A CONTRIBUTION TO THE PATHOGENESIS OF B. ABORTUS, BANG. — II.*

MARSHAL FABYAN, M.D.

(*From the Laboratory of Comparative Pathology, Harvard University Medical School, Boston, Mass.*)

HISTORICAL REVIEW.

That abortion in cattle was contagious in many instances, and might spread from animal to animal in a herd, was known to farmers and breeders in the early part of the nineteenth century. The veterinarians did not, however, at first accept this view. It was considered by many the most fatal disease, after tuberculosis, to which cattle were susceptible.

In 1826 Hutrel d'Arboval, a Frenchman, and in 1834 Youatt, an Englishman,[1] came to the conclusion that abortion often was contagious and not due to environment or accidents. Their work was confirmed by many investigators.

In 1878 Lehnert[2] was able to demonstrate this contagiousness by experiment. He caused abortion by introducing into the vagina of pregnant cows the vaginal discharge and placental tissue of aborted cases. In 1880 this was confirmed by Brauer[3] and others.

In 1886 Nocard[4] made extensive bacteriological investigations, studying the fetus and membranes of abortion cases in the hope of isolating the definite etiological factor. He was able to obtain a bacillus and a micrococcus in pure culture, but in neither of these was he able to produce abortion.

In 1894 Sand[5] also asserted the infectious character of the disease and gave various clinical data.

In 1895 Bang and Stribolt[6] were able to demonstrate the at present accepted etiological organism. They obtained a cow with all the symptoms of impending abortion and, having slaughtered her, removed the unopened uterus to the laboratory. This was opened with all aseptic precautions

* For the first paper see Centralbl. f. Bakt., 1 Abt. Orig. 61, 1912, pp. 549–555.

Received for publication April 30, 1912.

and an abundant yellowish, odorless exudate was found between the uterine mucosa and the chorion. This exudate contained numerous very small bacilli, apparently in pure culture. These were found singly, and in clumps; free, and often intracellular.

They were able to grow this organism in a solid medium composed of equal parts of .75 per cent agar, five per cent gelatine and sterile serum. The last was added after the agar had been liquefied and cooled to 45° C. The still liquid medium was then inoculated with the material, gently agitated, and placed in cold water to allow rapid solidification (this medium will be referred to as A. G. S.). At the end of two to four days' incubation at 37° C. they found that a zone of growth had developed, composed of very small colonies, the largest the size of a pinhead. This zone lay one-half centimeter below the surface of the medium and was of a thickness of one to one and one-half centimeters. If inoculated tubes, similar to the above, were placed in an atmosphere of oxygen, two zones of growth developed, one near the surface of the medium, the other close to the bottom. The bacillus might also be made to grow on the slanted surface by the introduction of oxygen. It would not develop in the ordinary atmosphere nor in the absence of oxygen as produced with alkaline pyrogallol. No growth followed incubating under the influence of carbon dioxide or nitrogen. Exhausting the air over the medium caused extension of the growth to the surface and this method was employed in making plate cultures but was not reliable. The organism might grow in serum glycerine bouillon. They concluded that the bacillus was neither an aërobe nor an anaërobe, but lay in an intermediate group requiring a pressure of oxygen less than air. By their method they were able to isolate B. abortus in a number of cases from the placenta. Abortion was produced with these isolated cultures by injecting healthy pregnant animals in various ways. The intravenous route was apparently the most certain.

In 1901 Preisz[7] at Budapest isolated a similar organism from the vaginal discharge of a case of abortion. He made

his inoculations directly on slanted ordinary agar made of meat infusion, peptone and salt, and after passing oxygen into these tubes he sealed them with wax and obtained a growth visible with a hand lens in three days. Inoculated into deep dextrose agar, the zonal growth appeared lying seven to fifteen millimeters beneath the surface of the medium. Stab cultures of the organism rarely reached the surface after prolonged incubation. He considered that his medium was as favorable as the A. G. S. of Bang. He was able, by using alkaline pyrogallol, to produce a growth and also by using acetylene gas. He believed that in these two instances growth resulted because oxygen was not wholly absent. He compared the organism to an anaërobe, but B. abortus differed in that it would also grow in pure oxygen. Small pregnant animals were injected according to Bang's method, but without positive results, and he concluded that his inoculations were made too early or his cultures had been grown too long artificially. He made note of the fact that no local lesion occurred from subcutaneous inoculation.

