand, one of the Board's Veterinary Inspectors, visited the farm on January 15th, and reported that up to the day of his visit 50 ewes (5 per cent.) had aborted about a month before full time, and we were subsequently informed that no cases of abortion had occurred afterwards.

The ewes, which were folded, had been carefully observed. The aborted lambs were in most cases putrid, but some of them were fairly fresh.

On the 15th January Mr. Brand slaughtered a ewe (Ewe L) and conveyed the uterus to the laboratory the same evening.

*Lesions.*—The uterus showed no external lesions. On opening into the cavity of the organ it was found to contain a considerable quantity of a dark reddish-coloured exudate between the mucous membrane and the chorion. The cotyledons presented a pulpy and anæmic appearance like what had been found in other cases of abortion. One lamb was present in the uterus.

*Microscopical and Cultural Examination.*—Smears made from the exudate contained a great

variety of microbes, but no vibrios or bacilli of cattle abortion could be found. No microbes could be found in the stomach fluid of the lamb. Tubes of culture media were inoculated in the hope that it might be possible after incubation to identify some definite organism. The cultures, however, all become very foul, and nothing definite could be obtained from them.

Mr. Brand also slaughtered a ewe which had aborted a few hours before he arrived at the farm. The uterus of this ewe was examined at the laboratory. Its wall was much thickened by œdema. On opening into the organ the membranes were found to be still attached. A small amount of reddish slimy exudate was present on the surface of the uterine mucous membrane. Many of the cotyledons were in a pulpy condition. On microscopical examination the exudate was found to be very foul, and no special bacteria could be identified in the preparations.

An attempt was made to infect a pregnant heifer (*see* Experiment 60) with some of the uterine exudate from Ewe L, but the result was negative.

##### Experiment 26, Ewes 184, 185, and 193.

Ewes 184 and 185 (cross-bred) were tupped on October 9th, 1908, and were expected to lamb early in March 1909. Ewe 193 (cross-bred) was tupped on October 26th, 1908, and was expected to lamb towards the end of March.

A dense emulsion of the uterine exudate from Ewe L (Flock 11) was prepared with sterile water, and each of the above ewes received 60 c.c. of the emulsion by the mouth and 10 c.c. by the vagina on January 16th, 1909, *i.e.*, about three months after service in each case.

Ewe 184 gave birth to a healthy lamb on March 4th, 1909, that is at full time. No vibrios or bacilli of cattle abortion could be found in preparations made from the foetal membranes.

Ewe 185 gave birth to two live lambs on February 28th, 1909, *i.e.*, a few days before full time. No vibrios or bacilli of cattle abortion could be found in the foetal membranes. One of the lambs, which was very weakly, died on the day after its birth.

Ewe 193 gave birth to two healthy lambs on March 25th, that is at full time. The membranes in this case were not recovered, so could not be examined.

##### *Summary of observations in connection with Flock 11.*

Proportion of abortions, 5 per cent.; no vibrios were demonstrated in material from the field.

Three ewes were put under experiment. All received natural material by the mouth; none became infected.

##### Flock 12.

Abortion was reported on the 18th January 1909 to be occurring in this flock, which was in Cambridgeshire. On January 19th Mr. Brand, one of the Board's Veterinary Inspectors, visited the farm, and obtained the following particulars. The flock was composed of 345 pedigree Southdown ewes, of which 31 (9 per cent.) had aborted. Most of the ewes aborted about a month before full time, and the shepherd expressed the opinion that the lambs must have been dead for some time before being aborted. The flock was folded on turnips, and was fed in the usual way which obtains in the county. Mr. Brand slaughtered a ewe (Ewe M) which the shepherd thought would probably abort. Another ewe (M 2), which had recently aborted, was also slaughtered, and the uteri from both cases were conveyed to the laboratory.

The uterus of Ewe M was found to be quite normal externally and internally, and no microbes of any kind could be found in scrapings from the uterine mucous membrane or cotyledons either by cultural or microscopical examination.

On opening into the uterus of Ewe M 2 (aborted) it was found to contain a reddish slimy exudate on the uterine mucous membrane between the cotyledons. The foetal membranes were still attached, and many of the cotyledons were in a pulpy condition. Microscopical examination of the exudate showed that it contained a variety of microbes, which, of course, was not unexpected, seeing that the animal had aborted some hours before being slaughtered.

## Experiment 27. Ewes 178 and 179.

Ewe 178 (cross-bred) was tupped on 11th November 1908, and was expected to lamb early in April.

Ewe 179 was tupped on 11th October 1908, and was expected to lamb early in March. A thin emulsion was prepared by mixing one ounce of the uterine exudate from Ewe M 2 (aborted) with sterile water. On 21st January 1909, *i.e.*, 71 days after service in the case of Ewe 178 and 102 days in the case of Ewe 179, each received 200 c.c. of the above emulsion by the mouth and 10 c.c. by the vagina. Both these ewes went off their feed, and showed signs of ill-health after receiving the exudate. This was probably due to the latter being foul, and it might be as well to note here that we have several times observed sheep show signs of serious intestinal trouble after receiving decomposing material of animal origin.

Ewe 178 was slaughtered on the 27th January, that is, seven days after receiving the uterine exudate of Ewe M 2. It was thought advisable to kill her at this early date, because she was distinctly unwell, and a small amount of yellow exudate was noticed on the fleece in the neighbourhood of the genital orifice. The uterus was cut out in the usual way and taken to the laboratory for examination.

*Lesions.*—It contained one fœtus, which seemed to have been dead for some little time. A dark chocolate-coloured exudate was present on the uterine mucous membrane, and on the chorion. The cotyledons were firmly attached, and when separated did not present that macerated appearance which had been so frequently found in connection with outbreaks of abortion in which the vibrio was found in the uterus. The uterine exudate contained a variety of microbes, but neither vibrios nor bacilli of cattle abortion could be found.

Culture tubes were sown with the exudate, but all of these became very foul. Material from this ewe was used for Ewe 177 (*see* Experiment 28).

On the assumption that the condition in the uterus of Ewe 178 had been produced by the material administered, it was thought advisable to kill Ewe 179 for the purpose of examining her uterus before it became open.

Ewe 179 was slaughtered on the 2nd February, that is, 12 days after receiving the exudate.

*Lesions.*—The uterus was pregnant and showed no external lesions. On opening into the organ one fœtus was found. The uterine mucous membrane, the cotyledons and the fœtus were all normal in appearance. No microbes of any kind could be found either by microscopical or cultural examination of material taken from the uterine mucous membrane, the cotyledons, or the stomach fluid of the fœtus.

## Experiment 28. Ewe 177 (cross-bred Cheviot).

It was thought advisable to test whether the exudate from the uterus of Ewe 178 (Experiment 27) would prove infective to another ewe.

Ewe 177 was tupped on 23rd October 1908, and was expected to lamb late in March. On 5th February 1909, that is 105 days after service, Ewe 177 received by the mouth 80 c.c. of washings from the uterus of Ewe 178 and 20 c.c. of the same material were injected into the vagina.

On 17th March, that is at full time, Ewe 177 gave birth to a live lamb. No vibrios or bacilli of cattle abortion could be found in preparations made from the membranes.

*Summary of Observations in connection with Flock 12.*

Proportion of abortions, 9 per cent.; no vibrios were demonstrated in material from the field. Three ewes were put under experiment. One ewe aborted after receiving natural material by the mouth and vagina, but no vibrios were found. The two other ewes did not abort or show other signs of being infected after receiving natural material by the mouth and vagina.

*Flock 13.*

In February 1909 abortion was reported to be occurring in this flock, which was located in Suffolk.

On 13th February Mr. Brand, one of the Board's Veterinary Inspectors, visited the farm, and obtained the following particulars. The flock consisted of 280 Suffolk ewes, which were due to start lambing on 9th March. At the date of his visit 38 ewes had aborted, and by the end of the lambing season the number of abortions amounted to 60, or 21.4 per cent.

The first abortion had occurred on 1st January, *i.e.*, about two months before full time. The flock was being fed on grass, dry food and turnips, and was allowed to graze for the evening feed. The owner stated that when he took the farm 28 years before he was warned not to put his breeding flock on a certain piece of black sand-land, as it was said to cause abortion. For the first time since his tenancy he had disregarded the warning, and had put his ewes on to this particular piece of land to allow them to graze on the remainder of a cabbage crop. Twelve days after grazing on this portion of the farm the ewes began to abort. The owner was inclined to attribute abortion to the animals having eaten chickweed, which was growing in abundance on this part of the farm.

Before Mr. Brand visited, Mr. Heatley, M.R.C.V.S., who consulted us about the outbreak, was good enough to select a suspicious ewe for slaughter, and he forwarded the uterus.

The uterus of this ewe (N 1), which had been slaughtered on the evening of the 7th February, arrived at the laboratory on the morning of February 8th. Mr. Heatley informed us that the ewe showed no distinctive symptoms. She did not appear to be quite well, and he had slaughtered her on chance. The organ had been packed in a large waterproof bag before despatch, and the uterine neck had been securely ligatured.

*Lesions.*—The uterine wall was very œdematous. After searing the surface, incisions were made at different parts of the wall. Through all these incisions it was easy to obtain a considerable amount of exudate for microscopical and cultural examination. On opening the organ it was found to contain one fœtus. The whole surface of the uterine mucous membrane and of the chorion was covered with exudate, which was of a dark chocolate colour. At some parts it was quite fluid, whilst at others it had the consistence of thick mucus. The cotyledons, which were macerated, were easily separated, and the separated surfaces presented a very anæmic appearance. The lamb was fully developed for the period of pregnancy, except that it was completely devoid of wool. It had, however, apparently been dead for a few days, as its skin looked somewhat macerated.

*Microscopical and Cultural Examination.*—Vibrios were very abundant in smears made from the exudate. They were the only microbes present, and actively growing pure cultures were easily obtained by inoculating culture media.

This outbreak was considered of great importance in the investigation of sheep abortion, as Ewe N 1 was one of the few cases from the field in which we had had the good fortune to kill an infected animal before distinct signs of approaching abortion had manifested themselves. The fact that this animal from the field showed the typical lesions connected with abortion, and that vibrios were found in absolutely pure culture in the exudate, supplied further strongly presumptive evidence that the vibrio which we had previously isolated and studied caused epizootic abortion in sheep.

Mr. Brand was instructed to slaughter another ewe in this flock, which he did. The uterus of this animal (Ewe N 2), however, turned out to be absolutely normal. Evidently the second ewe had not been infected.

## Experiment 29. Ewes 176 and 192.

Ewe 176 (cross-bred) was tupped on November 7th, 1908. Ewe 192 (cross-bred) was tupped on November 5th, 1908. Both were expected to lamb early in April.

On February 9th, that is 95 days after service in the case of Ewe 176 and 98 days in the case of Ewe 192, each received into the jugular vein 5 c.c. of a dense emulsion of the uterine exudate of Ewe N 1. No

symptoms followed the inoculation, except that for one day after the operation the temperature of each ewe was a degree above the normal.

Ewe 176 was slaughtered on February 18th, that is nine days after inoculation, as she showed a chocolate coloured discharge from her vulva. Before slaughter the discharge was examined microscopically, and vibrios were found to be present. The uterus was cut out in the usual way, and taken to the laboratory for examination.

*Lesions.*—Externally the organ showed no lesions. On opening into the uterine cavity no exudate could be found, and the uterine mucous membrane was quite normal in appearance. A fetus was present, which appeared to be quite normal. No vibrios or bacteria of any kind were obtained either by microscopical or cultural examination from the uterine mucous membrane or the cotyledons.

It is to be noted about this experiment that nine days after inoculation into the veins with pure, natural material containing vibrios, the vibrios were found in a discharge from the genital organs, although no uterine lesions could be found on post-mortem examination. Having regard to some of our former observations, in which, although the uterus was distinctly infected, we were only able to obtain vibrios from certain of the cotyledons, it seem not improbable that if we had examined a greater number of cotyledons in this case we might have found the organism. The result is particularly interesting when considered with that obtained in the fellow ewe (192), which was killed about a fortnight later.

Ewe 192 was observed to show a yellow watery discharge at the vulva on March 1st, *i.e.*, 19 days after infection, and about a month before full time. Vibrios were found in large numbers though not in pure culture in microscopical preparations made from the discharge. At 3 p.m. on the same day it became evident that Ewe 192 was aborting. The nose of the lamb was visible, and it was decided to deliver her by hand. The fetus had evidently been dead for some days, as it was somewhat macerated and shrunken. With the expulsion of the fetus a considerable quantity of chocolate-coloured mucoid exudate was also expelled. This contained vibrios in large numbers along with what appeared to be occasional chance organisms. The ewe was immediately slaughtered; the organ was excised in the usual way, and taken to the laboratory for examination.

*Lesions.*—Externally the organ showed no lesions, but on cutting into its cavity a fair amount of chocolate coloured mucoid exudate was found on the uterine mucous membrane. This exudate contained vibrios almost in pure culture.

#### Experiment 30, Ewe 175.

Ewe 175 (cross-bred) was tupped on November 9th, 1908, and was expected to lamb early in April. On February 9th, 1909, *i.e.*, 92 days after service, she received by the mouth 100 c.c. of an emulsion which had been prepared by bruising the fetal membranes and cotyledons from Ewe N 1.

On February 20th, *i.e.*, 11 days after receiving the material by the mouth, a discharge was noticed to be coming from the vulva. This discharge was examined microscopically and, although it was rather foul, it contained a considerable number of vibrios.

On April 3rd, that is practically at full time, Ewe 175 gave birth to a live lamb. When the membranes were recovered they were distinctly putrid, and no vibrios could be found in the cotyledons.

It is to be noted about this experiment that, although the pregnant uterus of a ewe which had been fed with material containing vibrios became invaded by this organism, the animal did not abort.

#### Experiment 31, Ewe 198.

Ewe 198 (cross-bred) was tupped on November 3rd, 1908, and was expected to lamb early in April.

On February 9th, that is 98 days after service, she received into the vagina 10 c.c. of the same material as was given to Ewe 175 by the mouth.

On March 31st, that is practically at full time, she gave birth to a live lamb. The fetal membranes were examined, but no vibrios were discovered. Apparently in this attempt to infect by the vagina the vibrio had failed to gain a footing in the pregnant uterus.

