

SPIRILLA ASSOCIATED WITH DISEASE OF THE FETAL  
MEMBRANES IN CATTLE (INFECTIOUS ABORTION).

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PLATES 81 AND 82.

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In the course of investigations into the pathology and etiology of abortion in cattle as it prevailed among the animals composing a certain group of herds under one management, and with more or less intercourse between individual herds, there was encountered a fair number of cases from which *Bacillus abortus* Bang could not be isolated from the fetus or placenta either in cultures or through guinea pigs or with both methods combined. These cases ranged themselves into several groups and comprised (*a*) the few fetuses yielding sterile cultures, (*b*) those yielding rapidly growing cultures of colon-like and other bacteria, and (*c*) those yielding pure cultures of a spirillum. It is this latter organism which will be dealt with in this paper.

After much of the work here reported had been done, a report of an English Committee on abortion in sheep came to the writer's notice.<sup>1</sup> The actual work is credited to Sir J. McFadyean and Sir Stewart Stockman. The direction of the investigation was in the hands of a larger committee of which the two authors mentioned were members. The brief report of this committee summarizes the results of the experiments reported in full in the appendix.

The microorganism is described as a vibrio and the more precise nomenclature left for future determination. The vibrio was cultivated on the well known medium consisting of agar, gelatin, and serum in deep layer as first used by Bang<sup>2</sup> for *B. abortus*, and it grew in much the same way as this bacillus. A careful reading of the statements concerning the cultural characters of the

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<sup>1</sup> Great Britain Board of Agriculture and Fisheries, Report of the departmental committee on epizootic abortion, London, 1913, 22.

<sup>2</sup> Bang, B., *Z. Thiermed.*, 1897, i, 241.

vibrio does not reveal any essential differences between this organism and the spirillum under discussion. Nor do we find any differences in their negative effects on small laboratory animals. Seven head of cattle were infected with exudate from aborting ewes either with or without the addition of pure cultures to the pathologic material. In two instances the mode of introducing the virus was by mouth, in the rest by intravenous inoculation. Two cases aborted, and in one of these the vibrios were detected. These results are as yet too meager and indefinite to answer the important question whether the vibrio of ovine abortion is the same as the spirillum of the bovine disease.

What is of more interest at the moment is a statement, quite buried in the report, of the isolation of vibrios from cases of abortion in cattle in a herd in Ireland and in Wales in 1911. A brief paragraph is all that is given of these findings and we are left to conjecture whether vibrios were found later in other herds.

#### *Lesions Associated with the Spirillum.*

In all, fourteen cases have come under our observation from which pure cultures of the spirillum have been obtained. At the same time the abortion bacillus of Bang was not met with in any of these cases.

Taking the cases consecutively as they occurred in the group of herds under observation since the earliest isolation of the spirillum, beginning June, 1917, and ending September, 1918, there were twenty-seven cases from which the bacillus of Bang was isolated and fourteen cases associated with spirilla. These figures are not to be considered accurate for the reason that a certain small number of cases were not studied and hence are not included. A certain number could not be studied so thoroughly as the rest and hence the associated organism may have been overlooked. Nevertheless, the figures may be taken as indicating that, in this group of animals as elsewhere, *Bacillus abortus* is at present the predominating organism in infections of the fetal membranes of cattle.

The cases examined indicate that the lesions associated with this organism are largely if not exclusively restricted to the fetal membranes and that the fetus suffers secondarily from a gradually increasing interference with the placental circulation. This is precisely what occurs when *Bacillus abortus* invades the chorion and cotyledons. We would therefore expect the changes in the fetus to be much the same in both etiological types. This is the case. It is impossible to

foretell whether a given fetus will yield cultures of *Bacillus abortus*, or of the spirillum, or none at all.

Unfortunately, the fetal membranes are either retained and discharged in a disintegrated and decomposed state, or if discharged with or soon after the fetus they are mostly in more or less advanced stages of autolysis, the death of the fetus having taken place some time previously. The material, therefore, which is the primary seat of the disease will be available in the best state only when cattle are slaughtered and the membranes examined fresh, *in situ*. Such cases are relatively rare and perhaps encountered only by accident. It may be that serological tests will enable us to select specifically affected animals during life.

The main lesions affecting the fetus are edema of the subcutaneous tissue and effusions into the large serous cavities. The fluids are, as a rule, more or less heavily tinged with blood. This effusion is frequently associated with delicate, loose, shreddy deposits of fibrin or more rarely heavy, whitish pseudomembranes in abdomen and much less abundantly on pleura and epicardium. The visceral changes are chiefly those referable to autolytic changes following death. Focal lesions are not present.

The stomachs usually contain considerable quantities of what appears to be swallowed meconium or perhaps, at least in some instances, material driven into the fourth stomach from the small intestines by antiperistaltic movements. The stomachs of normal fetuses contain a colorless, translucent, very thick, viscid fluid which in older fetuses may contain a few pellets of meconium and perhaps some hairs. The diseased fetus, however, almost invariably has in its stomachs a very turbid, thick, yellowish, flaky fluid. Not infrequently there are also found small, whitish, soft, disc-like masses which can be traced back to epithelial excrescences of the amnion and which have come away and been swallowed by the fetus with the amniotic fluid.

The lungs may or may not be inflated, depending on the age of the fetus. In some the air tubes contain fluid identical with that in the stomachs. This fluid may completely fill the trachea and bronchi.

The kidneys are usually surrounded by a thick bloody fluid which collects in a thin layer under the capsule and separates this from the cortex. The tissue about the kidneys is also frequently distended with fluid suffused with blood-coloring matter.

The study of sections of fixed and hardened tissues of fetuses has not brought out anything characteristic of the infection. In the digestive tract the epithelial coverings may be partly or wholly desquamated, probably not as the result of any disease process, but following death of the fetus. Focal lesions were absent here and from the other organs. The lungs in some cases presented collections of cells of undetermined type in the alveoli and smallest air tubes. This absence of active disease processes in the fetus is probably to be accounted for by facts to be submitted later on concerning the habitat of the spirillum.