In 1908 Nowak,[8] at the University of Krakau in Austria, took up the study of this organism, being attracted by its very interesting biological characteristics. He stated that its behavior towards oxygen was not without example, as was demonstrated by the work of Righi and Beijerinck. In studying pure material he found the method of Bang satisfactory, but where contaminations were present the growth might easily be killed out or masked. The broad surface which Petri dishes afforded was desirable to obtain isolated colonies. Preisz, by the use of alkaline pyrogallol, had been able to obtain growth on plates, but it was impossible to tell how much absorption had occurred and his results were uncertain. Nowak therefore sought to obtain an oxygen tension less than air by the use of a closed chamber containing the actively growing culture of an organism like B. subtilis, a method which had already been employed in the removal of oxygen in tetanus cultures. He placed tubes inoculated, some with B. subtilis and others with B. abortus, in a glass chamber closed with paraffine and placed this in

the incubator. Satisfactory results were obtained when the relation of the surface of B. subtilis was in proper proportion to the volume of the chamber. If too little of B. subtilis was present, no growth resulted; if too much, all the oxygen was absorbed and B. abortus checked. He found one square centimeter of surface growth of B. subtilis to fifteen cubic centimeters of volume the best proportion. Different varieties of B. subtilis gave similar results. The tubes inoculated with suspected abortion material were first incubated twenty-four hours in the large chamber of the incubator in order to develop any contaminations, and then incubated under the influence of B. subtilis, when typical colonies developed in the clear areas. He tested the purity of his cultures by the zonal growth of Bang. By gradually using less of B. subtilis, he trained B. abortus to grow in a normal atmosphere. In pure oxygen two out of six cultures grew. Under compression he obtained good results from three atmospheres but no growth at six atmospheres, although when this latter tube was placed under the influence of B. subtilis colonies rapidly developed. By his technic he was able to obtain many cultures from the fetus and vaginal discharge where other methods failed. He experimented only on small animals, and produced abortion with his cultures by the subcutaneous, intravenous, and intra-abdominal routes, but not by the vagina or by feeding.

In 1909 McFadyean and Stockman investigated contagious abortion in Great Britain and were able to isolate an organism similar to the one obtained by Bang in Denmark. They worked almost exclusively with the A. G. S. medium, but observed such departures from the ordinary cultural characteristics of B. abortus that they were obliged to test their cultures on animals to be sure that they were dealing with the same organism. Even the sub-surface growth in deep medium described by Bang as specific for B. abortus could not be relied on, for in one instance the tubercle bacillus grew in a like manner.

They considered B. abortus a strict aërobe, demonstrating that anaërobic conditions proved fatal to it. The organism

might develop in an atmosphere of coal gas, but this was explained by the fact that all the oxygen was not replaced. That the B. abortus does absorb oxygen was evident from the lessened internal pressure in the inoculated sealed flasks after prolonged incubation and this might destroy the flask. They, as well as Nowak, appreciated the fact that many of their tubes remained sterile although known to be inoculated with B. abortus. At times only a few of many tubes inoculated and incubated alike developed colonies. They produced abortion experimentally by injecting the exudate from cases studied and also the cultures isolated. The latter caused abortion more constantly. The injections were made subcutaneously and intravenously as well as by mouth and vagina. It is an interesting fact, in view of our work, that they inoculated each of two guinea-pigs with two cubic centimeters of emulsion of an exudate, one into the peritoneum, the other subcutaneously. The exudate was known to contain B. abortus both from cultural tests and further animal experimentation. However, "both guinea-pigs remained well for two months when they were killed and examined. No lesions of any kind were found." They therefore concluded that the exudate contained nothing pathogenic for non-pregnant guinea-pigs.

In 1910 Zwick [10] issued a preliminary report on the bacteriological investigation of contagious abortion made by the German Imperial Health Office. It was demonstrated that the Bang bacillus was the etiological factor in causing this disease in England, Germany, Holland, and Denmark. A series of cultures showed individual differences among different strains, but the organism could be grown readily on various ordinary laboratory media and they confirmed Nowak's observation that the organism could be made to grow readily in the ordinary atmosphere. In fact, in one of their cases the organism was grown aërobically from the animal body. They have met with success in causing abortion by inoculations.