#### Experiment 32, Ewes 194 and 196.

Ewe 194 (cross-bred) was tupped on November 13th, 1908, and was expected to lamb on April 8th, 1909.

Ewe 196 was tupped on the 28th October 1908, and was expected to lamb on March 24th, 1909.

On February 20th, 2 agar cultures made directly from the uterine exudate of Ewe N 1 were triturated with 30 c.c. of a culture in peptone broth obtained from the same source. The cultures on agar had been incubated for 10 days. The broth cultures, which were first subcultures, had been incubated for six days.

On the same day, that is 99 days after service in the case of Ewe 194 and 115 days after service in the case of Ewe 196, each ewe received intravenously 5 c.c. of the above mixture and 10 c.c. were injected into the vagina. No temperature reaction of any kind followed inoculation.

Ewe 194 was observed on March 2nd to be showing a blood-stained discharge around the vulva. This discharge was examined microscopically, and found to contain a large number of vibrios.

On March 3rd, that is 11 days after inoculation, 110 days after service, and 39 days before full time, Ewe 194 aborted a dead lamb. The membranes were recovered early, and scrapings made from the fetal cotyledons showed a considerable number of vibrios on microscopical examination. The lamb was well developed for the period of pregnancy. No macroscopic lesions could be found, and no vibrios could be demonstrated either by microscopical or cultural examination in the fluid from its stomach.

Ewe 196 aborted a dead lamb during the night of March 9th, that is 18 days after inoculation, 133 days after service and 16 days from full time. The membranes which were hanging from the vulva were recovered, and vibrios were found in preparations made from the fetal cotyledons. The lamb was up to time as regards development. It exhibited no macroscopic lesions, but vibrios were found in the fluid from its stomach.

This ewe (196) was slaughtered on the morning of the 10th March, the uterus was cut out in the usual way and taken to the laboratory for examination.

*Lesions.*—The uterine wall was very much thickened at different parts by œdema. The cavity of the organ contained a greyish mucoid exudate, which in some parts was tinged with blood. The uterine mucous membrane showed no macroscopic lesions. Some of the cotyledons which were still attached were pulpy, and easily separated. The separated surfaces showed a considerable amount of white, creamy juice, which was found to be composed mainly of leucocytes.

*Microscopical and Cultural Examination.*—The uterine exudate contained a considerable number of vibrios, but, as was expected, seeing that the uterus had been opened, they were not present in pure culture. Considerable numbers of vibrios were seen in preparations made from some of the separated cotyledons, but in smears made from others they could not be found. Pure cultures of vibrio were obtained in various media from the fluid of the lamb's stomach.

This experiment is interesting, as the results were positive after using an artificially grown pure culture of the vibrio.

#### Experiment 33, Ewe 83.

This experiment was undertaken for the purpose of keeping up the strain obtained from Flock 12 which had been continued by cultures through Ewe 196 (Experiment 32). It illustrates the comparative uncertainty of infection, especially when the infective material is administered by the natural orifices.

Ewe 83 was tupped on December 27th, 1908, and she was expected to lamb about the end of May 1909.

On the 13th March, that is 76 days after service, Ewe 83 received by the mouth 160 c.c. of an emulsion

prepared with sterile water from the cotyledons of Ewe 196.

On June 4th, that is at full time, Ewe 83 gave birth to a healthy lamb. The membranes were normal in appearance, and no vibrios could be found in preparations made from the fetal cotyledons.

#### Experiment 34, Ewe 70.

It is to be noted about this ewe that she had been successfully infected experimentally with cattle abortion in 1907, and that she had aborted a lamb on June 17th of that year, but that she gave birth to a live lamb on April 7th, 1908.

She was tupped on December 25th, 1908, and was expected to lamb towards the end of May 1909.

On the 13th March 1909, that is 78 days after service, she received into the jugular vein 5 c.c. of the following mixture: One agar culture of vibrio which had been incubated 10 days, and was a third subculture from the uterine exudate of Ewe N 1, and one broth culture (6 c.c.) which was a second subculture from the same source, and had been incubated nine days. No temperature reaction followed the inoculation.

On April 14th, that is 31 days after the inoculation, 109 days after service, and about 40 days before full time, Ewe 70 aborted a dead lamb. No vibrios could be found in the discharge which followed the act of abortion, nor could they be discovered either by microscopical or cultural examination of the fluid from the lamb's stomach. The membranes were rather putrid when they were recovered. Smears made from the cotyledons were very foul, and no vibrios could be identified with the microscope.

The result of this experiment is disappointing, for, although the ewe aborted following upon the administration of cultures of the vibrio, no definite evidence was obtained that the vibrio was responsible for the abortion in this case.

#### Experiment 35, Ewes 203 and 204 (Dorsets).

Ewe 203 was tupped on March 20th, 1909.

Ewe 204 was tupped on March 22nd, 1909.

Both ewes were expected to lamb about the middle of August. An agar culture, which was a sixth subculture from the uterine exudate of Ewe N 1 and had been incubated for 10 days, was triturated in a sterile mortar with 100 c.c. of a broth culture from the same source. The broth culture was a seventh subculture, and had been incubated for eight days.

On April 22nd, 1909, that is 33 days after service in the case of Ewe 203 and 31 days in the case of Ewe 204, each ewe received into the jugular vein 5 c.c. of the above mixture. No temperature reaction followed the inoculation.

On June 21st, that is 93 days after service in the case of Ewe 203 and 91 days after service in the case of Ewe 204, each received into the jugular vein 5 c.c. of the following mixture:—two agar cultures, which were ninth subcultures from the uterine exudate of Ewe N 1, and had been incubated 10 days, triturated with 10 c.c. of a broth culture from the same source, which was a tenth subculture, and had been incubated for 10 days. No temperature reaction followed inoculation.

On July 30th each ewe received into the jugular vein 5 c.c. of the following mixture:—two agar cultures, which were fifteenth subcultures from the uterine exudate of Ewe N 1, and had been incubated for 11 days, triturated with 10 c.c. of a broth culture from the same source; the broth culture was a sixteenth subculture, and had been incubated for four days.

As it was thought possible that vibrios might pass through the uterus, and create a certain amount of catarrhal discharge without causing early abortion, or without causing abortion at all (*see* Experiment 30), swabs were inserted into the vagina for several days after each injection, and microscopical preparations were made from the fluids thus obtained. In no case, however, could vibrios be found in preparations made from these swabs.

Ewe 203 gave birth to a live lamb on 17th August, that is, at full time. No vibrios could be found by microscopical examination of preparations made from the fetal cotyledons.

Ewe 204 gave birth to a healthy lamb on 17th August, that is, at full time. No vibrios could be found by microscopical examination of preparations made from the fetal cotyledons.

It is interesting to compare the positive results obtained in Experiment 32 (Ewes 194 and 196) by using first cultures of this strain, and the positive though somewhat incomplete result obtained in Experiment 34 (Ewe 70) by using second and third subcultures, with the negative results obtained in this experiment using subcultures of the same strain, which had been for a considerably longer time under conditions of artificial cultivation. The first culture, it is to be noted, was made on the 9th February; the earliest subcultures used in this experiment, which were sixth subcultures, were prepared on the 12th April, and were used for infection on the 22nd April. The earliest material, then, used in this experiment had been under conditions of artificial cultivation for 72 days.

#### Summary of Observations in connection with Flock 13.

Proportion of abortions, 21·4 per cent.; vibrios were demonstrated in material from the field. Ten ewes were put under experiment. Five of these aborted and showed vibrios. Of the five, two received natural material by the mouth and veins, one received natural material by the mouth, and two received culture by the vagina and veins. One ewe aborted and did not show vibrios after receiving culture by the veins. Four ewes did not become infected. Of the four, two received culture by the veins, one received natural material by the vagina, and one received natural material by the mouth.

#### LAMBING SEASON 1910.

##### Flock 14.

This flock, which consisted of 450 Hampshire ewes, was located in Hampshire. Mr. Medlock, one of the Board's veterinary staff, visited the farm on the 19th January, and obtained the following particulars: 308 ewes were due to lamb in January, 12 were due to lamb on February 24th, and 30 on February 19th. At the date of Mr. Medlock's visit 58 ewes had aborted (13 per cent.).

Mr. Medlock brought back some stomach fluid from a dead lamb (Lamb O 1) in sterile pipettes, but as no ewe appeared to be showing symptoms of ill-health he did not slaughter any. No vibrios could be found in the above-mentioned stomach fluid, which was very foul.

On January 26th the owner by arrangement slaughtered a ewe (Ewe O) he thought was going to abort, and sent the uterus to the laboratory in a waterproof bag which had been sent to him for the purpose. Ewe O had been slaughtered on the day before the uterus arrived at the laboratory.

*Lesions.*—Externally the uterus showed no macroscopic lesions. On cutting into the cavity a chocolate-coloured exudate was found on the uterine mucous membrane and on the chorion. One lamb (Lamb O 2) was present in the uterus. It had apparently been dead for some time, as its tissues were macerated, and the intestines had become converted into a cheesy mass. The whole of the uterine contents were putrid. No vibrios could be found in any of the material examined from the uterus, but hosts of putrefactive organisms were present.

As this material was too putrid to allow of inoculation into the veins, it was decided to administer it by one of the natural orifices. It was also thought advisable, seeing that no vibrios had been found, to again examine the possibility of the trouble being due to an ultra-microscopic virus.

#### Experiment 36, Ewes 217 and 222.

Ewe 217 (Dorset) was tupped on October 27th, 1909, and was expected to lamb at the end of March 1910.

Ewe 222 (cross-bred) was tupped on November 5th, 1909, and was expected to lamb early in April.

On January 26th each of these ewes received by the mouth an emulsion in water containing about 2½ grammes of the uterine exudate of Ewe O.

Ewe 222 aborted on February 7th, 1910, that is 12 days after receiving the material by the mouth, 94 days after service, and about 50 days before full time. The fetus which was found in the pen had been so much trampled upon that it was useless for examination. The ewe was slaughtered, its uterus was cut out, and taken to the laboratory for examination.

*Lesions.*—On opening into the organ it was found to contain a blood-stained, watery fluid between the chorion and the uterine mucous membrane. The fetal membranes were fairly firmly attached.

*Microscopical and Cultural Examination.*—On microscopical examination the fluid in the uterus was found to contain a very large number of putrefactive organisms. No vibrios or bacilli of cattle abortion could be found. So foul was this material that it was decided not to use it for further experiment.

Ewe 217 showed signs of approaching abortion on February 11th, 1910, that is 16 days after receiving the material from Ewe O, 33 days after service, and about 117 days before full time. A chocolate-coloured discharge was noticed to be coming from the vulva. Smears prepared from this discharge were examined by the microscope, but nothing other than the usual putrefactive organisms could be found. The ewe was slaughtered, the uterus was cut out in the usual way, and taken to the laboratory for examination.

*Lesions.*—The wall of the uterus was very œdematous, particularly in the neighbourhood of the neck. On opening into the organ a large amount of a brown-coloured and very tenacious exudate was found on the surface of the uterine mucous membrane and on the chorion. Two fetuses were present; one was quite putrid, but in the other putrefaction did not seem to be far advanced. Both, however, had evidently been dead for some days at least. The stomach fluid of the least putrid of the two was deeply blood tinged.

*Microscopical and Cultural Examination.*—Microscopical examination of the uterine exudate and the stomach fluid from the least putrid fetus showed a large number of ordinary putrefactive organisms, but neither vibrios nor bacilli of cattle abortion were present. Cultures sown from the material became very foul, and no special microbes could be identified in them.

It is to be noted about these two ewes that they were fed with material which came from an outbreak of abortion in the field, but which was very putrid, that they seemed to have become affected with abortion in consequence, and that none of the special microbes which had been found in other cases of abortion could be identified in the fluids or the diseased organs. It is also to be noted that the contents of the uterus in both cases had undergone putrefaction a comparatively short time after infection. It is possible, though improbable, that the putrefactive material itself was responsible for the results in these two ewes, but it has to be borne carefully in mind in trying to interpret these results, that the putrid material came from the uterus of an animal which had aborted, and it would not be unwarrantable to infer that an abortion-causing agent was also present. In consequence of these curious results those in the animals inoculated with the filtered material were looked forward to with great interest.

#### Experiment 37, Ewes 220 and 221.

Both these ewes, which were cross-bred, were tupped on November 5th, 1909, and were expected to lamb early in April.

A thin emulsion in water was prepared from the uterine exudate of Ewe O. This material was filtered through a Chamberland filter (F).

On January 26th, 1910, that is 82 days after service, each of these ewes received 20 c.c. of the above filtrate into the jugular vein.

Ewe 220 gave birth to two live lambs on March 29th, that is at full time. The membranes were quite normal.

Ewe 221 gave birth to a healthy lamb on the 27th March, that is about full time. The membranes in this case were also normal. It would appear from this experiment that the abortion-causing agent was retained by the filter.

#### Experiment 38, Ewes 233 and 234.

It was thought advisable to try the effect on other pregnant ewes of the material from the uterus of Ewe 217 (*see* Experiment 36) together with the mixed cultures which had been obtained from it.

Both of the ewes employed for this experiment were Cheviots and were tupped on January 20th, 1910. They were expected to lamb early in June.

On February 16th, 1910, each received by the mouth 100 c.c. of a dense emulsion of the uterine exudate of Ewe 217, to which two broth cultures from the same uterine exudate had been added. The cultures had been incubated for four days, and the uterine exudate had been stored in sterile broth since February 11th, *i.e.* five days.

Ewe 234 was slaughtered on the 26th February, 10 days after infection. The reason for slaughtering her at this early date after infection was that Ewe 222 (*see* Experiment 36), which had been infected with the original material, had aborted, and shown putrefaction of the uterine contents 12 days after infection, and it was hoped that by killing early we might have the opportunity of examining an affected uterus before putrefaction had supervened. The uterus, however, which was pregnant, was perfectly normal in every respect. No bacteria whatever could be found in scrapings from the uterine mucous membrane, fetal cotyledons, or the contents of the fetal stomach.