About the fetal membranes, little that is definitely known can be said until cases are available in which the fetus and membranes can be studied fresh and *in situ*. In general, however, it may be stated that the focal lesions of the cotyledons and of the chorionic membrane between cotyledons, and the exudate, which characterize the cases infected with *Bacillus abortus*, are absent in the cases associated with spirilla. The histological picture resembles in certain respects only that of the disease due to *Bacillus abortus*. The chorionic epithelium is destroyed and the tissue immediately below it is densely infiltrated in spots with cells, whose nuclei are in a state of fragmentation, as if a strong chemotactic influence were at work in the utero-chorionic space. There is, furthermore, edema of the chorion and extensive, rather loose cell infiltration into the edematous zone. The nature of these cells, necrotic in the cases examined, has not been definitely made out. In several instances there were dense collections of polynuclear leucocytes among the denuded villi of the cotyledons.

#### *Morphological and Biological Characters of the Spirilla.*

The only microorganism thus far encountered in films and sections of the fourteen cases is a spirillum, if we exclude certain mixed infections of the fetus with *Bacillus coli*, miscellaneous bacteria

attaching themselves to the placenta as it is discharged into the bedding and manure, and those swallowed by the fetus over 7 months of age during and just after birth.

Spirilla have been detected microscopically both in the fetus and in the few placentas available, but not in every case from which pure cultures were obtained. If we grant that the seat of the disease is in the fetal membranes, more particularly the chorion, the spirilla make their way into the amniotic fluid and thence into the digestive and respiratory tracts of the fetus. Spirilla have been detected in films of stomach contents in five cases and in sections of the small intestine in two. In the placental tissues they have been seen either alone or with miscellaneous filth bacteria. Their precise habitat in the chorion cannot be fixed upon with the meager material available. In one case the endothelium of certain capillaries of the chorion was crowded with spirilla (Fig. 7). This condition has not been seen in the few other cases examined. In one other case the necrotic cores of the villi contained dense colonies of minute organisms which were probably spirilla. In several other cases spirilla were loosely scattered through the necrotic tissues. Inoculation of guinea pigs in the two latter instances showed that *Bacillus abortus* was absent. It should be stated that the short forms of the spirillum are very minute and when packed together in dense masses only those on the periphery may be recognizable.

In form the spirilla found in fixed and hardened tissues vary more or less in length. The short forms, consisting of 1 and  $1\frac{1}{2}$  wave lengths or windings, predominate; those of 2 to 4 windings are less numerous. In the small intestines they occur in the desquamated epithelium and along the free margins of the villi (Fig. 1). There is evidence from the microscopic picture that still shorter forms exist which, owing to their minuteness, are not definable. The transverse diameter or thickness is probably not over  $2\ \mu$ , the windings about  $2\ \mu$  long, amplitude,  $0.5\ \mu$ .

The importance of a suitable staining agent in disclosing these forms cannot be overestimated. Thus far alkaline methylene blue has given satisfactory results for films. Giemsa's stain, now on hand, has been less so. In fixed material eosin-methylene blue has been used with good results. Bacteria in tissues and fluids may be in

various stages of active multiplication and degeneration and of encapsulation. A given dye may therefore pick out only certain stages. This is probably true of methylene blue. Films of stomach fluid stained 24 to 48 hours in one case showed a dense tangle of long, rather feebly stained spirilla with and without stained granules (Fig. 2), whereas the 10 minute preparation showed only small numbers of fairly short, well stained forms. The spirillum is Gram-negative.

Spirilla from cultures do not present any features not found in the microorganisms in the host tissues and fluids. In the earliest growth the short forms predominate. As the culture grows older longer spirochete-like forms appear and become most numerous. At the same time, deeply stained granules may appear at spaced intervals in some of the spirilla. These are also found in individuals from body fluids and only in the long forms. These facts point towards degenerative processes but further studies may tend to contradict this view.

If bits of tissues or particles of meconium or drops of stomach contents containing spirilla are added to slanted agar and the tube is hermetically closed with sealing wax, there will be seen after 3 or 4 days, along the lateral margins of the slanted surface, between agar and glass, a narrow grayish line extending up from the condensation water one or several centimeters. At the same time or some days later a very thin, barely visible film of growth will have extended from this line inward between agar and glass. In some instances this film continues to spread until it meets the film growing in from the opposite side. Rarely a thin film starts upward on the slope for a few millimeters from the condensation water. If a transfer to a fresh tube of agar is made after 4 or 5 days, a similar though feeble growth may be obtained. A second transfer is, however, apt to fail.

In casting about for media suitable for the continued cultivation of the strains it was found that while tubes containing bits of guinea pig or other mammalian tissue are suitable, the best method was to add a few drops of defibrinated horse blood to the condensation water of ordinary nutrient agar slants. This medium has served the purpose very well and at the time of writing strains from twelve of

the fourteen cases from which spirilla have been isolated in pure culture are still alive. Some have been transferred over 60 times. In general the older strains grow more profusely. After the strains had been under cultivation for some time they were tried on plain agar, sealed, with the result that the oldest strains have grown and are continuing to multiply in the absence of blood or bits of tissue (Figs. 3 and 4).

The growth on blood agar varies somewhat from strain to strain but the main features are the same. In the older strains, multiplication occurs in a thin layer over the sedimented corpuscles in the condensation water, between glass and agar, as a grayish layer on the slope starting from the condensation water and as isolated colonies on the slope. The latter may be absent. When present, they vary in size from mere points to 2 mm. in diameter and have the ordinary appearance of bacterial colonies. On plain agar the growth is much less vigorous as a rule, but even in this medium occasionally the amount of growth is unexpectedly large. Quite invariably the growth in the condensation water resembles a drop of thick translucent mucus and, owing to its viscosity, it may be difficult to pull it away from the inside of the tube in transferring to a fresh tube. Growth also occurs between glass and agar and less frequently on the slope.