In this country the disease has been known for some time

and has been studied in the Agricultural Experiment Stations of Kansas, Arizona, Connecticut, Illinois, and Wisconsin. Investigators in America had been unable to confirm Bang's researches, and in 1910 MacNeal and Kerr [11] studied eighteen cases, ten normal deliveries, and eight premature, of which six might be called, clinically, contagious abortion. Of these, two were cultured by Nowak's method with positive results.

More recently Larson [12] confirmed the presence of B. abortus, Bang, in this country, by complement-deviation tests made at the Wisconsin Agricultural Experiment Station. Five herds showed the presence of this organism. (Within a few weeks Larson has reported some interesting results from applying the complement-deviation tests to human serum.)

In 1911 Holth [1] described his method for isolating and cultivating B. abortus. Where contaminations were present he removed conditions favorable for their growth by using oxygen under tension in a sugar-free serum agar. He considered the method of Nowak as beset with more difficulties than sealing the tubes after the injection of oxygen, and described an apparatus for performing this. On suitable media the growth may at first be slow and poor with only a few organisms developing, but they may be readily educated to grow profusely. He demonstrated that material inoculated directly from the fetus to slanted serum agar gave no growth in two weeks' incubation, but if similar tubes were sealed with paraffine, growth might sometimes be observed after six days. The explanation offered was that the organisms by using the oxygen over and over again reduced it to the proper tension.

Bang considered the disease a uterine catarrh, the exudate being derived from the mucous membrane of the uterus. McFadyean and Stockman in their cases of abortion, produced experimentally and otherwise, described the cotyledons as soft, pulpy and of a gray, yellowish, anemic color. At times the exudate seemed to lie chiefly about

them, but in one instance, although the exudate was marked, the cotyledons appeared fairly normal. The membranes were extensively or only slightly involved. Once a square inch only, near the os, was diseased. Wall[13] has demonstrated that the uterine tissue first involved in this disease varies according to the entrance of infection. When it is by the vagina the cotyledons become the primary seat of the disease; when by way of the blood the mucous membrane of the uterus is first involved.

Bang noted the appearance of immunity after the first or second abortion.

Agglutination did not take place in normal serum in a dilution of one to twenty-five (McFadyean and Stockman) nor one to twenty (Zwick). In the diseased animals agglutination was obtained in a dilution of one to five hundred, one hundred and twenty-four days before term according to McFadyean and Stockman, and even from one to one hundred to one to ten thousand, according to Zwick. Gronsted,[14] working with Bang, was able to immunize rabbits until their serum had an agglutinative power of one to six hundred. He found that the serum of cows suffering from this disease had an agglutinative power from one to twelve hundred to one to six thousand seven hundred, occurring even two months before term. The technic of complement-deviation has also been developed and proves more delicate than the tests by agglutination.

McFadyean and Stockman, bethinking themselves of the mallein and tuberculin reactions, proceeded to make a similar filtrate which they called "abortin." They injected ten cubic centimeters subcutaneously and if at the eighth hour the animal's temperature was 104° F. or more, the test was considered positive. Zwick stated that his results in this line seemed encouraging, but to date were too limited to be of importance. Immunizing has been fairly successful. Hesse[15] has been able to obtain some favorable results by injecting the bacterial extract.

THE INOCULATION DISEASE IN GUINEA-PIGS.

In December, 1909, we received material from a valuable herd of dairy cattle. Abortion had occurred in two or three instances and material was sent to the laboratory in the hope that means of prevention might be found. A bit of cotyledon of the placenta of Cow 218 (number given the animal in this laboratory) was shaken in normal salt solution and one-half cubic centimeter of this fluid was injected subcutaneously into a guinea-pig. The animal to all appearances remained in perfect condition and, having gained forty per cent in weight, was killed eleven weeks later to furnish sterile tissue for some anaërobic culture media. It was then found that the spleen was abnormally large as were also the lymph nodes. The case was therefore investigated with some detail and besides cultures other guinea-pigs were inoculated with its tissues. Although the cultures on various media remained sterile, the guinea-pigs in every instance developed marked enlargement of the spleen and lymph nodes. The virus was thus kept alive by passage through guinea-pigs. All attempts to grow the etiological factor outside of the body failed, but in one instance a bit of spleen transferred to a fermentation tube of ordinary bouillon caused clouding of the media when incubated. A fairly large anaërobic bacillus was demonstrated, evidently a contamination. In fragments of the inoculated tissue, groups of a minute bacterium were seen, but in later transfers this organism could not be found. After four transfers extending over a period of eleven weeks and again after four more transfers, making a total period of twenty-seven weeks from the original culture, one cubic centimeter of this fluid injected into a guinea-pig caused typical lesions.