Ewe 233 was slaughtered on the 28th April, that is 98 days after service and 71 days after infection. The uterus, which was pregnant, was normal in every respect. No bacteria could be found in scrapings made from the uterine mucous membrane, the fetal cotyledons, or the stomach fluid of the fetus.

It is to be noted in connection with this experiment that, although the material used for infection was putrid, no abortion followed its administration. The result inclined us to think that possibly the agent which caused abortion in the cases of Ewes 217 and 222 (*see* Experiment 36) had lost its virulence, or that it had disappeared from the material owing to further putrefaction. The agent might have been the vibrio, but the material was too putrid to enable any particular organism to be identified.

#### Summary of Observations in connection with Flock 14.

Proportion of abortions, 13 per cent.; no vibrios demonstrated in material from the field.

Four ewes were put under experiment.

Two of these, which aborted, received natural material by the mouth. The two others, which received natural material by the mouth, did not abort.

#### Flock 15.

This flock, which was in Sussex, consisted of 319 Southdown ewes.

The owner reported early in February that abortion was occurring amongst his ewes. On February 4th, 1910, Mr. Medlock, one of the Board's veterinary staff, visited the farm and obtained the following particulars. Thirty-five of the ewes were due to lamb in February, and 284 in March. At the date of his visit 37 ewes had aborted. At the end of the lambing season the total abortions amounted to 50 (16.6 per cent.). The ewes were folded, and were being fed on cow cabbage, rape, and roots. The first abortion took place on January 1st, but the owner did not pay much attention to the occurrence. At the time of Mr. Medlock's visit none of the ewes were showing what might be called premonitory symptoms of abortion, so he did not slaughter any of the animals for examination. One ewe, however, had recently brought forth a dead lamb. The membranes were still attached in this ewe, and Mr. Medlock was able to make smears from the cotyledons. These, along with the dead lamb, were brought to the laboratory. The smears contained vibrios, but the lamb itself turned out to be too putrid for a satisfactory examination to be made.

On February 9th the owner by arrangement slaughtered a ewe (Ewe P) which in his opinion seemed likely to abort. The neck of the uterus was ligatured, the organ was cut out, packed in a waterproof bag, and sent to the laboratory, where it arrived on the same evening.

*Lesions.*—The wall of the uterus was very œdematous. After searing the surface, incisions were made at various points, and it was possible to obtain a considerable amount of exudate for microscopical and cultural examination. On opening into the organ a brownish, mucoid exudate was found on the uterine mucous membrane and on the chorion. The cotyledons were undergoing softening, but they were not so pulpy as in many of the other cases examined. Two fetuses (P 1 and P 2) were present. Both appeared to be well up to time, and showed no macroscopic lesions. Their appearance indicated that they were probably alive at the time the ewe was slaughtered.

*Microscopical and Cultural Examination.*—The uterine exudate contained a large number of vibrios which were present in pure culture. No vibrios, however, could be found by microscopical examination of the fluid from the stomachs of the fetuses. Pure cultures of the vibrio were obtained on fluid and solid media from the uterine exudate and also from the stomach fluid of the fetuses, although in the latter the microscope had failed to reveal their presence. This outbreak derives additional importance from the fact that again we had the good fortune to obtain from an infected flock a uterus which had not been open, although it was in an aborting condition, and to find vibrios present in pure culture in the uterine exudate.

On March 1st the owner of this flock sent up another lamb which had been aborted, and an examination of its stomach fluid revealed the presence of vibrios in large numbers.

#### Experiment 39, Ewe 225.

Ewe 225 (cross-bred) was tupped on December 6th, 1909, and was expected to lamb early in May 1910.

On February 9th, that is 65 days after service, she received by the mouth 200 c.c. of a thick emulsion in water of the uterine exudate from Ewe P.

On April 30th, 1910, that is 145 days after service, a few days short of full-time, and 80 days after infection, Ewe 225 gave birth to a live lamb. The membranes were examined, but no vibrios or bacilli of cattle abortion could be found. It was concluded that this was possibly a normal parturition.

#### Experiment 40, Ewes 226 and 227.

Ewe 226 (cross-bred) was tupped on December 10th, 1909. Ewe 227 (cross-bred) was tupped on December 13th, 1909. Both were expected to lamb early in May 1910.

On February 9th, 1910, that is 61 days after service in the case of Ewe 226, and 58 days in the case of Ewe 227, each of these ewes received by the jugular vein 10 c.c. of a dense emulsion in sterile broth of the uterine exudate from Ewe P.

Ewe 226 on February 15th, that is 6 days after infection, 67 days after service, and 83 days from full time, showed a brown discharge from the vulva. On examining this discharge it was found to contain a large number of vibrios. As the ewe was showing signs of approaching abortion she was immediately killed. On cutting out the uterus it was found that the fetus had already reached the vagina. The organ was taken to the laboratory for examination.

*Lesions.*—Hæmorrhage had taken place into the pelvic tissue. The uterus was thickened and very œdematous, particularly in the region of the neck. The fetus had a macerated appearance, and had probably died a short time after infection. On opening into the uterus a small quantity of brown-coloured, mucoid exudate was found on the mucous membrane and on the surface of the chorion. Each of the horns contained about an ounce of this material.

*Microscopical and Cultural Examination.*—Large numbers of vibrios were found in the exudate by microscopical examination. Pure cultures of the vibrio were obtained in fluid and solid media from the material found in the uterine horns.

Ewe 227 on February 21st, that is 12 days after infection, 70 days after service, and 80 days before full time, aborted a fetus. The fetus was macerated and dark brown in colour. The membranes, which

came away with the fetus, were found soon after abortion had taken place. Smears made from the fetal cotyledons showed a considerable variety of microbes, but no vibrios could be identified. The ewe was slaughtered a short time after it was known that she had aborted. The uterus was cut out in the usual way and taken to the laboratory for examination.

*Lesions.*—The organ had almost completely contracted. On cutting into the cavity, however, a very small amount of a dark chocolate-coloured exudate was found on the uterine mucous membrane. On examining this material with the microscope it was found to contain a large number of vibrios, but many other kinds of bacteria were also present. The latter had apparently invaded the uterus after abortion. No cultures were attempted from this exudate owing to its contaminated condition. An interesting feature of this experiment is that no vibrios could be found in the cotyledons of the placenta, which had been expelled and exposed to gross putrefaction, but that they were easily found in the exudate inside the uterus, which was less putrid.

#### Experiment 41, Ewes 223 and 232.

Ewe 223 (Cheviot) was tupped on November 6th, 1909, and was expected to lamb early in April 1910.

Ewe 232 was tupped on January 19th, 1910, and was expected to lamb early in June.

On February 15th, that is 101 days after service in the case of Ewe 223 and 26 days in the case of Ewe 232, each received by the mouth five ounces of a fairly dense emulsion in water of the uterine exudate of Ewe 226 (see Experiment 40).

As the experiment in this case was to test the effect of exudate containing vibrios when administered by the mouth, it was thought advisable to complete it by another designed to test the virulence of the material by intravenous inoculation (see Experiment 42, Ewe 1203). Both the ewes concerned in this experiment at the time of inoculation were showing symptoms of some nervous trouble, but we were compelled to use them, as our stock of pregnant ewes was becoming exhausted.

Ewe 223 was slaughtered on the 22nd February, that is seven days after receiving the uterine exudate of Ewe 226. She was slaughtered, as it was thought improbable that she would live much longer, and it was decided to make the best use of her we could by examining her uterus in the fresh state.

*Lesions.*—The uterus, which contained two lambs, was quite normal in appearance. No vibrios could be found by microscopical or cultural examination of material from the uterine mucous membrane, the fetal membranes, or the stomach fluid of the fetus.

Ewe 232 had to be slaughtered on May 22nd, that is 96 days after receiving the emulsion from Ewe 226, as she seemed unlikely to live much longer.

*Lesions.*—The uterus, which contained one fetus, was normal in every respect. No bacteria of any kind could be found by microscopical or cultural examination of scrapings from the uterine mucous membrane and the fetal membranes. The stomach fluid of the fetus was sterile.

#### Experiment 42, Ewe 1203.

Ewe 203 (Dorset) was tupped on October 17th, 1909, and was expected to lamb about the middle of March 1910.

On February 15th, that is 121 days after service, she received into the jugular vein a fairly dense emulsion in sterile salt solution of the uterine exudate from Ewe 226 (see Experiment 40). For three days after inoculation the ewe suffered from intestinal symptoms accompanied by tympany. By the 18th February she seemed to have recovered, but she was found dead on the morning of the 21st, that is six days after inoculation, and about three weeks before full time. The uterus was cut out in the usual way, and taken to the laboratory for examination.

*Lesions.*—On opening into the organ a dark brown exudate was found in certain parts of the uterine mucous membrane and on the chorion. The cotyledons were breaking down, and the uterus presented the appearance typical of those which we obtained from the field, and which were infected with the vibrio.

Three fœtuses were present, one of which had apparently been dead for some days.

*Microscopical and Cultural Examination.*—Microscopical examination showed a large number of vibrios to be present in the uterine exudate, but apparently the uterus had become invaded by other microbes, probably after death, as several varieties were also present. No vibrios could be found in the stomach fluid of any of the fœtuses. Cultures of the vibrio were obtained from the uterine exudate, but, as was not unexpected, they were all contaminated by other microbes.

Taken with the results in Experiment 41, this experiment demonstrates the greater activity and certainty of action displayed by the virus when introduced intravenously.

#### Experiment 43, Ewes 228 and 229.

Both ewes, which were cross-breds, were tupped on January 8th, 1910, and were expected to lamb early in June.

Although vibrios had been found in the uterine exudate from one of the ewes of this flock (15) in which abortion was prevalent, it was thought advisable to again examine the possibility of the trouble being due to an ultra-microscopic virus. A fairly rich emulsion in water was prepared from the uterine exudate of Ewe P. This emulsion was filtered through a Chamberland filter (F), and each of the above ewes received 10 c.c. of the filtrate into the jugular vein on the 9th February, that is 32 days after service.

Ewe 229 was slaughtered on April 28th, that is 78 days after inoculation, 110 days after service, and 40 days before full time. At the time she was slaughtered she was showing no signs of approaching abortion, but it was thought that if she had become infected as the result of the inoculation, lesions ought to be present at this date. The uterus was cut out in the usual way, and taken to the laboratory for examination.

*Lesions.*—It contained one fœtus, and was perfectly normal in appearance. The fœtus was well developed for the period of pregnancy, and showed no lesions whatever. No bacteria of any kind could be found by microscopical or cultural examination of material taken from the uterine mucous membrane, the fetal cotyledons, and the stomach of the fœtus.

Ewe 228 on June 1st, that is at full time, gave birth to a living, healthy lamb. The fluid from the vagina showed no vibrios or bacilli of cattle abortion. The membranes were not recovered, and could not, therefore, be examined.

The result of this experiment was entirely negative.

It would appear, then, that the abortion-causing agent was retained by the filter.

#### Experiment 44, Ewes 230 and 231.

Ewe 230 (cross-bred) was tupped on January 9th, 1910.

Ewe 231 (cross-bred) was tupped on January 13th, 1910.

Both ewes were expected to lamb early in June.

On February 12th, that is 34 days after service in the case of Ewe 230, and 30 days in the case of Ewe 231, each ewe received into the vagina 10 c.c. of a dense emulsion in sterile broth of uterine exudate from Ewe P. The exudate used in this case had been stored in tubes of sterile broth since February 9th (3 days).

Ewe 230 was slaughtered on February 26th, that is 14 days after infection. At the time of slaughter she was showing no symptoms of approaching abortion, but it was thought advisable to kill her, as some of the other ewes which had been infected with the same virus had aborted at an early date after infection. The uterus was cut out in the usual way, and taken to the laboratory for examination.

*Lesions.*—Externally no macroscopic lesions were apparent. On cutting into the organ it was found to contain a fœtus. No exudate could be found on the uterine mucous membrane.

*Microscopical and Cultural Examination.*—No microbes of any kind could be demonstrated either by microscopical or cultural examination of scrapings made from the uterine mucous membrane or the fetal

membranes. The stomach fluid of the fœtus contained no microbes.

Ewe 231 gave birth to a healthy lamb on the 12th June, that is at full time. No vibrios could be found in preparations made from the fetal cotyledons.

It would appear from this experiment that infection per vaginam after pregnancy has begun is not a certain method, and in this connection it is interesting to compare the results obtained by the same method in cows, using the bacillus of cattle abortion (see Report I. and Appendix).

#### Experiment 46, Ewe 1204.

Since our experience had been that the vibrio seemed to lose its virulence at a comparatively early date when subjected to the conditions of artificial cultivation, and since the duration of virulence of the infective material outside the body is an important point in epizootiology, it was thought that it might be interesting to enquire whether the uterine exudate containing vibrios also lost its virulence when preserved for some time in sterile broth in the laboratory.

Ewe 1204 (Dorset) was tupped on October 16th, 1909, and was expected to lamb about the middle of March.

On March 1st, that is 135 days after service, Ewe 1204 received into the jugular vein 5 c.c. of a dense emulsion in broth of the uterine exudate from Ewe P. The exudate had been preserved in broth at the temperature of the laboratory since the 9th February, that is 20 days. Three days after inoculation the ewe showed signs of serious systemic disturbance, which, in the light of the final result of the experiment, may have arisen from the death of the fœtus in utero. She refused food, her breathing was very rapid, and she suffered from what appeared to be attacks of tympany.

Ewe 1204 aborted a dead lamb on March 6th, that is five days after inoculation, 140 days after service, and about 10 days before full time. The lamb was quite fresh, and showed no macroscopic lesions, but vibrios were found in the fluid from its stomach. They were very few in number, and their presence was overlooked at the first examination. They were only detected some days afterwards on re-examining portions of the stomach fluid, which had been preserved in sterile pipettes.

The ewe was slaughtered, the uterus was cut out in the usual way, and taken to the laboratory for examination.

*Lesions.*—Externally the organ showed no macroscopic lesions. On cutting into the cavity it was found that the fetal membranes were still attached. A considerable amount of a dark brown, mucoid exudate was found on the uterine mucous membrane, particularly around the cotyledons.