The usual cultural media, including carbohydrate media in fermentation tubes, have been tried without success. It is not improbable that after prolonged cultivation in sealed tubes of plain agar it may be possible to obtain cultures in bouillon, milk, etc.

In young cultures the spirilla are in active motion. The short forms, 1 to  $1\frac{1}{2}$  windings long, move with great rapidity across the field of the microscope. The longer, more sluggish individuals of two to four windings move in a straight line, revolving about the longitudinal axis at the same time. The very long forms, often bent or curved, are very sluggish and move in irregular manner, about one end, or only revolve. Cultures more than a week old may contain no active individuals.

With this method of cultivation the spirilla have been isolated in pure culture, and quite uniformly so, from the fourth stomach, meconium, and lung tissue of the fetus. They were isolated not so

frequently from spleen, liver, and kidneys. In all cases bits of tissue as large as peas were transferred to the culture tube of plain agar. The invasion of the body generally is thus not the rule and may be due to accidentally favorable conditions.

The action of this organism on the small laboratory animals so far tried is apparently without harmful effects. Of the forty-odd guinea pigs inoculated subcutaneously and intraperitoneally with body fluids and tissues containing cultivable spirilla, all have thrived like normal animals. Several rabbits treated repeatedly with large doses injected into the abdominal cavity have remained well. No anaphylactic symptoms have developed. Rats and mice are also refractory to large doses of cultures. Birds have not yet been tested.

The resistance of the culture forms to spontaneous disintegration is evidently low. Cultures renewed after a period of a week may be lost. On the other hand, some resist. Thus of two original cultures from body tissues and fluids, one was still alive after  $2\frac{1}{2}$  months. It was kept at room temperature and protected from drying by sealing wax. Another culture under the same conditions and only  $3\frac{1}{2}$  weeks old was dead.

Owing to the time required to bring the various strains of spirilla to grow actively in media free from blood and tissues, serological tests to determine whether the strains react alike to immune sera are still under way and they will be described in a later publication. Preliminary tests with the serum of rabbits repeatedly inoculated with large doses of living spirilla indicate a close relationship of the fetal strains.

*Probable Relation of the Spirillum to the Disease Process.*

The proof of any direct etiological relation between a given microorganism and a definite disease process is brought by reproducing the latter with pure cultures of the microorganism in question. This should not be a difficult undertaking were it not that infectious abortion is widespread. To utilize for crucial tests animals from infected herds may lead to errors of interpretation in two directions. The inoculated animals may abort on account of spontaneous infection or they may have acquired more or less resistance and fail to



abort after inoculation. The experimental tests with the spirillum are therefore waiting for suitable subjects. In the meantime, the presumption that this microorganism bears a relation to abortion similar to that borne by *Bacillus abortus* is supported by several facts.

The spirillum is isolated in pure culture from fetuses under precisely the same conditions as is *Bacillus abortus*. The cultural peculiarities of the two in requiring a reduced oxygen tension are the same. The distribution of both organisms topographically is the same. Both occur quite regularly in the gastrointestinal and respiratory tracts of the fetus, more rarely in other viscera. Both occur in the placental fluids but *Bacillus abortus* is more readily demonstrable because of the susceptible guinea pig, which eliminates in itself the miscellaneous filth bacteria in the discharged fetal membranes.

The cases in which the spirillum was demonstrated did not harbor *Bacillus abortus*. None of the guinea pigs inoculated with fetal material or placenta has acquired the specific lesions produced by it. On the other hand, the spirillum was absent in the cases from which *Bacillus abortus* has been isolated. In the many hundreds of cultures prepared in this laboratory the bacillus of Bang develops after 5 to 10 days. This period gives the spirillum ample opportunity to multiply if present. No such mixed infection has been observed. It appears, therefore, that these organisms are mutually exclusive for reasons not known at present. A further significant fact which cannot be discussed until the cases associated with *Bacillus abortus* have been analyzed and presented is the limitation of the spirillum to second and later pregnancies thus far.

Another fact which supports the etiological rôle of the spirillum is the discovery of vibrios or spirilla in the abortion of sheep and in two instances in herds of cattle in Ireland and Wales, as has been stated above. Although the method of cultivation was different in our work from that used by the English workers, the evidence is sufficiently strong to warrant the inference that they are closely related.

TABLE I.

Cow No.	No. of sire.	Age of calf at birth.	Date of breeding.	Date of abortion.	No. of pregnancy after purchase.	Cultures.						Guinea pig inoculations.					
						4th stomach.	Meconium.	Lung.	Spleen.	Liver.	Kidney.	Fetal fluids.	4th stomach.	Lung.	Meconium.	Placenta.	
67	?	<i>in.</i>	(Purchased Jan. 6 1918)	Jan. 6 1918	?							+					
90	?	6½	(Purchased Sept. 2, 1917.)	June 2 1917	?	++	--*			+							
95	1	28½	(Purchased Nov. 29, 1916.)	" 14 1916	2nd			++									
149	1	16½	Nov. 13 1917	Oct. 27 1917	2nd			++									
159	2	16¾	June 10	Nov 7	3rd	+		++	-								
179	3	29	Apr. 23	" 26 1918	2nd	Heavy growth.		+-									
192	36		May 30	Jan. 23 1918	3rd	?		++									
213	4,5	21	Aug. 4	Mar. 18	4th	++		++									
246	2,6	7½	" 31 1918	May 28	2nd			+-									
251	2	17½	Jan. 11	June 10	2nd	++		++									
256	6	19	" 24	" 18	4th	++		+									
258	7	27	" 14 1917	" 29	2nd	?		+									
263	3	20	Dec. 16 1918	July 30	3rd	++		++									
267	4	23	Feb. 9	Aug. 12	3rd	++		++									

\* Small intestine.