It became of interest to know whether we were dealing with an ultramicroscopic virus and filtrates from shaken suspensions of infected organs were injected, but no disease developed.

At this time the work of MacNeal and Kerr[11] came to our notice and a culture of B. abortus was obtained from them

in order to determine if the etiological factor of this guinea-pig disease was related to it. Agglutination tests showed no clumping with normal guinea-pig serum, even in a low dilution of one to ten, but in the serum of a diseased pig the clumping was marked in dilutions up to one to one thousand. MacNeal's Culture I. (our laboratory number for this culture) was injected into guinea-pigs and caused the same characteristic lesions already noted. About this time pure cultures of a similar organism were obtained from both the spleen and the liver of two of the original series (Culture II. a and b). One of these animals had been inoculated with the contaminated culture, the other directly from the source of that culture. Further agglutination tests of these organisms thus isolated confirmed our previous results, for the clumping was complete in the serum of the animal inoculated with B. abortus I. in a one to one thousand and slightly in one to fifty thousand dilution. Thus we were dealing in all probability with B. abortus. We will now go somewhat more into detail concerning the material studied.

Cow No. 218. — This cow aborted at the eighth month and portions of the placenta were not received at the laboratory until six days afterwards. An odor of creolin or some other disinfectant was perceptible. Several of the cotyledons had a yellowish appearance but were still firm. Others were much softened. Smears showed the presence of three or more species of bacteria. The cells were very fatty. From this case Cultures II. a and II. b were obtained.

Cow No. 232. — The placenta in this case came from an abortion at seven months and was only forty-eight hours old. It was the second case which had occurred in a herd in a week. The veterinarian was uncertain whether to call this "Contagious Abortion" or not, as the cow gave birth to three calves (triplets), the first abortion having occurred at three and one-half months. The cotyledons were soft, yellowish, and easily scraped to pieces. Smears showed the presence of a fungus, numbers of minute coccus-like organisms, and some large bacilli. B. coli and a streptococcus were isolated by culture. To avoid confusion it might be said that B. subtilis was not employed in cultures from this case, and guinea-pigs inoculated with the tissue gave no characteristic lesions. We considered this abortion not due to B. abortus.

Cow No. 233. — Abortion occurred at the seventh month. The material consisted of a considerable part of the placenta and a small amount of turbid fluid. The odor suggested certain kinds of cystitis, but there was

no putrefaction. Smears from the cotyledon showed several groups of minute bacilli after much search. No other bacteria were seen. Culture III. came from this case.

Cow No. 235. — The placenta came from a six months' abortion. The tissue presented a very edematous, gelatinous appearance and contained about fifteen cotyledons; odor, inoffensive. The cotyledons varied in size and in the amount of injection and infiltration of the margins. The central portion of the cotyledon presented a yellow fringe of villi, but in some this had disappeared and there was a punched-out ulcer with a "shorn beard" base. Two or three cotyledons were markedly injected. Between these numerous superficial ulcers one centimeter in diameter appeared, slightly opaque and having a yellow granular appearance. Smears showed a few minute organisms and a bacillus present. From this case Culture V. was isolated.

Cow No. 234. — This specimen was the vaginal discharge following an abortion at eight months, and consisted of tenacious mucus of brownish hue containing débris such as straw and hair. Few organisms could be seen in smears but a staphylococcus was isolated. From this case Culture VI. was obtained.

In addition to these cultures there were studied a culture obtained from Professor W. J. MacNeal, of the University of Illinois, growing on a tube of blood agar which was designated Culture I., and a culture from Professor Bang of Copenhagen, Denmark, in capillary tubes of serum bouillon and also on serum agar. This was designated Culture IV.