*Microscopical and Cultural Examination.*—On examining smears from the uterine exudate under the microscope no vibrios or other bacteria could be found. A few days after the post mortem, however, a further microscopical examination was made of portions of the uterine exudate, which had been stored in sterile broth, and kept in the laboratory, and in some of these preparations a few distinct vibrios were discovered by staining with toluidin blue. One could also see a number of dimly stained objects, which might be referred to as the ghosts of vibrios. There were also present a considerable number of granular bodies, such as one finds in old cultures of the vibrio. Culture media sown with the exudate and the fluid from the fetal stomach gave no growth of any organism whatever.

It is not improbable that the early result of inoculation in this case was due in some measure to Ewe 1204 being in a very advanced stage of pregnancy at the time of infection, and it is of interest to compare the result in this experiment with that in Ewe 1203 (see Experiment 42), which was also infected at a late stage of pregnancy. Both these experiments are of particular interest, since they demonstrate that as the result of infection with the vibrio abortion may occur with great rapidity even in the late stages of pregnancy, and they possibly throw some light on what is observed in practice, viz., that by far the greater number of abortions in ewes take place towards

the end of pregnancy. It may be that at this period pregnant ewes are more susceptible to the action of the vibrio.

The experiment also demonstrates that the material from the uterus may remain virulent for some time. This, along with the fact that abortion in sheep may occur a few days after infection, especially in the advanced stages, probably explains how the disease may run through a flock owing to heavy infection of the pastures towards the end of pregnancy.

#### Experiment 47, Ewe 205.

As explained in the previous experiment (46) the uterine exudate of Ewe 1204 and the fluid from the stomach of the fœtus were thought to contain no vibrios when first examined, and vibrios were only found a few days later as the result of a further examination. Seeing that Ewe 1204 had aborted subsequent to inoculation with natural material from Ewe P containing the vibrio in apparently pure culture, the result of the first microscopical examination of the exudate of Ewe 1204 was very difficult to interpret. It was accordingly decided to experiment further with the material from the uterus of this Ewe 1204, and to again inquire into the possibility of an ultra-microscopic virus being responsible for abortion in sheep.

Ewe 205 (Dorset) was tupped on 3rd February 1910, and was expected to lamb early in July.

Ewe 205 on March 8th, that is 33 days after service, received into the jugular vein a dense emulsion in broth of the uterine exudate of Ewe 1204, which had been stored in the laboratory in sterile broth since the 6th March (2 days). No temperature reaction followed the inoculation.

On June 6th, that is 90 days after infection, 123 days after service, and about 27 days before full time, Ewe 205 seemed somewhat unwell, and it was decided to slaughter her. The uterus was cut in the usual way, and taken to the laboratory for examination.

*Lesions.*—Externally the organ showed no macroscopic lesions. On cutting into the uterus it was found to contain two lambs which were well developed for the period of pregnancy. The lambs were normal in every respect. The uterine mucous membrane and the fœtal membranes were also normal in appearance. No uterine exudate was present, and no bacteria could be found either by microscopical or cultural examination of scrapings from the uterine mucous membrane or the cotyledons. The uterine exudate of Ewe 1204, therefore, had apparently failed to infect Ewe 205.

#### Experiment 47A, Ewe 240.

Ewe 240 (cross-bred) was tupped on February 12th, 1910, and was expected to lamb early in July.

On March 9th, that is 25 days after service, she received into the jugular vein 18 c.c. of a filtrate which was obtained by passing through a Berkefeldt filter (V) a fairly dense emulsion in broth and physiological salt solution of the uterine exudate of Ewe 1204. The filter was tested at the time of filtration by adding to the unfiltered material a virulent culture of the bacillus of fowl cholera, and inoculating two drops of the filtrate to a mouse. The mouse unfortunately succumbed to fowl cholera, showing that the filter used permitted the passage of bacteria of microscopic size, but, as the mouse did not die for two days after inoculation, the filtrate had in the meantime been used to inoculate Ewe 240.

Ewe 240 was observed to be straining on June 14th, that is 97 days after inoculation, 122 days after service, and about 28 days before full time. A thick brown discharge was seen around the vulva. On microscopical examination this discharge was found to be very foul, and no vibrios could be identified in it. The ewe was slaughtered, the uterus cut out in the usual way, and taken to the laboratory for examination.

*Lesions.*—Externally no macroscopic lesions were present. On cutting into the organ a dark reddish-brown exudate was found on the uterine mucous membrane and on the chorion. One fœtus was present. It had been dead for some time, and was undergoing putrefaction.

*Microscopical and Cultural Examination.*—On microscopical examination, the uterine exudate was found to

contain a great variety of bacteria, but no vibrios could be seen. The stomach-fluid of the fœtus was also very foul, and contained no vibrios. Culture media were sown with the exudate from the fluid of the fœtal stomach in the hope that, although the material was very foul, it might be possible to identify some special organism in the culture. Nothing could be found, however, in the cultures except a multitude of putrefactive organisms.

This experiment is very difficult of interpretation. It cannot be accepted as a positive result following upon inoculation with a filtrable virus, as the filter had given proof that it was pervious to bacteria of microscopic dimensions. On the other hand, there is no definite evidence that the vibrio passed through this faulty filter into the filtrate, as no vibrios were found in the uterine exudate or in the fœtus. It is possible, of course, that the vibrio may have been primarily responsible for the result, and that owing to the invasion of the affected uterus by extraneous microbes, the vibrio had disappeared.

Lastly, one must not neglect to consider another possibility, namely, that the result was an accidental abortion due to some mechanical or other injury to the fœtus in utero, followed by an invasion of the uterus by putrefactive bacteria.

#### Experiment 48, Ewes 218 and 243.

Ewe 218 (Dorset) was tupped on January 18th, 1910, and was expected to lamb towards the middle of June.

Ewe 243 (cross-bred) was bought out of a flock in which abortion was unknown. She was one of a lot which had to be purchased late in the lambing season, as our stock of pregnant ewes at the laboratory was nearly exhausted. She was expected to lamb in April.

On March 1st, 1910, each of these ewes received into the jugular vein 10 c.c. of the following mixture, containing cultures of the vibrio, obtained originally from the uterine exudate of Ewe P—three agar cultures which were second subcultures, and which had been incubated for eight days, and 20 c.c. of broth culture, which was a third subculture and which had been incubated for four days.

Ewe 243 on March 12th, that is, eleven days after inoculation and probably from a fortnight to three weeks before full time, gave birth to a lamb which was alive, but very feeble. The lamb died on the following day. Smears made from the cotyledons showed a large number of vibrios.

Ewe 218 was slaughtered on April 28th, that is 58 days after inoculation, 100 days after service, and about 50 days before full time. The uterus was cut out in the usual way, and taken to the laboratory for examination.

*Lesions.*—Externally it showed no lesions. On cutting into the organ it seemed to be quite normal. No exudate was present, and no microbes of any kind could be found by microscopical or cultural examination of scrapings made from the uterine mucous membrane and cotyledons. The uterus contained two lambs, both of which were normal for the period of pregnancy. No vibrios could be found in the stomach fluid of the fœtuses. This experiment further demonstrates the uncertainty of infection with artificially grown cultures.

#### Experiment 49, Ewes 244 and 245.

Ewes 244 and 245 were both cross-bred, and were obtained from a flock in which no outbreak of abortion had occurred. The date of tupping was not known exactly, but they were expected to lamb in April 1910.

On March 1st, 1910, each ewe received by the mouth and the vagina the following mixture of cultures of the vibrio, which were subcultures from the original culture obtained from the uterine exudate of Ewe P: three agar tubes, which were second subcultures, and had been incubated for eight days, 20 c.c. of a broth culture which was a third subculture, and had been incubated for four days, and 600 c.c. of sterile water; 290 c.c. of the mixture were given to each ewe by the mouth, and 10 c.c. were injected into the vagina.

Ewe 244 was slaughtered on March 14th, that is 13 days after infection, as she was showing signs of

approaching abortion. Before slaughter a discharge was apparent around the vulva, and in this discharge a considerable number of vibrios were found by microscopical examination. The uterus was cut out in the usual way and taken to the laboratory for examination.

*Lesions.*—The organ showed no external lesions, but on cutting into the cavity a considerable amount of dark brown-coloured exudate was found on the uterine mucous membrane and on the chorion around the cotyledons. The exudate contained inspissated particles such as one finds in connection with abortion in cattle. The cotyledons were attached fairly firmly, but on separation the surfaces showed a moist milky juice. One fœtus was present, and it had apparently been dead for a day or so, as the peritoneal cavity contained fluid which was blood-stained, and the contents of the stomach were also blood-stained. The fœtus, however, was quite fresh.

*Microscopical and Cultural Examination.*—The uterine exudate contained a few vibrios, but nothing could be found in the fluid from the stomach of the fœtus. Broth tubes were sown with the uterine exudate, but for some unexplained reason no growth was obtained.

The uterine exudate of this ewe was inoculated to Ewe 251 (see Experiment 51).

Ewe 245 was slaughtered on March 19th, that is 18 days after infection, as she was showing signs of approaching abortion. The uterus was cut out in the usual way, and taken to the laboratory for examination.

*Lesions.*—Externally the wall, especially about the neck, was œdematous and thickened. On cutting into the organ a considerable amount of a blood-stained, mucoid exudate was found evenly distributed over the surface of the uterine mucous membrane and on the chorion. It was particularly abundant in the neighbourhood of the cotyledons. On separation, some of the cotyledons were found to present a yellow anæmic appearance. One fœtus was present, and it was well developed for the period of pregnancy. It was quite fresh, but it had apparently been dead for a day or so, as the abdominal muscles were infiltrated by a blood-stained œdema, and fluid was present in the peritoneal and pleural cavities.

*Microscopical and Cultural Examination.*—The uterine exudate showed a large number of vibrios on microscopical examination. A considerable number of vibrios were also found in the inter-muscular œdema, the fluid from the pleural and peritoneal cavities, and the stomach fluid of the fœtus. Pure cultures of vibrio were obtained from the uterine exudate by sowing fluid and solid media.

The importance of this experiment is that both ewes became infected with abortion after the administration of pure cultures of the vibrio by two of the natural orifices (mouth and vagina), and that the tissues of the fœtus in one case were invaded by the vibrio in pure culture.

#### Experiment 50, Ewe 219.

As a control to the virulence of the cultures used in the above experiment (49) it was thought advisable to inoculate subcultures of the same date from Ewe P to another ewe by the veins.

On July 15th, 1910, Ewe 219 received into the jugular vein, one agar culture, which was a second subculture from Ewe P, and which had been incubated for 14 days, and one potato-broth culture, which was a third subculture from Ewe P, and which had been incubated for eight days.

This ewe gave birth to twin lambs at full time. The membranes were not recovered, so could not be examined, but there was no reason to believe that the parturition was in any way abnormal.

#### Experiment 51, Ewe 251.

As the exudate found in the uterus of Ewe 244 (Experiment 49) contained very few vibrios, which were stainable at least, it was decided to test its pathogenic power by further experiment.

Ewe 251 (cross-bred) was one of a lot obtained from a flock in which abortion was unknown. The ewe was expected to lamb some time in April 1910.

On March 15th, that is probably a month before full time, she received into the jugular vein 10 c.c. of an emulsion in broth of the uterine exudate of Ewe 244. No temperature reaction followed the inoculation.

On April 28th, which must have been about full time, Ewe 251 was slaughtered for the purpose of examining the uterus and its contents. The organ was cut out in the usual way, and taken to the laboratory for examination.

*Lesions.*—No external lesions were present. On cutting into the organ it was found to contain two lambs which were well developed and apparently at full time. No exudate or other abnormality was found in connection with the uterine mucous membrane or the fetal envelopes. No bacteria could be found by microscopical examination of scrapings from the uterine mucous membrane and the cotyledons.

Apparently the uterine exudate of Ewe 244, which was not rich in vibrios, had failed to infect Ewe 251.

#### Experiment 53, Ewes 252, 253, 255, and 256.

This experiment was performed for the purpose of further testing the ineffective properties of pure artificial cultures of the vibrio administered by the mouth.

Five agar tubes of the vibrio, which were second subcultures from the uterine exudate of Ewe 245 (see Experiment 49), and which had been incubated for six days, were triturated in a sterile mortar with 30 c.c. of a broth culture, which was a second subculture from the same source, and which had been incubated for six days. To this mixture 460 c.c. of sterile water were added.

All the above ewes were purchased from a flock in which abortion was unknown, and they were expected to lamb towards the end of April 1910.

On April 4th each ewe received by the mouth 100 c.c. of the above mixture of cultures.

On April 15th, Ewes 252, 255, and 256 again received by the mouth 100 c.c. of a mixture of cultures. The mixture was in every respect similar to the material formerly used to infect, except that the cultures used in this case were third subcultures and had been incubated for 10 days.

Ewe 253 on April 10th, six days after infection, gave birth to a living lamb. No vibrios could be found by microscopical examination of smears prepared from the fetal cotyledons. The time between infection and natural lambing was very short in this case; the animal might have become infected had the date of normal parturition been more remote.

Ewe 255 on April 20th, that is 16 days after administration of the first dose of culture and five days after the second, showed signs of approaching abortion. Around the vulva a discharge was present, and upon microscopical examination it was found to contain vibrios. This ewe was immediately slaughtered, the uterus was cut out in the usual way, and taken to the laboratory for examination.

*Lesions.*—Externally no macroscopic lesions could be found. On cutting into the organ a yellow mucoid exudate was found on the uterine mucous membrane and on the chorion. The uterus contained one lamb, which was well developed and apparently had not been very long dead, as it was quite normal in appearance.

*Microscopical and Cultural Examination.*—Microscopical examination of the uterine exudate showed that it contained a large number of vibrios in apparently pure culture. The stomach fluid of the lamb also contained vibrios. Cultures of the vibrio were obtained on different media from the uterine exudate and from the stomach fluid of the lamb.

Ewe 252 gave birth to a live lamb on April 18th. No vibrios could be found in smears made from the discharges or membranes.

Ewe 256 unfortunately turned out not to be pregnant. She had never been noticed to abort.