† Liver.

## SUMMARY.

Spirilla of identical morphological and cultural characters have been isolated in pure culture from the fetuses of fourteen cases of abortion. The condition of the fetus is much the same whether spirilla or the bacilli of abortion are present. This condition is probably due in both cases to interference with the placental circulation. The injurious action of the etiological factor when spirilla are present is limited to the fetal membranes, more particularly the chorion. Definite lesions of the fetus were not detected. The spirilla gain access to the digestive and respiratory organs of the fetus when the latter swallows the amniotic fluid. More rarely they are disseminated through the body, probably through the circulation. The spirilla will grow in certain culture media only under reduced oxygen tension, readily secured by sealing the ordinary culture tubes with sealing wax. Laboratory animals (mammals) are refractory. The precise relation of the spirillum to the pathologic process remains to be more definitely formulated. Since the spirillum was first isolated, twenty-seven cases have been found associated with *Bacillus abortus* and fourteen with the spirillum. In none was a mixed infection with both organisms detected. The spirillum has been isolated only from the second or succeeding pregnancies.

Table I summarizes the data collected thus far. It gives by number the male and shows that the spirillum is not associated with any one bull. The spirillum has been found in fetuses of various ages as shown in Column 3. The distribution of spirilla as shown by cultures is given, the sign + indicating pure cultures, the sign - no growth. The guinea pig inoculations are shown to be uniformly negative as regards *Bacillus abortus*.

## APPENDIX.

Inasmuch as no data have thus far been published concerning the occurrence of spirilla in bovine abortion it has seemed best to publish the following brief notes on the individual cases.

*Cow 90.*—Cow purchased November 29, 1916. Aborted June 2, 1917. Placenta discharged soon after. Both examined June 4 after having been refrigerated.

Male fetus, length 57 cm. Hairless, except on lips. Marked general subcutaneous edema with slight staining of fluid with hemoglobin. In abdomen and pleural and pericardial sacs considerable amounts of blood-tinged fluid together with thin elastic fibrinous deposits on liver, omentum, lungs, and heart. All organs are more or less soft, putty-like in consistency.

Stomachs contain a turbid, bile-stained, flaky viscid fluid. Cultures made by adding a few drops of stomach contents and a bit of liver to agar and sealing. In these, active spirilla found in pure culture.

Two guinea pigs inoculated with 1.3 cc. of a suspension in salt solution of cotyledons, ground in sand. Chloroformed after 2½ months. No lesions of *B. abortus*. Cultures from spleen remain sterile.

Sections of the placenta fixed in Zenker's fluid and stained in eosin-methylene blue show a complete loss of the epithelium covering chorion and villi of cotyledons and the presence of groups of polynuclears at the roots of villi. In certain blood vessels of chorion from 10 to 120 $\mu$  in diameter the endothelial cells are crowded with minute bacteria, not resembling *B. abortus* and in a situation where the latter is not found (Fig. 7). The minute organisms are probably short spirilla, for on the thin margin of groups, where they lie in single layer, short curved forms are recognizable. Endothelial cells within the lumina of these vessels are also crowded with the same forms. Where chorion and amnion are fused there is considerable edema with infiltration of cells no longer identifiable (necrotic).

*Twin Fetuses 94 and 95.*—Dropped on pasture June 14, 1917. Placenta was discharged some hours later but unfortunately was not brought to the laboratory with the fetuses.

*Fetus 94.*—Male fetus with good coat of hair, black and white; length 72 cm. No hemorrhagic discoloration or edema of subcutis. Mouth soiled with earth. Stomachs contain a thick, very viscid, dark greenish, opaque, flaky fluid. Liver large, rather pale; kidneys soft, with numerous cortical hemorrhages. Lungs indicate that some air has entered. Nothing noteworthy otherwise. Cultures from this fetus as follows: Those from spleen, kidney, and lungs remain free from growth. From this case no spirilla isolated. Sections from fixed tissues show nothing noteworthy beyond congestion.

*Fetus 95.*—Twin of No. 94. Abdominal organs, except spleen and liver, eaten out by some animal on pasture. Lungs contain a trifle air, but are otherwise heavy and full of blood.

Of five tubes prepared with bits of lung tissue, three developed into pure cultures of a spirillum. The two remaining contained spore-bearing aerobes.

Histological examination of the lung tissue shows presence in air tubes and alveoli of groups of cells of uncertain character. Interlobular tissue broadened and lymph spaces filled with blood.

*Cow 149.*—Cow aborted October 27, 1917. Due to calve March 19, 1918. Calved normally in 1916. Placenta now retained.

Fetus  $16\frac{1}{2}$  inches long, male. Skin readily pulled from underlying tissues exposing axillary and inguinal regions as if dissected by *B. welchii*. All muscles soft and readily torn away from attachments. Blood-tinted fluid in abdominal cavity. Liver macerated and tissue readily crushed. In the stomachs a pinkish fluid. The layer of epithelium detached and carried out by incision with the fluid like sheets of tissue paper.

Blood-tinted fluids in pleural sacs; lungs with slight interlobular edema. No odors of bacterial decomposition.

Pure cultures of a spirillum obtained from bits of lung tissue and stomach fluid. Cultures from other organs not made.

Two guinea pigs inoculated with suspensions of lung tissue and one with stomach contents. They were chloroformed after 7 weeks. No lesions found and all cultures from spleens remain sterile.

*Cow 158.*—This case is reported because it also was probably associated with spirilla, but cultures were not made. *B. abortus* was absent.

Cow purchased February 16, 1917. Calved normally March 10 and aborted November 6.

Fetus and membranes discharged together, the two still connected by umbilical cord. No odor of decomposition. Fetus  $12\frac{1}{2}$  inches long, female. In subcutis of ventral aspect and especially over pubis a translucent, only in part blood-tinted edema. Blood-tinted fluid in abdomen. Liver very friable. Thorax contains considerable blood-tinted fluid.