The material to be inoculated was prepared in the following manner: In the case of a placenta one or two cotyledons were removed and washed thoroughly in normal salt solution, after which they were placed in sterile Petri dishes. With a sterile knife the yellow villi and basement membrane were scraped off and added to a small amount of salt solution. This suspension was shaken and was then ready for inoculation. The vaginal discharge was diluted by adding a small bit to salt solution, and was then well shaken. In the case of infected guinea-pigs, pieces of the spleen and liver and sometimes the kidney and lymph nodes were removed aseptically and ground with salt solution in an agate mortar or Latapie machine, and injected. Filtrates were obtained from the ground tissues by shaking the suspension for one hour and then filtering it through a Berkefeld filter.

With tissue suspensions one-half to five cubic centimeters were injected and gave positive results in practically every instance (four exceptions mentioned later). Of course the

actual number of B. abortus injected was very uncertain as the prevalence of the organism in the tissue was not known. If contamination with other bacteria was suspected, smaller amounts more diluted were injected in the hope that the animal's resistance would dispose of the few extraneous organisms inoculated.

With cultures the amounts could be better standardized. One cubic centimeter of a twenty-four to forty-eight-hour bouillon culture was usually injected. In one instance one-tenth cubic centimeter of a forty-eight-hour culture gave a typical pathological picture in ten weeks. Two cubic centimeters have also been injected. In a few cases a concentrated suspension of a culture was prepared for injection by scraping off the growth from a forty-eight-hour agar slant with a platinum wire and adding two and one-half to five cubic centimeters salt solution. Within certain limits the larger the dose the more marked the lesions. In our tissue suspensions a minute amount caused hardly any lesions, while five times that amount resulted in a marked disease. Naturally the injections with cultures were more uniform. However, after a period of eight to ten weeks the lesions were similar whether one or two cubic centimeters of a twenty-four-hour culture had been injected.

Most of our inoculations have been intra-abdominal (70 per cent), but the subcutaneous route has been used in many instances (25 per cent). Apparently the latter is slightly more severe and the initial loss of weight is greater. Intra-thoracic injections (4 per cent) were made to study lesions occurring especially in the lungs. The development and course of the disease is not influenced by the site of inoculation. Suspensions of cultures introduced by mouth have also caused lesions.

Signs and symptoms. — In a few instances a local lesion developed from a subcutaneous inoculation in which the amount of culture injected was very large and once or twice from a direct injection of cotyledon tissue. In these cases an induration appeared, sometimes extending over an

area two or more centimeters square. This later came to a head and discharged, or on section presented a yellow, granular, creamy mass consisting chiefly of mononuclear cells. If an ulcer did not form the mass usually became partially absorbed, but never much incapsulated. Ordinarily injections with pure cultures subcutaneously have not been followed by any local lesion except for a small swelling lasting a few days.

The animal may lose five to thirty and even one hundred grams in the first week — the larger the animal the greater the initial loss of weight. If the dose has not been too large this loss is slowly made good and the increase thereafter is normal or somewhat retarded. A considerable portion (72 per cent) of our animals showed some loss from their best weight when they were killed. This was generally a question of ten to fifty grams, — in one case four hundred and forty in an animal weighing seven hundred grams when inoculated. She died after thirty-six weeks.

A few days after inoculation the temperature rises usually about a degree, and continues uninterruptedly for twelve to twenty weeks or more, and then subsides. Much handling tends to increase the temperature one or two degrees. An animal which has a temperature of 103° or 104° F., by being handled may run a temperature of 105° to 106° F. Near death it becomes subnormal.

As a rule the animal presents no characteristic appearance. Three of our cases suffered from blindness. Swellings in the extremities especially about the carpal joints were noted. Weakness in the posterior limbs developed, associated with constipation and soiling of the parts. Recovery usually followed. These signs in one or two instances might be attributed in part to involvement of the joints or enlargement of the adjacent lymph nodes interfering with the movements of the limbs. In one case already referred to as losing so much weight, an inguinal lymph node became two centimeters in diameter and very firm, and the posterior limb was held abducted ten to twenty

degrees. After several weeks this swelling entirely disappeared. There was one case of paraplegia. The various lesions are apparently painless.