Although the date at which these ewes were tupped was not definitely known, it seems probable that in the cases of Ewes 252 and 253 parturition took place at full time. The interval between infection and lambing was short, and that may explain the negative results in these cases. It is important to note, however, that one out of the three pregnant ewes, which were infected by pure cultures of the vibrio by one of the natural orifices,

the mouth, was starting to abort when slaughtered, that lesions identical with those found in ewes aborting in the field were present, and that the uterine exudate and the stomach fluid of the fetus contained vibrios in pure culture.

Experiment 54, Ewes 257, 258, 259, and 260.

This experiment was designed to test the action of cultures of the vibrio when introduced by the vagina.

All the ewes employed for the experiment were purchased from a flock in which no abortion was known to have occurred, and they were expected to lamb towards the end of April. It may be mentioned, however, that one ewe purchased with this lot was observed on arrival at the laboratory to be unwell, and as it was thought that the illness might be connected with some uterine trouble, she was not included in the experiment. She was killed for examination, and a dead fetus of about three months development was found in her uterus. It is also important to note that the uterus of this ewe contained no exudate whatever. The fetus had a sour smell, but there was no odour of putrefaction. It has never been our experience to meet with appearances of this kind in connection with abortion in ewes, and it was concluded that this case was the result of the fetus having been killed in utero by an accident.

On April 4th, 1910, each of the three ewes employed for this experiment received into the vagina 10 c.c. of a mixture containing pure cultures of the vibrio obtained from the uterine exudate of Ewe 245 (see Experiment 49). The cultures used were five agar tubes, which were second subcultures and had been incubated for six days, and 40 c.c. of a broth culture from the same source, which was a second subculture and had been incubated for six days.

On the 15th April each ewe again received 10 c.c. of a mixture of cultures, which was in every way similar to the above, except that the cultures were third subcultures and had been incubated for eight days.

This experiment was unfortunate, as all the ewes turned out to be non-pregnant, and, so far as one could learn from post-mortem examination, they had not been pregnant for months. These ewes, which were stated to be pregnant, had to be purchased from an outside flock, as our stock of ewes which had been tupped at the laboratory was exhausted at this late date, and we were anxious to get as much experimental evidence as possible before the end of the lambing season, since we had previously found that the vibrio under artificial conditions of culture did not retain its virulence until the following tupping season.

*Summary of Observations in connection with Flock 15.*

The proportion of abortions was 16.6 per cent.; vibrios were found in material from the field.

Twenty-one ewes were put under experiment, four of which were barren. Of the 17 pregnant ewes, eight aborted and showed vibrios. Five of the eight received natural material by the veins, two received culture by the mouth and vagina, and one received culture by the mouth. Nine ewes did not become infected. Of these three received natural material by the mouth (one of these was slaughtered soon after infection), two received natural material by the vagina, three received natural material by the veins, and one received culture by the mouth.

C.

SPECIAL OBSERVATIONS DESIGNED TO DETERMINE THE NATURAL MODE OF INFECTION BY USING PURE CULTURES OF VIBRIO.

The experiments detailed in this section were carried out at an early stage of the inquiry, towards the end of 1907, as at this time there was at least a considerable amount of evidence in favour of the vibrio being a specific cause of abortion in sheep.

Owing to the absence of fresh material which could be utilised for infection, we had to fall back on cultures obtained originally from Ewe 29 (see Experiment 15, Flock 4). Ewe 29 was slaughtered on the 14th June 1907, on which date the first cultures were made from her cotyledons. These cultures were kept up in the laboratory by subculturing, and at the date of the experiments about to be described, 27th November 1907,

we had arrived at the 31st subculture, and the strain of vibrio had been under conditions of artificial cultivation for 166 days. In the light of our information regarding the effect of artificial conditions of upkeep on the virulence of the vibrio, which was mainly acquired after the date of these special experiments, it is possible that the negative results of the latter are, to some extent, attributable to that cause. In considering this point, however, one must also take into account some of the apparently positive results obtained in Experiment 65 with what was practically the same material, and which was used on the same date for the purposes of infection.

The material used for infection was composed as follows: 1,200 c.c. of broth culture of the vibrio, which was a 31st subculture from Ewe 29, and had been incubated for ten days, together with seven agar cultures, which were 31st subcultures from the same source and had been incubated for ten days.

Experiment 55, Ewes 139 and 138.

These ewes (cross-bred) were tupped on the 27th and 28th September 1907, and were expected to lamb towards the end of February 1908.

On November 27th each received into the vagina 10 c.c. of the above mixture of cultures from Ewe 29.

Ewe 139 on February 22nd, 1908, that is about full time, gave birth to one live lamb and one dead one. The live lamb was weakly, and died on the day after birth. The membranes were normal, and no vibrios could be found either by microscopical or cultural examination of material taken from the fetal cotyledons or the stomach fluid of the fetus.

Ewe 138 gave birth to a live lamb on March 10th, 1908, that is at full time. The lamb was weakly, and died the day after birth. The membranes were normal, and no vibrios could be found by microscopical examination of preparations made from the fetal cotyledons.

Experiment 55A, Ewes 136 and 137.

Both these ewes (cross-bred) were tupped on September 21st, 1907, and were expected to lamb towards the end of February 1908.

On November 27th each received by the mouth 50 c.c. of the mixture of cultures from Ewe 29 used in the previous experiment.

Ewe 136 gave birth to two healthy lambs on February 19th, 1908, that is at full time. The membranes were normal. No vibrios could be found by microscopical examination of preparations made from the fetal cotyledons.

Ewe 137 gave birth to a live lamb on February 21st, 1908, that is at full time. The lamb was not very strong, and died a week after birth. The membranes were normal, and no vibrios could be found by microscopical examination of preparations made from the fetal cotyledons.

As it was anticipated that the foregoing experiments might possibly give negative results simply on account of the channels whereby the infecting material had been administered, the following experiment was performed as a control to the virulence of the cultures.

Experiment 56, Ewes 134 and 135.

Both these ewes (cross-bred) were tupped on the 21st September 1907, and were expected to lamb towards the middle of February 1908.

On the 27th November 1907 each received into the jugular vein 10 c.c. of the mixture of cultures from Ewe 29 used in the previous experiment.

Ewe 134 gave birth to two lambs, one living and one dead, on February 10th, 1908, that is a few days before full time. The dead lamb was fully formed; the living one was weakly, and died five days after birth. The membranes were normal. No vibrios could be found by microscopical examination of the fetal cotyledons, nor could any be found either by microscopical or cultural examination of the fluid from the stomach of the lamb which was born dead.

Ewe 135 gave birth to two lambs, one living and one dead, on February 15th, 1908, that is at full time. The dead lamb was fully developed; the living one was weakly, and died the day after its birth. The fetal membranes were normal in appearance, and no vibrios could be found by microscopical examination of prepara-

tions made from the foetal cotyledons. No vibrios could be found either by microscopical or cultural examination of material taken from the stomach of the dead lamb.

#### Experiment 57, Ewe 147.

As a supplement to the previous experiment this one was performed with particularly large doses of culture of vibrio from the strain of Ewe 29. Only a liquid culture was used, however, and it had been incubated for five weeks.

Ewe 147 was tupped on October 6th, 1907, and was expected to lamb early in March.

On November 29th, 1907, she received 80 c.c. of the above culture into the jugular vein and 300 c.c. by the mouth. No temperature reaction followed inoculation.

On March 6th, 1908, that is at full time, Ewe 147 gave birth to a healthy lamb. The membranes were normal. No vibrios could be found by microscopical examination of preparations made from the foetal cotyledons.

As the results of these experiments with pure cultures of this strain, which had been under conditions of artificial cultivation for a considerable time, were entirely negative, it seemed inadvisable to pursue the inquiry further on the same lines. It was also considered possible that we might have the opportunity of investigating this point in connection with future outbreaks and under more favourable conditions (compare Experiments 65 and 66).

#### D.

#### EXPERIMENTS FOR THE PURPOSE OF TESTING WHETHER CATTLE AND OTHER DOMESTICATED ANIMALS ARE SUSCEPTIBLE TO THE VIRUS OF SHEEP ABORTION.

It was only possible to do a limited number of experiments under this head, because we could only obtain natural material for experiment during a short period in each year, and it was exceedingly difficult to have a stock of pregnant females of the various kinds of domestic and other animals available over such a short period as the lambing season. It was thought at first that probably the susceptibility of other animals could be tested on a considerable scale by making use of cultures, but, as has already been pointed out, these cultures appeared to lose their virulence after a short period of upkeep under conditions of artificial cultivation. Since the experiments are not numerous, those performed with natural material and with artificial cultures have both been grouped in this section.

#### Experiment 57A, Heifer 79.

This heifer was bulled on 21st January 1907. On 16th February, that is 26 days after service, she received into the jugular vein 10 c.c. of a mixture of two parts of the fluid from the stomach of Lamb D 1 (Flock 4) in one part of broth, and 20 c.c. of the same material were injected into the vagina. No temperature reaction followed the inoculation.

On 20th February she received into the jugular vein 10 c.c. of a fairly dense emulsion in broth of the uterine exudate of Ewe E (Flock 5). This material had been preserved in sterile broth at the laboratory for five days. By consulting the particulars in connection with Flock 4 it will be seen that the materials used in this experiment were rich in vibrios.

On 4th May, that is, 77 days after the first inoculation, Heifer 79 was slaughtered. The uterus was pregnant, and perfectly normal in every respect, except that a small area, about one inch square, in the neighbourhood of the neck showed what looked like an exudate. Microscopical examination, however, of preparations made from this material revealed no bacteria. Nothing could be found in the fluid of the foetal stomach.

#### Experiment 58, Heifer 78.

Heifer 78 was bulled on 28th January 1907. On 22nd February, that is, 25 days after service, she received into the jugular vein 80 c.c. of a fairly dense emulsion from the uterine exudate of Ewe F (Flock 6). By consulting the history of Flock 6 it will be observed

that the exudate was used on the day of its arrival. A temperature reaction from 103° to 105°·4 followed inoculation and lasted for five days.

On May 8th, that is 75 days after inoculation, Heifer 78 was slaughtered. She was showing no signs of approaching abortion at the time, but it was thought that sufficient time had been allowed for lesions to develop in the uterus.

*Lesions.*—The uterus, which was pregnant, was normal in every respect externally and internally. The foetus was well developed for the period of pregnancy, and showed no macroscopic lesions. No bacteria of any kind could be found by microscopical or cultural examination of material taken from the uterine mucous membrane, the cotyledons, or the stomach fluid of the foetus.

#### Experiment 59, Goat 55.

Goat 55, which had been served on November 12th, 1906, received into the jugular vein 4 c.c. of a dense emulsion in water of the slimy exudate from the membranes of Lamb H 1 (Flock 8), on the 28th February, that is 108 days after service. The exudate was proved by experiment to contain vibrios (see Exp. 21, Ewe 103). No temperature reaction or illness followed inoculation.

On March 4th, that is 38 days from full time and 4 days after inoculation, Goat 55 aborted three kids. The foetuses showed no lesions, and no bacteria of any kind could be found in the fluid from their stomachs. The goat was killed a few hours after aborting.

*Lesions.*—There was no exudate in the uterus, but, of course, a certain amount of fluid which one would expect shortly after a normal parturition was present. The uterine membrane was normal in appearance.

*Microscopical and Cultural Examination.*—Tubes of culture media were sown with fluid from the uterus. These developed mixed growths of bacteria which had probably invaded the organ after parturition. Nothing resembling the abortion bacillus of cattle or the vibrio was found by microscopical or cultural examination.

#### Experiment 60, Heifer 181.

Heifer 181 was bulled on November 29th, 1908. On January 16th, 1909, that is 48 days after service, she received by the mouth 160 c.c. of an emulsion in water of the uterine exudate of Ewe L (Flock 11) and 150 c.c. of the same emulsion were injected into the vagina.

On September 4th, that is at full time, and 230 days after infection, Heifer 181 gave birth to a dead calf. The calf showed no lesions, and had apparently perished during the parturition. The foetal membranes were normal, and neither vibrios nor bacilli of cattle abortion could be found in preparations made from the foetal cotyledons.

#### Experiment 61, Heifer 200.

Heifer 200 was bulled early in December 1908. On February 3rd, that is 45 days after service, she received by mouth 350 c.c. of washings made from the uterus and foetal membranes of Ewe 178 (see Experiment 28, Flock 12), and 50 c.c. of the same material were injected into the vagina.

On August 28th, that is at full time, she gave birth to a dead calf which was fully formed. Neither vibrios nor bacilli of cattle abortion could be found in preparations made from the foetal cotyledons or the stomach fluid of the foetus.

#### Experiment 62, Heifer 46.

Heifer 46 was bulled on September 1st, 1908. On March 13th, 1909, that is 193 days after service, she received into the jugular vein 10 c.c. of a mixture containing:—a thin emulsion of the exudate from the foetal membranes of Ewe 196 (see Experiment 32), one agar culture of vibrios which was a third subculture from the uterine exudate of Ewe N 1 (Flock 13), and which had been incubated for 10 days, and 6 c.c. of broth culture from the same source, which was a second subculture, and had been incubated for nine days. By referring to the case of Ewe 196 it will be seen that she aborted, and vibrios were found in the discharge from

her genital organs. It is also to be noted that the material from the cotyledons was contaminated. Three days after the inoculation the heifer was very uneasy. She seemed unable to co-ordinate her movements; her respiration was accelerated, but she showed no rise of temperature. She appeared to recover on the fourth day, but on the tenth day after inoculation the temperature rose to  $105^{\circ}\cdot 4$ , and remained above the normal for three days. During the febrile period a slight mucous discharge was occasionally noticed from her vagina, but no vibrios or bacilli of cattle abortion could be found in the material.

On March 26th, that is 206 days after service, and 13 days after infection, Heifer 46 gave birth to a calf, which appeared to be so well matured that it was thought there had been some mistake regarding the date of service. The calf, however, was very weakly, and died six days after birth. The membranes were retained, and when recovered were too foul to be satisfactorily examined. No vibrios or bacilli of cattle abortion, however, were found in the discharge from the genital organs after parturition.

#### Experiment 63. Cow 46A.

Cow 46A was bulled on October 18th, 1909. On February 10th, that is 115 days after service, she received into the jugular vein 10 c.c. of an emulsion in sterile broth of the uterine exudate of Ewe P (Flock 15). Immediately after inoculation she showed toxic symptoms which took the form of unsteady gait. On February 11th she had an attack of tympany, which had passed off by the next day. There was no temperature reaction.