The placenta shows a glass-clear gelatinous edema between chorion and amnion. No liquid obtainable by incision into this layer. Cotyledons whitish; the villi long and slender when floated in water.

Three guinea pigs inoculated with 1 cc. of a turbid suspension of cotyledons ground up in salt solution were kept 8 weeks. They were then chloroformed and cultures prepared from bits of spleen tissue. All remained free from growth. The guinea pigs were normal.

Sections of fixed and hardened cotyledons show extensive collections of polynuclears between the villi denuded of epithelium. The villi are represented only by the necrotic connective tissue stroma. In this stroma there are exceedingly dense masses of minute bacteria which in places thin out and are resolved into short wavy forms.

*Cow 159.*—This cow was purchased in April, 1914. Calved normally in 1915. Aborted February 23, 1916 and November 7, 1917.

The fetus of 1916 was 3 to 4 inches long and discharged with membranes. It had undergone considerable maceration, the entire body being soft, semitranslucent and only head, limbs, and ribs recognizable. Odor peculiar but not suggestive of bacterial decomposition. Two guinea pigs inoculated with placental tissues remained well and were chloroformed after 10 weeks. No lesions were found and spleen cultures remained indefinitely free from growth. Sections of the placenta show groups of polynuclears among villi, but no spirilla among other bacteria.

The fetus and placenta of 1917 were obtained together. Fetus  $16\frac{3}{4}$  inches long, male. Blood-tinted fluid in subcutis, in abdomen, perirenal tissue, thorax, and pericardial sac. A thin deposit of fibrin shreds on heart surface. Rumen contains a thick, viscid, pinkish fluid and a mass resembling the color and consistency of yolk of egg. Fourth stomach contains a similar fluid but no suspended matter.

The placenta received only in part. After removing shavings and other bedding, the cotyledons were found to be gray to whitish. There was no edema of the fetal membranes. The amniotic fluid still remaining was thin, slightly blood-tinted, with fine flakes in suspension.

Cultures were made from contents of fourth stomach and amniotic fluid. Only one from stomach contents showed growth in the form of a pure culture of spirilla.

Sections of fixed and hardened tissues of fetus presented nothing noteworthy. Spirilla were detected in contents of small intestine. Sections of the chorion, including portions of cotyledons, showed infiltration of cells into the wall of the small blood vessels. The cells are probably mononuclear but the bizarre form of the nuclei makes it impossible to define them. On the periphery of the cell infiltration are many curved forms, of one to two windings, very minute. They are not in dense groups but scattered singly through the tissue. None is seen in the endothelium of the vessels as is the case in No. 90. There is some fibrin in the vascular walls and also in the nearly nude villi of the cotyledons. Polynuclears are absent but this may be due to the washing of the fetal membranes necessitated by the filth attached to them. Miscellaneous bacteria are, as might be expected, attached to the free exposed surfaces of the fetal membranes.

Three guinea pigs were inoculated, one with suspension of ground up cotyledons, one with contents of fourth stomach, and one with amniotic fluid. After 8 weeks they were chloroformed. Cultures from the spleens remained free from growth.

*Cow 179.*—Cow aborted November 26, 1917. The fetus was placed in refrigerator and examined November 28. Placenta retained.

Fetus 74 cm. long, with a good coat of hair. Surface of body stained yellowish. Some shavings from bedding in mouth. Abdomen somewhat distended.

Stomachs filled with a yellowish, only slightly viscid fluid, holding in suspension abundant soft yellowish brown masses. Large intestine well distended with meconium. Liver slightly enlarged, yellowish. Spleen on section shows some lighter areas. Lungs not air-distended. Trachea and bronchi filled with stomach contents.

Cultures were made directly from fetal tissues. Three tubes inoculated respectively with contents of fourth stomach, spleen, and kidney tissue remained sterile. In one kidney and lung tube heavy growths appeared of several species. In one tube of stomach contents a pure culture of spirilla developed. In one liver tube a small capsulated bacillus appeared. Sections of various organs, fixed and hardened, show nothing characteristic or noteworthy.

Two guinea pigs inoculated, one with a suspension of ground up lung tissue, the other with stomach contents, were chloroformed after 7 weeks. Lesions absent. Cultures from spleen negative.

*Cow 67.*—Fetus, within membranes. Discharged January 6, 1918, and placed at once into refrigerator. Examined January 7.

Fetus about 17 cm. long. Head resting on abdomen and directed caudad. Cervical vertebrae separated and only skin and sternomastoid muscle holding head to body. Tissues soft and partly autolyzed. Allantoic and amniotic fluids turbid, reddish in color.

A culture from amniotic fluid added to slanted agar developed into a pure culture of a spirillum.

No guinea pigs inoculated from this case.

*Cow 192.*—Cow aborted January 23, 1918. Due March 18. Calved 1914 and 1917. Fetus received, frozen, at laboratory. Placenta retained. Length 91 cm., weight 45 pounds. The only noteworthy features of this case are as follows:

The stomachs contained much very viscid light brownish fluid, suspending pieces of meconium up to 7 cm. long, and some hairs. Colon and rectum distended with very dark, tenacious meconium.

Lungs inflated with exception of the left ventral lobe.

Films from surface of mucosa of fourth stomach and rectum negative as regards bacteria.

Cultures from the various organs gave the following result. Two liver, two spleen, and two kidney tubes remained free from growth. Three tubes containing lung tissue and two containing meconium developed into pure cultures of a spirillum. Both tubes containing stomach contents developed heavy growths with gas formation. (Evidently the fetus had breathed and swallowed at birth.)

Two guinea pigs inoculated, one with lung tissue, the other with meconium, were kept 5 weeks, then chloroformed. Lesions and spleen cultures negative.