As regards the course of the disease the animals divide themselves into three groups. Some run a gradually downward course especially after the injection of a large dose, become much emaciated and die. The larger animals hold their weight relatively better. With an ordinary dose, such as one cubic centimeter of a twenty-four to forty-eight-hour bouillon culture, the animal usually gains, but often slowly. In other cases the normal physiological increase in weight continues. Some of our animals lived months and were then disposed of in good condition. At present we have one in apparently good health which was inoculated with virulent material sixteen months ago.

Undoubtedly certain guinea-pigs have more resistance to B. abortus than others. In our experiments four animals which were injected with suspensions of infected tissues had comparatively slight lesions at autopsy, and the organism could not be isolated from their tissues, although further inoculations from two of the four cases produced characteristic lesions.

Of the fifty-eight animals considered, twelve died. Death in two was due to rupture of the spleen; in four, to subsequent injection of toxins of the abortion bacillus, and in the remaining six it followed emaciation and exhaustion, kidney disease being extensive in two and pneumonia in one. It might be said, therefore, that only in five cases (8 per cent) did death occur as the uncomplicated result of inoculation with B. abortus.

Gross pathological changes. — These refer to animals, fifty-eight in number, inoculated with the original material, tissues of infected pigs, and cultures and which lived at least six to eight weeks.

The external appearance of the body may be normal or markedly emaciated. The opacity of the cornea of one or

both eyes and the swelling of the extremities have been already mentioned.

On reflecting the skin over the ventral surface there was usually fat tissue in slight amount. In large animals it was marked in some cases. In the emaciated every particle may have disappeared, exposing most beautifully the minute structures, such as nerves and vessels.

The lymph nodes were enlarged in fifty-six cases, or ninety-five per cent. The enlargement was chiefly universal, but occasionally some groups of nodes were more distinctly enlarged than others. The superficial lymph nodes, as a rule, were always larger than the rest; sometimes those in the inguinal region, sometimes those in the axillary or the cervical region were the most conspicuous. Of the internal nodes the retroperitoneal were always conspicuous and in some cases markedly enlarged. The nodes were perhaps more readily visible on account of the diminution in the fat tissue. They were frequently one centimeter in length, two or three millimeters in cross-section, and weighed .15–.20 gram. The color was a delicate, translucent pink. On section no definite structure could be made out. The cut surface was moist and succulent. In a few instances slightly opaque areas were suggested, focal in character, or extending along the periphery, and undefined extraneous blackish pigment was occasionally present. The mesenteric lymph nodes were enlarged and presented the characteristics already described. Those along the spinal column were of a much lighter color than the others.

Even before the abdominal cavity was opened in many instances the much enlarged spleen could be readily detected, occasionally extending well toward the pelvis. In a few cases it could not be seen until the organs were manipulated, being bound down by adhesions of a firm character. These were to the abdominal wall, liver, stomach, pancreas, and in two cases to the kidney. These adhesions occurred only in cases injected intraperitoneally.

The organ was enlarged in fifty-seven cases, or ninety-eight per cent. The one case in which the spleen was

normal in size lived thirty weeks before being chloroformed and showed other lesions of the disease. Enlargement was seen as early as the second week. In general, the spleen was of normal shape, averaging about 4.5 centimeters in length, 2.5 centimeters in width, and 4.6 millimeters in thickness. Instead of a normal weight of .4–.7 gram it averaged 2–4 grams, — once 9.9 grams. In many the distention of the capsule was conspicuous, the organ fairly bursting; in fact, in two instances, once while the cage was being cleaned out and once when the animal was being weighed, death resulted in a few minutes as a result of rupture. The surface of the organ may be normal or present numerous fine, grayish, pin-point elevations. Rarely there was an irregular cauliflower appearance as if from some internal new growth. This appearance was undoubtedly masked now and then by the great distention of the organ. Occasionally a few or numerous grayish, opaque, pin-head foci could be distinguished just beneath the capsule. The color was darker than normal. On section, the surface was very moist; the pulp usually swollen, soft, obliterating in part the normal markings. Seldom were distinct foci seen. When the disease became chronic the spleen appeared only moderately enlarged and rather flabby.