On March 21st she again received into the jugular vein 6 c.c. of a fairly dense emulsion from the uterine exudate of Ewe 245 (see Experiment 49). No temperature reaction followed inoculation. By referring to Ewe P (Flock 15) and to Ewe 245, it will be seen that the material used for inoculation in each case contained vibrios in pure culture.

On April 8th, that is 172 days after service, 57 days after the first inoculation, and 186 days after the second inoculation, Cow 46A aborted a fetus. The fetus, which was hairless, appeared to have been dead for some little time before abortion took place. The fluid from the fetal stomach showed a small number of vibrios to the microscope. Cultures of the vibrios were also obtained by sowing media with this material, but they were not pure. Only a small amount of discharge was present after the act of abortion, and no vibrios could be found in it. The fetal membranes were removed from the uterus by manipulation. They presented a dry appearance, and the cotyledons showed the yellow anemic condition which has been described in connection with cattle abortion. A considerable number of vibrios were found by microscopical examination of preparations made from the cotyledons. No bacilli of cattle abortion could be found.

There seems little doubt that this animal aborted as the result of infection with material containing vibrios in pure culture.

#### Experiment 64. Heifer 266.

This heifer was purchased from a herd in which abortion was not known, and when put under experiment she was believed to be about six months pregnant.

On April 17th, she received into the jugular vein a mixture containing one agar culture of vibrio, which was a third subculture from the uterine exudate of Ewe 245 (see Experiment 49), and which had been incubated for ten days, and 6 c.c. of a broth culture from the same source. The broth culture was a fifth subculture, and had been incubated for four days. At the same time she received by the mouth five agar first cultures from the stomach fluid of the fetus of Cow 46A, together with 30 c.c. of broth culture from the same source. By reference to Cow 46A (see Experiment 63) it will be seen that the cultures given by the mouth were not pure, although they contained vibrios. No temperature reaction followed the inoculation.

On June 15th, at full time, Heifer 266 gave birth to a strong, healthy calf. The membranes were immediately recovered, but no vibrios could be found in preparations made from the cotyledons.

It will be observed that attempts were made to infect pregnant cattle with material from the uteri of aborting ewes in five cases, one of which was positive. Vibrios were found in material from the latter. Another pregnant heifer, which was infected with a mixture of natural material and culture, aborted, but no vibrios could be found in the material from her uterus. One pregnant heifer was infected with pure cultures of vibrio with negative result.

One pregnant goat aborted a few days after receiving natural material into the veins, but no vibrios could be found in the material from her uterus.

It is also important to note that no bacilli of cattle abortion were discovered in connection with these animals, all of which are susceptible to that microbe. The positive result obtained in Guineapig 451 (Experiment 8) should also be considered under this section.

Lastly we think it very important to record that Mr. Norris, M.R.C.V.S., of the Department of Agriculture and Technical Instruction for Ireland, who underwent a special course of instruction at the Board's laboratory, and was well acquainted with the characters of the vibrio and the bacillus of cattle abortion, found the former in connection with a case of cattle abortion in Ireland when the latter was entirely absent. Mr. Norris, through the late Mr. Hedley, Chief Veterinary Inspector of the Department of Agriculture and Technical Instruction for Ireland, sent us some of the stomach fluid from the calf, and we were able to confirm Mr. Norris's observation by finding large numbers of vibrio in the material. Further, we have had the good fortune to investigate a natural outbreak of vibronic abortion among cows on premises in Wales.

Between May and September of 1911, four cows cast their calves on this establishment. Nothing further happened until February 1912, when another abortion took place and the fetus was sent to the Board's laboratory. No bacilli of bovine abortion were found in the stomach fluid of this calf, but vibrios were present in pure culture, and cultures were obtained in artificial media. Nothing further happened until December 1912, when another fetus from a cow was sent to the laboratory. The bacilli of bovine abortion were absent from the fetal stomach, but vibrios were present in pure culture. The owner stated the cows went to pasture and had run with sheep, but that no sheep breeding was, or had been conducted on the place for two years before the abortion started amongst the cows. He further said that when sheep were bred on the place he had not been particularly troubled with abortion in his ewes. Abortions, however, had occurred amongst the ewes. If we assume that infection had persisted on the premises from the time sheep breeding was stopped (two years), an interesting problem arises regarding the manner of its persistence. We have not lost sight of the possibility that rabbits and other vermin may keep up the infection, and having regard to the nature of the causal micro-organism it is also possible, that it may have a carrier amongst the lower forms of life—a tick, for example—as in some forms of spirochaetosis. There seems to be little doubt that cattle can become infected with abortion due to the vibrio, but there is a good deal of experimental evidence and field observation in favour of the view that infection by this microbe is rare.

#### E.

#### DIAGNOSIS.

In a considerable number of cases it is possible and easy to make the diagnosis by examining with the microscope smears made from the cotyledons and from the discharge from the genital passages. The vibrios can be stained very well with dilute carbol-fuchsin or methylene blue, but they can also be seen moving about the field of the microscope in unfixed and unstained preparations of exudate diluted with sterile fluid. In stained preparations the granules referred to in Section A can sometimes be made out when no vibrios can be seen.

An examination of the fluid from the stomach of the fetus also admits of the diagnosis being made in many cases. It frequently happens, however, particularly when the material evacuated is undergoing putrefaction, that no vibrios can be found, and other methods are required to enable a correct diagnosis of

the nature of the abortion to be arrived at. In the Appendix to Report, Part I, reference was made to the agglutination test, and details were given of the test by fixation of the complement and of the reaction test, in so far as they were applicable to bovine abortion. It is difficult to obtain sufficiently luxuriant

cultures of the vibrio, and to cultivate it in such a way that the cultures can be made use of for all these tests. We have hopes of getting over this difficulty, but so far we have only been able to investigate the possibilities of the agglutination test as an aid to diagnosis before and after the act of abortion.

Observation (1).—Ewe 217 (*see* Experiment 36) infected with natural material by the mouth, slaughtered while aborting sixteen days after infection, vibrios found in discharge and uterus. The blood was withdrawn at the time of slaughter, and the serum was tested two days afterwards with the following result:—

Dilution.	Result in Test Tube.	Micro-examination.		Remarks.
		After Staining.	In Hanging Drop.	
1-1	Tube quite clear	Marked clumping	Some motile vibrios.	The tubes were left in incubator for 17 hours before judging. To the microscope the vibrios were swollen, granular and distorted. No change in controls.
1-10	Not quite clear	" "	" "	
1-25	Slightly clear	" "	" "	
1-50	No change	Distinct	" "	
1-100	"	"	" "	

Observation (2).—Ewe 226 (*see* Experiment 40) infected with natural material by veins, slaughtered while aborting six days after infection, vibrios found in discharges and uterus. The blood was withdrawn at time of slaughter, and kept on ice for eight days, when the serum was tested.

Dilution.	Result in Test Tube.	Micro-examination.		Remarks.
		After Staining.	In Hanging Drop.	
1-10	Quite clear	Marked clumping	Some motile vibrios.	After six hours there was no change to the eye. The tubes were finally judged after 17 hours in the incubator. The controls showed no change to the eye.
1-25	" "	" "	" "	
1-50	" "	" "	" "	
1-100	" "	" "	" "	
1-500	Nearly clear	" "	" "	

Observation (3).—Ewe 234 (Experiment 38) infected by the mouth with natural material and culture; slaughtered ten days after infection, no signs of infection were found. The blood was withdrawn at time of slaughter, kept on ice for five days, when serum was tested.

Dilution.	Result in Test Tube.	Micro-examination.		Remarks.
		After Staining.	In Hanging Drop.	
1-10	Quite clear	—	—	The tubes were judged after 19 hours in the incubator. The culture was seven days old. Controls unchanged.
1-25	No change	—	—	
1-100	"	—	—	
1-500	"	—	—	

Observation (4).—Ewe 227 (Experiment 40) infected with natural material by veins, aborted 12 days after infection, vibrios found in uterus. Blood was withdrawn on day of abortion, de-fibrinated, and serum separated in the centrifuge, and used for test the same day.

Dilution.	Result in Test Tube.	Micro-examination.		Remarks.
		After Staining.	In Hanging Drop.	
1-10	Quite clear	Marked clumping	—	The culture was seven days old. Tubes were judged after 19 hours. Controls showed no change.
1-50	" "	" "	—	
1-100	" "	" "	—	
1-500	Distinctly clear	" "	—	
1-1,000	" "	" "	—	

Observation (5).—Ewe 230 (Experiment 44) infected with natural material by vagina, slaughtered 14 days after infection, and showed no signs of being infected. The blood was withdrawn at time of slaughter, and the serum was used four days afterwards. Agglutination was complete in all 1-10, -25, -100, -500 and -1,000.

Observation (6).—Ewe 204 (Experiment 35) infected twice with cultures by intravenous injection, but gave birth to a healthy lamb. The serum was tested nine months after the second inoculation in the following dilutions:—1-100, -500, -1,000. The tubes were judged after being in the incubator for 17 hours. All were quite clear.

Observation (7).—Ewe 244 (Experiment 47) infected by the mouth and vagina with cultures, slaughtered while aborting thirteen days after infection. The blood was withdrawn at slaughter, and the serum was tested the following day in dilutions of 1-50, -100, -500, -1,000. The tubes were judged after being 17 hours in the incubator. No tube was clear, but all showed a slight degree of clearing.

Observation (8).—Heifer 46 (Experiment 62) infected by the veins with culture of vibrio, but did not abort. The serum was tested one year after infection, using dilutions of 1-50, -100, -500, -1,000, and -1,500. All the tubes up to 1-1,000 were quite clear; the tube 1-1,500 was practically clear. The culture employed was not dense.

Observation (9).—The serum of five ewes which had aborted on a farm in Scotland was tested in dilutions of 1-100, -300, -500, -1,000 with absolutely negative results. No vibrios could be found in the membranes or fœtuses of these ewes.

Observation (10).—Ewe 240 (Experiment 47A) inoculated into veins with filtrate (through Berkefeldt V) of an emulsion made with uterine exudate containing vibrios. The filter was afterwards found to be defective. The ewe aborted and the contents of the uterus were found to be very foul; no vibrios were found. The serum was tested the day after slaughter in dilutions of 1-50, -100, -300, -500, -1,000. No agglutination occurred.

## F.

### ATTEMPTS TO IMMUNISE EWES BY INJECTING LARGE DOSES OF LIVING CULTURES OF THE VIBRIO.

Since it was possible that the injection of living cultures of the vibrio might infect the pregnant uterus, it was necessary to administer the immunising dose of such cultures some time before the ewes became pregnant, in order to allow time for the living vibrios to be destroyed in the body.

The ram was not put with the ewes under experiment until about seven weeks after the immunising inoculation, but, as will be understood, the ewes did not all take the ram at the same date. Throughout the experiment the immunised ewes, along with the controls, were allowed to run in a grass paddock about half an acre in size, and whatever grazing they could get was supplemented by dry forage and roots.

The cultures used for immunisation were from the strain originally obtained from Ewe 29, which was started on the 14th June 1907.

Experiment 65, Ewes 114, 115, 116, 117, 118, 119, and as controls, Ewes 127, 128, 129, 131, 132, and 133.

On the 10th July 1907 each of the six ewes which were to be immunised received under the skin of the hind leg 100 c.c. of an actively growing broth culture of vibrio, which was a third subculture from the cotyledons of Ewe 29, and had been incubated for ten days. Several of the ewes showed a certain amount of lameness for a couple of days after the inoculation, and all of them for a period of five days showed a similar temperature reaction, varying from 103 to 105.

On July 20th each again received under the skin of the opposite leg 100 c.c. of a broth culture of vibrio, which was a fifth subculture from the uterine exudate of Ewe 29, and had been incubated for five days. Some of the ewes were slightly lame after the second inoculation, and all of them again exhibited a temperature re-action of the same severity as in the first case.

The immunised ewes took the ram on the following dates in 1907:—Ewe 114 on October 5th; Ewe 115 on September 25th; Ewe 116 on October 3rd; Ewe 117 on September 27th; Ewe 118 on September 30th; and Ewe 119 on October 18th.

On November 27th each of the immunised ewes which were then believed to be pregnant were infected in the following way. Each received into the jugular vein 10 c.c. of a mixture composed of a broth culture from the strain from Ewe 29, which had been incubated for eight days, and three agar cultures, which had been incubated for the same time; by the vagina, 10 c.c. of a mixture composed of 200 c.c. of broth culture from the strain obtained from Ewe 29, and which had been incubated for eight days, and three agar cultures from the same source, which had been incubated 10 days; by the mouth, 50 c.c. of a mixture composed of 1,000 c.c. of broth culture from the strain obtained from Ewe 29, and which had been incubated for nine days, and four agar cultures from the same source which had been incubated for 10 days. All the cultures used for infection were 31st and 32nd subcultures.

Ewe 114 (immunised) aborted a fœtus, which was found in the paddock on the 29th November, that is 132 days after the last immunising dose, two days after infection, and 55 days after service. The fœtus had evidently been dead for some time before abortion, as its intestines were pulpy. It must certainly have been dead before Ewe 114 received the infecting dose. A swab was passed into the vagina of this ewe, and smears were made from this swab. Microscopical examination of these smears revealed a multitude of bacteria, but no vibrios could be seen. The ewe was immediately slaughtered, the uterus was cut out in the usual way, and taken to the laboratory for examination.

*Lesions.*—The cotyledons were still attached, but they could be easily separated by traction. Smears made from the separated surfaces showed a considerable number of vibrios, but no cultures were attempted, as it was difficult to obtain uncontaminated material. It is difficult to say whether the vibrios which had undoubtedly reached the cotyledons belonged to the immunising dose or entirely to the infecting dose. If to the latter it is most improbable that they were responsible for the death of the fœtus, which seemed to have occurred before the date of the test inoculation.

Ewe 115 (immunised) showed a brown watery discharge around her genital organs on December 26th, 1907, that is 159 days after administration of the last immunising dose, 29 days after infection, and 92 days after service. No vibrios could be found by microscopical examination of smears made from the discharge. The ewe was immediately slaughtered, the uterus cut out in the usual way, and taken to the laboratory for examination.