*Cow 213.*—Cow calved in 1914, 1915, and 1916. Aborted March 18, 1918. Due June 9. Placenta discharged soon after.

Male fetus, 53 cm. long, hairless. Pelvis unusually narrow; nature of deformity not investigated. Muscular and subcutaneous tissue slightly edematous. Abdomen contains some blood-stained fluid.

Stomachs not overdistended. Rumen contains a thick, tomato sauce-like fluid, viscid, holding in suspension whitish flakes 2 to 3 mm. in diameter, consisting of squamous epithelium (small cell masses from inner surface of amnion). Films show a few spirilla of three to four windings. Fourth stomach contains a still homogeneous, very viscid, slightly amber-colored, transparent fluid, which had to be cut with scissors on account of viscosity, in order to get some for cultures. Rectum distended with small cylindrical masses of dry meconium packed side by side.

Liver of a coarsely mottled yellowish appearance. Spleen several times normal size, flabby. Only one kidney present, this with unusually broad cortex.

Thorax half full of blood-stained fluid. Lungs not inflated. Slight interlobular edema.

Placenta covered with foreign matter and emitting a strong fecal odor. Cotyledons in the main of normal appearance. Some are grayish to whitish. The outstanding feature of the chorion is the presence of slightly elevated patches of an irregular nodular surface usually 3 to 4 cm. in diameter. The nodules are very firm, not readily crushed or pulled away from underlying tissue. Subchorionic tissue edematous. Films from different cotyledons and patches show several varieties of bacteria including spirilla of  $1\frac{1}{2}$  to 4 windings. These are more numerous than the other miscellaneous bacteria. Of the cultures prepared two kidney, two spleen, and one liver tube remain sterile. Pure cultures of spirilla are obtained from two lung and two fourth stomach tubes.

Three guinea pigs inoculated respectively with suspensions of lung tissue, contents of fourth stomach, and meconium were chloroformed after 6 weeks and found normal. Of the spleen cultures two tubes contain spore formers, the rest remain sterile.

Microscopic examination of fixed tissues of fetus presents nothing noteworthy. Sections from various regions of placenta show marked edema of chorion and a zone of cell infiltration and cell necrosis between chorion and amnion. The epithelium of chorion has disappeared and the bared margin is densely infiltrated with cells in localized areas. The cells are evidently necrosed, for the nuclei are pyknotic and of bizarre shapes. Some smaller vessels of chorion are nearly closed by lamellated thrombi. Bacteria not detected in these sections. Fibrils of fibrin abundant in tissue spaces and vessels and mask or simulate the presence of bacteria. On the denuded surface of chorion some thick bacilli, and seen only once, a dense mass of spirilla attached to a projecting shred of tissue. The villi of cotyledons not appreciably altered, but most of epithelium has disappeared.

*Cow 246.*—This cow was purchased September 1917 and aborted in November. This fetus was not obtained. She aborted again during the night of May 27, 1918. Fetus obtained early next morning and refrigerated until May 30.

Fetus still within unbroken membranes. About 20 cm. long. A thick, chocolate-colored fluid within amnion. Skin of fetus easily drawn away exposing partially macerated muscular tissue. Organs in same condition, the liver resembling thick cottage cheese in consistency.

Tubes were inoculated only from liver and lungs. In these pure cultures of spirilla developed.

Two guinea pigs inoculated with a suspension of lungs and liver mixed were chloroformed after 6 weeks. Lesions and spleen cultures negative.

*Cow 251.*—This cow aborted June 10, 1918. Due October 21. The fetus was found in the manure drop in the morning. Placenta retained.

Fetus female, hairless, 44 cm. long, weight 2,510 gm. Some coils of small intestine hanging out of opening about umbilicus from which blood-tinted fluid escapes. Universal blood-tinted edema of subcutis, of the musculature, and of perirenal space. Liver large, tissue discolored, partly macerated. Stomachs



contain a small quantity of a very turbid yellowish fluid containing whitish particles, composed of squamous cell masses. Large intestines contracted, empty. Thorax half full of blood-stained fluid. Air tubes free from aspirated matter.

Of the tubes inoculated pure cultures of spirilla developed from contents of fourth stomach, small intestine, and lungs. Lung tubes also contained heavy growths in condensation water.

Two guinea pigs were inoculated with suspension of lung tissue and contents of fourth stomach respectively. Chloroformed after 6 weeks, they were found normal and spleen cultures remained sterile.

*Cow 256.*—Cow purchased in 1914. Calved normally in 1915, 1916, and 1917. Aborted June 17, 1918. Due October 23. Placenta retained.

Fetus, male, length 47 cm., weight 3,925 gm. Hairless. General slight subcutaneous edema, blood-tinted. Large bleb on back containing 95 cc. of blood-stained fluid. 240 cc. of bloody fluid in abdomen and about 50 cc. in pleural sacs. Stomachs contain a thick turbid yellowish fluid. Spirilla detected in films (Fig. 5). Nothing noteworthy about remaining viscera.

Tubes inoculated with bits of tissue and fluids yielded the following results. Two tubes with stomach contents developed mixed cultures of spirilla and other bacteria. Two spleen tubes developed into pure cultures of spirilla. The same is true of two kidney, one lung, and two liver tubes. One other lung tube had a very heavy growth.

Two guinea pigs inoculated, one with stomach contents, the other with lung tissue, were kept 5½ weeks, then chloroformed. They were normal and the spleen cultures remained free from growth.

Sections of fixed and hardened tissues show the presence of numerous short spirilla in four different regions of small intestines. They were limited to the contents.

*Cow 258.*—Cow purchased August, 1917. Calved in same year. Aborted July 1, 1918. Due September 24. Placenta retained.