The liver was diseased in forty-three cases, or seventy-five per cent. In two or three instances there were adhesions to the surrounding tissues, again following intraperitoneal injection. The size of the organ did not seem to be definitely affected by the disease, and in chronic cases it may be small, although occasionally it had a tense, congested appearance similar to the spleen. Scattered over the surface were few or many grayish, translucent, pin-point foci just visible, or two or three millimeters in diameter extending into the substance of the organ. When newly formed these foci had a glistening, pearly appearance and later assumed a yellowish, opaque color. Rarely they had an opaque center and translucent periphery. The surface might also present a yellowish tracery or even distinct scarring. The gall-bladder

appeared normal. The hepatic lymph node was sometimes conspicuously enlarged.

The pancreas showed no marked changes. The islands of Langerhans were often quite conspicuous as yellow opaque dots of irregular size.

The kidneys were diseased in seventeen, or twenty-nine per cent of the cases. Of these, two had been inoculated with Culture I., twelve with Culture II., one with Culture V., and two with Culture VI. It is interesting that five of the twelve cases were injected with tissue from one guinea-pig. The inoculations were by both the intraabdominal and subcutaneous routes, and even with as small a dose as one-half cubic centimeter of a twenty-four-hour bouillon culture. Lesions were noted as early as the sixth week. The size of the organ varied only slightly from the normal as a rule. On stripping the capsule in a few instances definite round, grayish foci, similar to those in the liver, were noted. These protruded slightly, and were of a white color. In other cases they were much more indefinite and diffuse. On section there was rarely any radiating from these foci toward the medulla. Other round foci were seen in the substance of the cortex. In one instance both kidneys were distinctly enlarged, colorless, and apparently very diffusely and completely diseased.

No macroscropic lesions were found in the adrenals.

In only one instance was any definite lesion seen in the female genitals. In this case, at a point on the left horn about a centimeter above the bifurcation, there was a distinct nodule one centimeter in diameter bulging into the peritoneal cavity, with a surface closely resembling the cauliflower appearance seen on the spleen. The ovaries are not affected in our cases.

The testicles showed changes in ten, or forty per cent of the males injected. Five of these animals were inoculated subcutaneously and five intraabdominally and even one-half cubic centimeter of a twenty-four-hour bouillon culture caused lesions, the earliest found, occurring nine weeks after inoculation. All the strains caused the disease except No. V.

The testicles were adherent to the sac in three cases, all intra-abdominal inoculations, and associated with other abdominal adhesions. The testis proper was fibrous in two cases. The epididymis was involved in eight cases; the right in three, the left in three, and both in two cases. On removing the testicle from its sac, the epididymis when diseased was distinctly enlarged and presented one or more yellowish areas which on section allowed soft, yellow, granular material to escape. This opaque material was seen twice, in part of the seminal vesicles of the affected side.

In the lungs lesions were noted in thirty-eight, or sixty-five per cent of the cases. These did not involve the pleura but lay just beneath. They might take the form of pin-point, translucent, grayish dots more or less thickly sown, and resembling colonies of a pure culture, or a tracery of irregular shape, or distinct solid areas often contracted and depressed, involving a third of a lobe. On section the surface was fairly normal, disclosing firm, irregular, grayish areas only in a few cases. In one case the pleura was affected secondarily from disease of a rib.

The bones were involved in sixteen cases (27 per cent). Five of these had been inoculated subcutaneously and with only one-half cubic centimeter of a twenty-four-hour bouillon culture in two instances. Slight lesions developed in the ribs in one case after three weeks, in the extremities in another after five weeks.

The vertebræ were involved in two cases. The lesion in one of these occurred at the junction of the lumbar and sacral portion without causing any apparent deformity, and was discovered on investigating a yellow discoloration beneath the dura of the spinal canal. On section of the bone a small amount of yellow pus oozed forth. The other involvement was in the body of the sixth dorsal which bulged three to four millimeters into the thoracic cavity. Upon section a small amount of yellow, granular material was contained behind a shell of bone.