*Lesions.*—Externally no lesions were apparent, but the uterus was exceedingly small for the period of pregnancy. On cutting into the organ it was found to contain two fœtuses, which were little more than an inch in length. These fœtuses appeared to have been arrested in their development about a month after the commencement of pregnancy, or possibly about the time the infecting dose was administered. The fœtal membranes had a softened appearance, and many of the cotyledons were separating. No exudate, however, was present on the uterine mucous membrane, but there was a certain amount of thick brown fluid material around the degenerated cotyledons. A great number of smears were prepared from the cotyledons and examined, but no vibrios or other bacteria could be found by microscopical examination. Culture media which were sown with scrapings from the cotyledons gave a growth resembling bacillus subtilis, but no vibrios could be identified.

Ewe 116 (immunised) showed a yellow discharge around the genital organs on 31st December, that is, 164 days after administration of the last immunising dose, 34 days after infection, and 89 days after service. No vibrios could be found by microscopical examination of smears made from this discharge. The ewe was immediately slaughtered, the uterus cut out in the usual way, and taken to the laboratory for examination.

*Lesions.*—Externally the organ showed no lesions. On opening into the uterus it was found to contain a well-developed fetus, which was quite fresh. No exudate was present on the uterine mucous membrane, and the fetal membranes were normal in every respect. No vibrios could be found by microscopical or cultural examination of material taken from the uterine mucous membrane, the fetal cotyledons, or the stomach fluid of the fetus.

It seems probable that this ewe was not in reality showing signs of approaching abortion at the time she was slaughtered.

Ewe 117 (immunised) gave birth to a live lamb on 9th March 1908, that is at full time. The fetal membranes were quite normal, and no vibrios could be found by microscopical examination of smears made from the fetal cotyledons.

Ewe 118 (immunised) aborted twin fetuses on 20th November 1907, that is, 132 days after administration of the last immunising dose, 2 days after infection, and 60 days after service. The fetuses were found in the paddock and were enclosed in their membranes. A yellow mucoid exudate was present on the chorion of both fetuses. Smears prepared from this exudate were found on microscopical examination to contain a considerable number of vibrios.

Ewe 119 (immunised) showed a yellowish discharge around the genital organs on 2nd January 1908, that is, 67 days after infection, and 76 days after service. She was immediately slaughtered, the uterus was cut out in the usual way and taken to the laboratory for examination.

*Lesions.*—The organ showed no external lesions. On opening into the cavity it was found to contain two fetuses, which were well developed for the period of pregnancy, and had probably been alive at the time the ewe was slaughtered. A good deal of mucus was found on the uterine mucous membrane in the neighbourhood of the cotyledons. The cotyledons were very soft and easily separated. The fetuses showed no lesions whatever.

*Microscopical and Cultural Examination.*—Microscopical examination of smears made from the above-mentioned mucus revealed no vibrios, but a few cocci were found; these were evidently the result of external contamination. Vibrios could not be found in the fluid from the fetal stomachs. No vibrios developed in culture media, which were sown with the uterine mucus, but several colonies of cocci and large sporing rods appeared. It was apparent, however, that these had nothing to do with the condition of the uterus. Culture media sown with the fluid from the fetal stomachs remained sterile. It seems probable that the discharge observed at the vulva of this ewe during life was not a premonitory symptom of abortion.

Ewe 127 (control, not immunised) was tupped on September 15th, 1907, and was expected to lamb about the middle of February 1908.

On December 11th, 1907, that is 14 days after infection, and 84 days after service, a small amount of blood-stained, yellow discharge was seen around the vulva. No vibrios could be found by microscopical examination of smears made from this discharge.

Ewe 127 was immediately slaughtered, the uterus was cut out in the usual way, and taken to the laboratory for examination.

*Lesions.*—Externally the organ showed no macroscopic lesions. On cutting into the organ it was found to contain a fetus, which had apparently been dead for some days, as it was becoming decomposed. A considerable amount of yellow mucoid exudate was found on the uterine mucous membrane. The cotyledons were softened, and easy to separate. The condition was very like what had been found in connection with other cases of abortion in ewes.

*Microscopical and Cultural Examination.*—No vibrios could be found either by microscopical or cultural examination of the uterine exudate, or the stomach fluid of the fetus.

Ewe 128 (control, not immunised) was tupped about the middle of September 1907, and was expected to lamb about the middle of February 1908.

On February 23rd, 1908, that is about full time, she gave birth to a live lamb. The membranes were normal in appearance, and no vibrios could be found in preparations made from the fetal cotyledons.

Ewe 129 (control, not immunised) was tupped on September 15th, 1907, and was expected to lamb towards the end of February 1908.

On December 27th, that is 30 days after infection, and 103 days after service, a dark brown membranous material was observed hanging from the vulva. No vibrios could be found by microscopical examination of smears made from this material. The ewe was immediately slaughtered, the uterus was cut out in the usual way, and taken to the laboratory for examination.

*Lesions.*—The organ was very small for the period of pregnancy, and a considerable amount of oedema was present in the neighbourhood of the neck. On cutting into the cavity it was found that the fetal cotyledons had completely separated from the maternal ones, and the latter had shrunk almost to nothing. A shrunken fetus, about two inches long, was present. The uterine mucous membrane was covered in places by a small amount of thick brownish material, which may possibly have arisen from liquefaction of the fetal membranes.

*Microscopical and Cultural Examination.*—No vibrios could be found in smears made from the uterine mucous membrane, or from the external surface of the fetus. Culture media were sown with scrapings from the uterus. Most of the culture tubes, however, remained sterile, but one or two gave a growth of staphylococci. These seemed to be chance organisms, which had invaded the open uterus.

It is to be noted about this ewe that she was slaughtered immediately the uterus showed signs of being open, and that that possibly accounts for the comparatively uncontaminated condition of the uterine contents. It is a question, of course, whether the fetus died as the result of the experimental infection, or whether we operated unknowingly upon a ewe which at the time of infection contained a dead fetus. The probabilities are in favour of the fetus having died very shortly after infection, and its size, although it was shrunken, was indicative of this being the case. Even if we assume that the vibrio was not responsible for the death of the fetus, its absence from the uterus still remains unexplained, as there can be no doubt that the vibrio possesses the power of growing in utero, and it has been found to persist for some time, at least, after the death of the fetus.

Ewe 131 (control, not immunised) was tupped on September 19th, 1907, and was expected to lamb about the middle of February 1908.

On the 3rd February 1908, that is 68 days after infection, and about 12 days before full time, a slight amount of brown-coloured discharge was noticed around the vulva of Ewe 131. Microscopical examination of smears made from this discharge showed that it contained vibrios. It was decided, however, not to slaughter this animal, but to wait, and observe if she would abort. On the 4th February the discharge had stopped. On the 5th February no discharge could be found, and as the ewe did not appear to be taking kindly to confinement in the loose box in which she had been put to facilitate observation, she was turned out again into the paddock. From February 6th to 16th she was carefully watched, and visited several times a day. During this period the same discharge was noticed to be present intermittently around the vulva, but no vibrios could be found in it by microscopical examination.

At 11 a.m. on the 16th February, that is practically at full time, she aborted a mummified fetus about 1½ inches in length. From the appearance of the fetus it must have died at an early period of pregnancy, and it is not improbable that death took place shortly after infection. After abortion a fair amount of discharge oozed from the genital organs. Microscopical examination of this material showed it to contain a variety of organisms, which is not surprising, seeing that the discharge had been going on for several days. No vibrios, however, could be found in this foul

material. The ewe was not slaughtered at the time she aborted, as it was thought she would be useful for a further observation later on, and it was considered hopeless to expect results from the examination of her uterus, which by this time was evidently in a very foul condition.

She was slaughtered on March 30th, 1908, that is, 42 days after abortion, and at this date the uterus presented a normal appearance externally and internally. No vibrios or other microbes could be found by microscopically examining scrapings from the uterine mucous membrane.

Ewe 132 (control, not immunised) was tupped on September 20th, 1907, and was expected to lamb towards the end of February 1908.

On December 29th, 1907, that is 32 days after infection, and about 49 days before full time, this ewe was noticed to be showing signs of uneasiness; her back was arched, and she was disinclined to feed. She was carefully observed until the 31st December, during which time she did not improve, but no discharge could be found in connection with her genital organs.

On January 1st, 1908, a slight discharge was noticed around the vulva. It was dark brown in colour, and had a mucoid consistence as in the case of other aborting ewes. No vibrios could be found by the microscope in smears made from this discharge. Ewe 132 was slaughtered on this date. The uterus was cut out in the usual way, and taken to the laboratory for examination.

*Lesions.*—Externally the neck of the organ was slightly cedematous, and a clot of brown exudate was in process of being squeezed into the vagina. Smears were prepared from this clot, but no vibrios could be found in them. On opening into the uterus, the fetal membranes were found to be of a dirty brown colour. The cotyledons were flattened, and easy to separate. The exposed surfaces of the latter after separation were bloodless, dry, and of a dirty brown colour. Two fetuses were present, both of which were in a pulpy condition. They appeared, in fact, to be undergoing the process of mummification, but the liquid matter had not been fully absorbed or discharged. The uterine mucous membrane was covered at places by a dark brown, liquid material, which might possibly have arisen from liquefaction of the fetus and its membranes.

*Microscopical and Cultural Examination.*—No vibrios could be found by microscopical examination of the material found on the uterine mucous membrane. Culture media were inoculated, but the tubes all remained sterile. Owing to the condition of the fetus in this case it was difficult to say at what time death had taken place. It is quite possible, however, that it followed shortly after infection. The difficulty with regard to explaining the disappearance of the vibrio also arises in the case of this ewe.

Ewe 133 (control, not immunised) was tupped on September 20th, 1907, and was expected to lamb towards the end of February 1908.

On December 21st, 1907, that is 24 days after infection, and 92 days after service, a mucous discharge was found to be coming from the vulva. This material was not examined microscopically. The ewe, however, was immediately slaughtered, and a preliminary examination was made in the slaughterhouse.

*Lesions.*—The organ was cedematous in the neighbourhood of the neck. It also seemed to be slightly distended by gas. One fetus was found in the vaginal passage, another being contained in the uterus. When the organ was opened, it was found to contain a very considerable amount of putrid exudate. The cotyledons were separated, and undergoing putrefactive changes. The whole of the uterine mucous membrane was in a catarrhal condition. Smears made from the uterine contents showed a variety of putrefactive organisms, but no vibrios; nor could vibrios be found by examining smears from the separated cotyledons. The fetuses were not examined, as putrefaction was so far advanced. Culture media were sown with the uterine material, but the tubes became very foul, and no vibrios could be identified in the growths.

When summing up the result of Experiment 65 it would appear that Ewe 114 (immunised) should be excluded, although vibrios were present in the discharges, as it seemed highly probable that her fetus was dead in utero at the time of infection. The exclusion of Ewe 114 leaves five immunised animals and six controls for consideration. Of the five immunised ewes, two aborted, one lambed at full time, and two were slaughtered on suspicion 34 and 76 days after infection, but were found to be normal.

Of the two ewes which aborted, vibrios were found in one.

Of the six control ewes which were not immunised before infection, one lambed at full time and one aborted. Four were slaughtered on suspicion 14, 30, 32, and 24 days after infection. Vibrios were found in the discharges from the ewe which aborted. In each of the four controls slaughtered the uterus was found to be in an aborting condition, but no vibrios were present. In two of them, however, putrefaction of the uterine contents was advanced.

These 12 ewes were put under experiment at an early stage of the inquiry and merely as a preliminary observation. It seemed to us that the only conclusion which could be drawn from the experiment was that, owing to the irregularity of the results following infection with cultures of the vibrio, it would be hopeless to expect to obtain information of much practical importance by operating on such a number of pregnant ewes as one could conveniently employ at a laboratory, and that for further information on the value of preventive inoculation one would require to undertake extensive observation on flocks exposed to natural infection in the field. Our subsequent observations serve to confirm this opinion, as we found that the irregularity of the results of experimental infection with the vibrio showed itself when other strains were used. Apart from the question of immunisation, however, several points of interest arise out of the above experiment. There is a considerable amount of evidence in support of the view that the vibrio soon loses its pathogenic properties under conditions of artificial cultivation. If this be so, however, it is difficult to explain the positive results in this experiment (65) following inoculation with culture which had been kept up on artificial media for several months (*see* Ewes 118 and 131). If it be not the case that the vibrio soon loses its pathogenic properties under such circumstances, it might be permissible to attribute abortion in the cases of Ewes 115, 127, 129, 132, 133 to the experimental infection with cultures. The difficulty of explaining the disappearance of the vibrio from their uterine contents, however, would still remain, but, of course, it is not improbable that this may be accounted for by the invasion of the uterus by putrefactive bacteria some time before the act of abortion took place.

With reference to infection, the fact that vibrios which had been excreted by Ewes 114 and 118 were present on the pastures of the paddock at an early date (29th November), must not be lost sight of, for it is also quite conceivable that the vibrios excreted had gained in virulence by passing through these ewes, and that Ewes 115, 127, 129, 132, and 133 were infected from the pasture.

The positive result obtained in Experiment 66 by contact gives support to this possibility (*see* Experiment 66).

## G.

### INFECTION AT PASTURE.

Experiment 66, Ewes 34 and 150.

The object of this experiment was to test whether pregnant ewes would become infected by grazing in the paddock (about half an acre) with those which were used in the previous experiment. It does not come under the head of immunisation, but it is convenient to give the details in this part of the Appendix as some of the details are intimately related to Experiment 65. It will be observed by consulting the last experiment (65) that several of the ewes aborted in the paddock, and that vibrios were found in the discharges of some

of them. We may take it, then, that the pasture had become contaminated by the vibrio.

Ewe 34 (Dorset) was tupped on September 6th, 1907, and was expected to lamb early in February 1908.

Ewe 150 (Cheviot) was tupped on October 8th, 1907, and was expected to lamb early in March 1908.

Until they were brought in for the experiment, both ewes had been kept in a field at a considerable distance from the laboratory premises.