Fetus hairless, male, length 57 cm., weight 22 pounds. Abdomen prominent. Testicles swollen, edematous. Subcutaneous edema very slight. In abdomen about 500 cc. of a turbid light reddish fluid, depositing on staining a white sediment made up of leucocytes. Covering liver and adjacent organs is a heavy elastic whitish pseudomembrane, spongy, 1 to 2 mm. thick on liver, and easily stripped off. It contains many cells, appearing as mononuclears in films. Stomachs contain a very viscid fluid, suspending yellowish brown flakes and masses of squamous cells. Liver rather large, yellowish, friable.

Small amount of blood-tinted fluid in pleural sacs. Scattering light yellowish foci in lungs, 2 to 3 mm. in diameter, air-containing. The rest airless. Pericardial cavity contains a turbid, nearly colorless fluid. Epicardium covered with a thin, whitish roughened exudate, giving the heart a furry appearance. Films show spirilla in contents of fourth stomach and rectum.

Tubes of agar inoculated with bits of organs and with fluids from stomachs, etc., gave the following results. Two tubes from fourth stomach and one from

lungs heavily overgrown. Two liver, one lung, two spleen, two kidney, and two meconium tubes develop into pure cultures of spirilla.

Four guinea pigs inoculated, two with meconium and two with lung tissue, were kept 6 weeks and then chloroformed. All were normal and spleen cultures remained free from growth.

In sections of the various organs no noteworthy changes. Spirilla not detected.

*Cow 263.*—Cow purchased in September 1915. Calved in 1916 and 1917. Aborted July 30, 1918. Due November 18.

Fetus 51 cm. long, hairless except on lips and chin. Cord remains about 12 cm. long, swollen, edematous. A piece of meconium 5 cm. long protrudes from rectum.

Abdomen contains blood-tinted fluid. Liver slightly softened. Under capsule of kidneys a thin layer of a thick bloody fluid.

Mouth contains a little yellowish, flaky material. Stomachs normally distended with a viscid fluid suspending soft yellowish granules and whitish flakes representing cellular excrescences of amnion. Films from contents show some short and long spirilla.

Thorax partly filled with a bloody fluid. Trachea contains fluid like that in stomachs.

Cultures from different organs give the following results. Two spleen and two kidney tubes remain sterile. Two stomach, two liver, three lung, and two meconium tubes develop into pure cultures of spirilla.

Three guinea pigs inoculated respectively with contents of fourth stomach, meconium, and lung tissue kept 6 weeks were free from lesions when chloroformed. Cultures from spleens negative.

*Cow 267.*—Cow purchased July, 1916. Calved 1916 and 1917. Aborted August 12, 1918. Due November 23. Placenta retained.

Fetus, male, hairless, 59 cm. long, weight 6,618 gm. Marked enlargement of abdomen. General subcutaneous edema causing swollen appearance of limbs. Testicles swollen, edematous.

Abdomen contains 650 to 700 cc. of a turbid reddish fluid. White, elastic pseudomembranes cover liver almost entirely and extend over omentum and mesenteries. Easily pulled away.

Stomachs contain a thick, turbid, not very viscid, yellowish fluid. Films show presence of spirilla. Large intestine well filled with meconium. Spirilla present.

Kidneys contain about a dozen cysts, 2 or 3 mm. in diameter, at base of medulla. Spleen with pulp rather soft.

Pleural sacs contain about 50 to 75 cc. of a deeply blood-stained fluid. Minute shreds of whitish exudate sprinkled over all surfaces of the lungs. The air tubes contain a yellowish mucoid fluid like that in stomachs. Spirilla present (Fig. 6).

Cultures of various tissues show the following results. Two lung, two kidney, two spleen, two liver, two fourth stomach, and two meconium tubes develop into pure cultures of spirilla.

Three guinea pigs inoculated respectively with contents of fourth stomach, lung tissue, and meconium and kept over 5 weeks remained well. When they were chloroformed no lesions were found and spleen cultures remained sterile.

## EXPLANATION OF PLATES.

All films are stained in alkaline methylene blue.

## PLATE 81.

FIG. 1. Section of small intestine, Fetus 256. The figure includes only the contents made up of desquamated cells and three spirilla in focus. Many others, not in view, are present in the cell mass. Eosin-methylene blue.  $\times 1,000$ .

FIG. 2. Film from contents of trachea of Fetus 267, containing large numbers of long spirilla, brought out by prolonged staining (48 hours). The material consists of regurgitated and aspirated stomach contents.  $\times 1,000$ .

FIG. 3. Fresh agar culture of spirilla from Fetus 179. Eleventh transfer.  $\times 1,000$ .