On December 13th, 1907, that is after some of the ewes in the previous experiment had aborted, Ewes 34 and 150 were placed in the same paddock.

Ewe 34 aborted one fresh lamb and one mummified lamb on January 28th, 1908, that is 46 days from the commencement of contact, and about five days before full time. The membranes and the stomach fluid of the fresh lamb were examined, but no vibrios could be found. Smears prepared from the vaginal discharge were also examined with negative results. Culture tubes sown with the stomach fluid of the fresh fœtus remained sterile.

Ewe 150 showed a dark brown coloured discharge from the vulva, and aborted two fœtuses on January 22nd, 1908, that is 40 days after the commencement of contact, and 43 days from full time. Immediately before abortion took place she evacuated from her vulva about 50 c.c. of a yellow exudate. Microscopical examination of smears made from this exudate showed it to contain an immense number of vibrios, and very few other organisms. The fœtal membranes were macerated in appearance, and were very fragile. All the cotyledons were covered by yellow exudate, which was very tenacious and difficult to scrape off. Scrapings from the fœtal cotyledons showed large numbers of vibrios to the microscope. The fœtuses were very dark in colour, and somewhat emphysematous; they had apparently been dead for some little time, and only a small quantity of fluid was found in their stomachs. The fluid was dark red in colour, and contained vibrios, but a few other microbes were also present. This ewe was not slaughtered until the 14th April, at which date the uterus was quite normal in appearance.

It is to be observed that both these ewes aborted subsequently to being put to pasture on a paddock contaminated by vibrios from the uteri of at least two ewes, and that the observation lends support to the view that infection from vibrios is contracted from the fields in which ewes have previously aborted.

#### H.

#### EXPERIMENTS TO TEST THE POSSIBILITY OF USING THE SERUM OF A HYPER-IMMUNISED ANIMAL AS A PROTECTIVE AGENT.

It is well known that the injection of serum cannot be expected to endow the animal organism with immunity against a bacterial disease for more than a period of from 10 to 20 days. This being so, and the period of pregnancy in ewes being about 150 days, it would have been absurd to attempt to immunise ewes by injecting a dose of serum before they became pregnant. Moreover, from a practical point of view it did not appear that any remedy, which would have to be administered to a flock of pregnant ewes at several periods during pregnancy, would recommend itself to the sheep farmer. Nevertheless, we thought it advisable in the first instance to inquire whether repeated injections of protective serum to pregnant ewes which had been infected would prevent them aborting, for it must not be forgotten that it is possible that the majority of abortions in the field arise from infection contracted only a short time previous to the act. The experiments about to be described were carried out during the lambing season of 1907-8, at a time when the experiments performed up to date with the vibrio had furnished a considerable amount of evidence that that microbe was responsible for outbreaks of abortion in sheep.

The serum used in the experiments was obtained from Heifer 54, which had been repeatedly subjected to subcutaneous inoculation with large doses of liquid cultures of the vibrios.

It may be here stated that cultures of the vibrio are not at all toxic to cattle when administered

subcutaneously, and one can begin with an injection of 1,000 to 2,000 c.c. of actively growing cultures of vibrio without causing any ill effects.

A month before serum was drawn from Heifer 54 for this experiment she had received one dose of 8,000 c.c. of actively growing culture of the vibrio.

#### Experiment 67, Ewes 140, 141, 142, 144, 145, and 146.

All these ewes (cross-bred) were tupped between the last week in September and the first week in October 1907. They were therefore expected to lamb about the middle of March 1908.

On October 27th, 1907, each ewe received 10 c.c. into the vagina and 10 c.c. by the jugular vein of the following mixture of cultures from Ewe 29 (*see* Experiment 15): 400 c.c. of broth culture, which had been incubated for eight days, triturated with six agar cultures which had been incubated for 10 days. At the same time each ewe received by the mouth 50 c.c. of a broth culture which had been incubated nine days, triturated with four agar cultures which had been incubated 10 days.

All the ewes were allowed to run in a special grass paddock during the experiment.

The strain of culture used for experiment was the same as in the case of Experiments 55, 55A, 56, and 57.

It had been under conditions of artificial cultivation for 135 days.

Ewe 140 on 12th December 1907 received subcutaneously 20 c.c. of the above serum from Heifer 54, and a similar dose was injected the day following. On this day, however, 13th December, a blood-stained discharge was noticed at the root of her tail.

On 14th December, that is, 48 days after infection, she was seen lying down isolated from the others, and a big clot of mucus was found on the ground behind her vulva. As this was thought to be a case of early infection she was immediately slaughtered. The uterus was cut out in the usual way, and taken to the laboratory for examination.

*Lesions.*—On opening into the organ it was found that one horn contained a considerable amount of chocolate-coloured exudate between the mucous membrane and the chorion. The cotyledons of this horn when separated had a juicy and degenerated appearance. The uterus contained one fœtus, which had probably been dead for a few days, as its tissues had a macerated appearance; it was not putrid. The other horn was empty, but it had all the appearance of having recently been pregnant. No fœtus, however, could be found after a careful search in the paddock.

*Microscopical and Cultural Examination.*—A large number of smears made from the uterine exudate were examined, but no vibrios could be found. In fact, the material seemed to be free from bacteria of any kind. Culture media were sown with the exudate from the pregnant horn, but no growth of vibrios was obtained.

Ewe 141 received subcutaneously 20 c.c. of the serum of Heifer 54 on the 13th, 14th, 15th, 16th, and 17th December. After an interval she again received subcutaneously 20 c.c. of the same serum on the 30th and 31st January, and on the 1st, 2nd, 3rd, 4th, and 5th February 1908.

On 24th March 1908, that is, at full time, Ewe 141 gave birth to a healthy lamb. No vibrios could be found by microscopical examination of the fœtal cotyledons.

Ewe 142 received subcutaneously 20 c.c. of serum from Heifer 54 on 12th, 13th, 14th, 15th, 16th, and 17th December. After an interval she again received 20 c.c. of serum of Heifer 54 on 30th and 31st January, and on 1st, 2nd, 3rd, 4th, and 5th February.

On 27th February 1908, that is at full time, Ewe 142 gave birth to a strong, healthy lamb. No vibrios could be found by microscopical examination of the fœtal cotyledons.

Ewe 144 received subcutaneously 20 c.c. of serum from Heifer 54 on December 12th, 13th, 14th, 15th, 16th and 17th.

On 24th February 1908, that is a few days short of full time, she gave birth to two dead lambs. Both

lambs were well developed, and had evidently died at birth. No vibrios could be found in the fluid from the stomachs by microscopical examination or in smears from the fetal cotyledons.

Ewe 145 received subcutaneously 20 c.c. of serum from Heifer 54 on December 12th, 13th, 14th, 15th, 16th and 17th.

On 16th March 1908, that is at full time, Ewe 145 gave birth to a live lamb. No vibrios could be found by microscopical examination of smears made from the fetal cotyledons.

Ewe 146 received subcutaneously 20 c.c. of serum from Heifer 54 on December 12th, 13th, 14th, 15th, 16th and 17th.

On 30th March 1908, that is at full time, Ewe 146 gave birth to a live lamb. No vibrios could be found by microscopical examination of smears from the fetal cotyledons.

It is to be noted about these ewes that they were kept in a different paddock from those which were the subjects of Experiments 65 and 66, and that they were not exposed to infection on the pastures. It is, of course, open to question whether the injections of serum cut short the action of the vibrios which were used to infect. It seemed to us that the most useful experiment with serum from a practical point of view, and having regard to the uncertainty of obtaining positive results with cultures, would be to inject a considerable number of ewes about a month before time with serum, and afterwards either inject them intravenously with uterine exudate containing a pure culture of vibrios, or expose them to heavy natural infection in the field. An opportunity of doing this has been looked for, but unfortunately none has as yet presented itself. It is an opportunity which the Board of Agriculture and Fisheries might continue to look for.

## DESCRIPTION OF THE MICRO-PHOTOGRAPHS ON PLATE 1.

- FIGURE 1.—A smear of uterine exudate from Ewe 226 (*see* p. 16), showing the vibrio in pure culture.  $\times 2,000$ .
- .. 2.—A smear from a scraping from a cotyledon of Ewe 29 (*see* p. 6), showing the vibrio in pure culture.  $\times 2,000$ .
- .. 3.—Preparation from a three days' old culture of the vibrio in broth.  $\times 2,000$ .
- .. 4.—Preparation from a four days' old culture of the vibrio on the surface of agar-potato medium, which had been incubated in a rarefied atmosphere.  $\times 2,000$ .
- .. 5.—Preparation from a pure culture of the vibrio on the surface of agra-potato medium, which had been incubated for a month. Some of the vibrios stain less deeply than in the case of young cultures, and a number of granules are present.  $\times 2,000$ .
- .. 6.—Preparation from a month old culture of the vibrio on the surface of potato-agar, incubated in a rarefied atmosphere. Few vibrios are present but there are an enormous number of granules like cocci. This culture was proved to be pure by sub-culture.  $\times 2,000$ .
- .. 7.—Culture of the vibrio of sheep abortion in upright agar, inoculated when liquid and solidified. The culture was obtained from an outbreak of vibrionic abortion amongst cows (*see* p. 22). No surface growth appeared in this tube but was apparent in other tubes from the same source.
- .. 8.—Culture of the vibrio of sheep abortion in upright agar, inoculated when liquid and solidified. The culture was obtained from Ewe 29 (*see* p. 6). The tube shows both the surface and the sub-surface growth.

Our thanks are due to Mr. A. L. Sheather, B.Sc., M.R.C.V.S., for preparing these photographs.

FIG. 1.

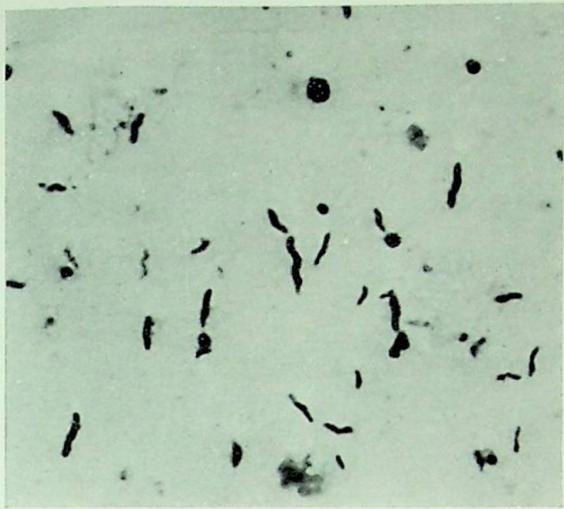


FIG. 2.

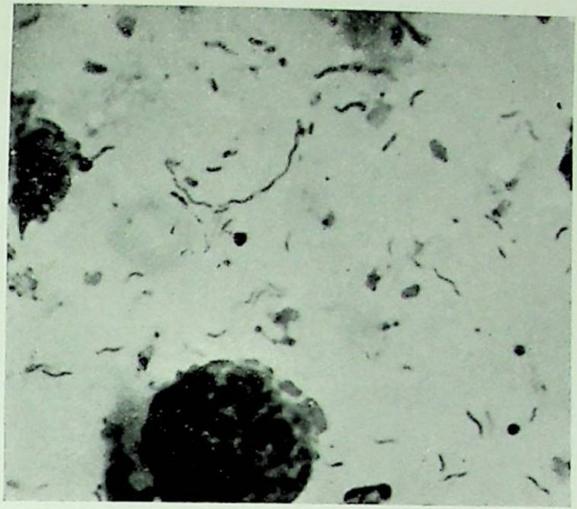


FIG. 3.



FIG. 4.



FIG. 5.

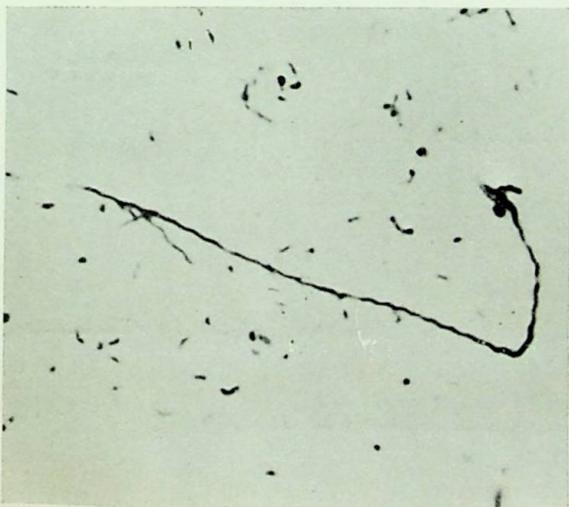


FIG. 6.

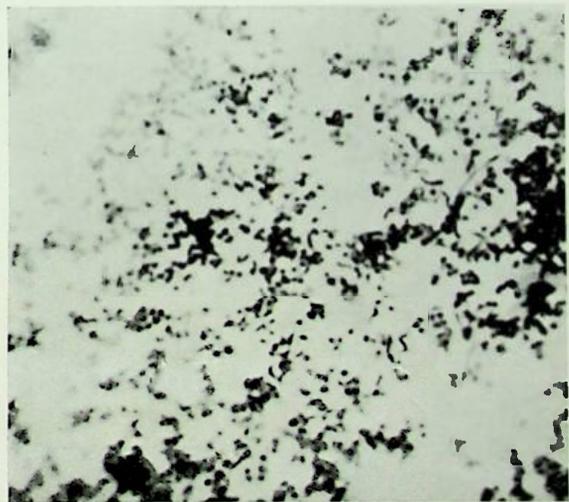


FIG. 7.

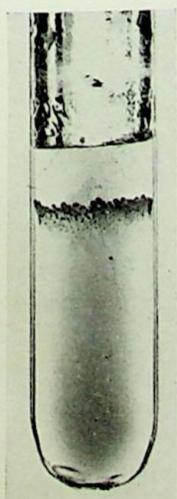
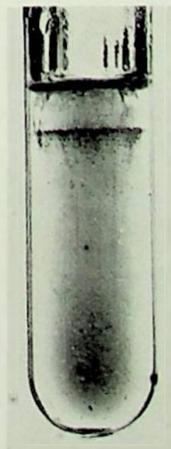


FIG. 8.



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