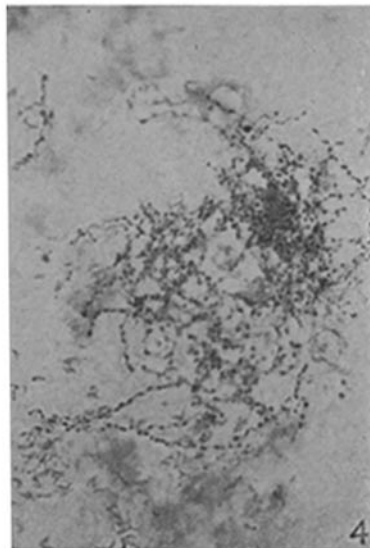
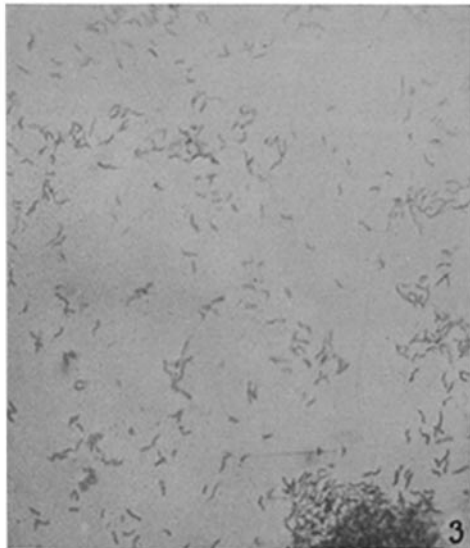
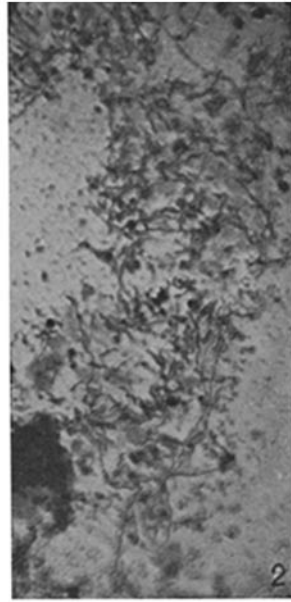
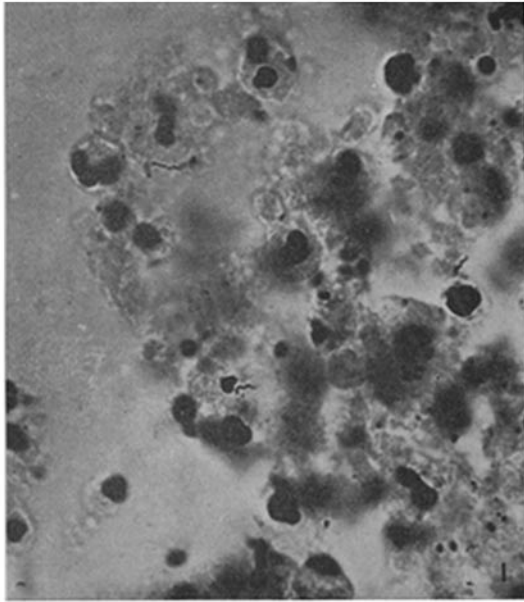
FIG. 4. Old agar culture of spirilla from Fetus 149. Fourteenth transfer. The spirilla are long and beset with deeply stained coccus-like bodies.  $\times 1,000$ .

## PLATE 82.

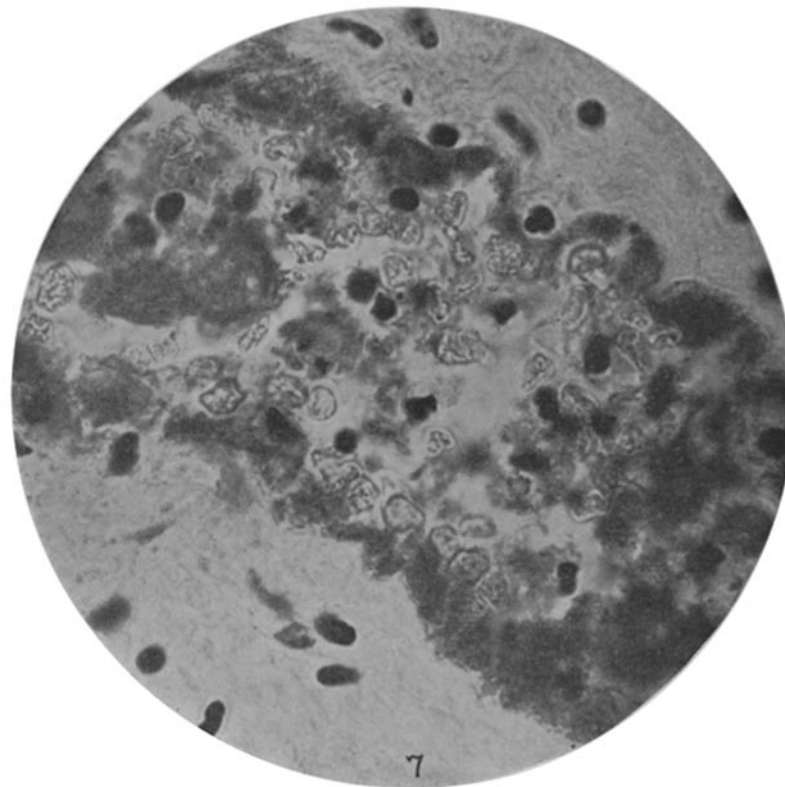
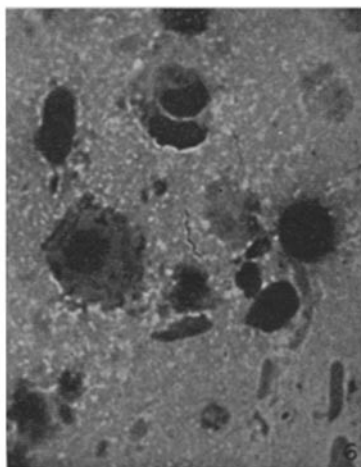
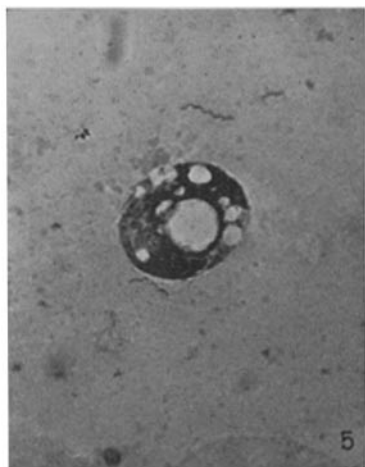
FIG. 5. Stained film of contents of rumen, Fetus 256, four spirilla and one degenerated cell in view.  $\times 1,000$ .

FIG. 6. Two spirilla in film from air tubes of Fetus 267.  $\times 1,000$ .

FIG. 7. Transverse section of chorion of Fetus 90, showing the endothelial cells of a blood vessel packed with bacteria which in thin spots have been identified as short spirilla. Cells within lumen of vessel are densely packed with them. Eosin-methylene blue.  $\times 1,000$ .



(Smith: Spirilla in infectious abortion of cattle.)



(Smith: Spirilla in infectious abortion of cattle.)