The disease of the ribs was most interesting. It was caused by one strain (No. II.) in seven of the eight cases,

and, as in the disease of the kidney, several were the result of inoculations from one infected guinea-pig. Strain No. 5 caused the eighth case. There were from one to five ribs attacked in any one case. These might be on the same side, adjacent or not, or on opposite sides. As a rule they lay between the fourth and seventh ribs. The disease was characterized by swelling which seemed to have its origin at the epiphysis adjoining the sternal cartilage. From this, extension and enlargement of the rib occurred, reaching in some cases to the vertebral column, but only once involving it. Different stages might be seen in the same animal (Plate XXI., Fig. 1). The swelling at the cartilaginous extremity, usually pink, was sometimes yellowish and occasionally it was on the point of rupturing into the thorax. Similar yellow swellings were occasionally seen along the course of the rib. The diameter of a diseased might be several times that of a normal rib (Plate XXI., Fig. 2). On section the medullary portion appeared larger than the whole of a normal rib. The tissue about the diseased rib seemed to be dense and fibrous; the rib itself less brittle than normally. The marrow instead of having a pink normal color was yellow and quite soft. Involvement of the pleura from a discharging rib was seen once as already mentioned; in another case the lung presented a depressed area at the diseased point.

In ten cases the extremities were involved; the carpal region in eight, the tarsal in one, and the knee in two cases. One animal after fourteen weeks had disease of the vertebræ, ribs, knee, and carpi. The lesions of the extremities appeared as rounded, fusiform swellings, and on removing the skin presented a smooth, pale, translucent, fibrous surface, except in a few instances in which yellow points protruded between the tendons (Plate XXI., Fig. 3). Section through the joint showed that the lesion was chiefly one of the distal portion of the bone and the cartilage, involving the joint only by extension. In one instance of disease of the knee joint the leg was rigidly extended. Section of this joint showed no destructive process but a dense, fibrous thickening about the joint.

Blindness was observed in three cases, one of the left, one of the right, and one of both eyes; all from Strain No. II. The cornea was grayish opaque. The earliest case occurred after thirteen weeks. A few cases of weakness in the posterior limbs associated with constipation and soiling of the parts developed, but usually this was only a temporary condition and the animal recovered. No nerve lesions were seen. The one case of paraplegia developed seven weeks after inoculation with original material and was chloroformed five days later. On exposing the lower cord, the lumbar enlargement seemed swollen. The dorsal median blood vessel was markedly dilated. The remaining portion of the cord and brain seemed normal. No cases have shown brain lesions.

No lesions of the muscles, heart and digestive tract have been seen.

Histological changes. — To avoid repetition it seems best to describe first the cellular elements common to the various lesions. It may be said that the essential changes associated with this disease are of a chronic, inflammatory character, and resemble those of tuberculosis, often to a startling degree, both in character and focal dissemination. The lesions are small, usually microscopic, and occur chiefly in the perivascular areas in most of the viscera of the body.

The focal lesion consists of a group of epithelioid elements, *i.e.*, cells with large vesicular, usually oval nuclei, poor in chromatin. The cytoplasm stains very poorly and is ill-defined. Among these cells may be a few nuclei with large peripheral blocks of chromatin and probably lymphoid cells.

The numerical relation between these two kinds of cells varies from case to case and from organ to organ. Some foci are composed mainly of large cell elements, while the periportal and perivenous cell masses are largely of lymphoid elements. In some foci, plasma and giant cells may be noted. Occasionally polynuclear leucocytes are seen. Mitoses are numerous. In the more chronic cases the tissue

changes have progressed beyond the stages described and signs of organization into connective tissue are present.

In the lymph nodes foci of epithelioid cells may make their appearance as early as the tenth day after inoculation. Later they become more profuse and resemble tuberculous tissue closely. These groups of cells seem to push aside the lymphoid elements which remain to the last at the periphery (Plate XXI., Figs. 4, 5). Occasionally a focus of these cells lies directly under the capsule, bulging it slightly. Extension into the surrounding tissue may occur (Plate XXII., Figs. 1, 2). An occasional giant cell and groups of plasma cells may be seen. The connective tissue hyperplasia becomes very pronounced in the lymph nodes, the fibers forming a network about the nests of epithelioid cells but later occupy the greater part of the node, especially the central portion. Different nodes in the same animal may present different stages of infiltration or organization. Rarely focal necrosis is present.

In the spleen one is attracted by the great dilatation of the blood sinuses which might be expected from the macroscopic appearance. Cellular proliferation is active as may be seen by the frequent mitoses. The epithelioid cells occur in groups as has been described and involve the organ more or less extensively. These may invade the Malpighian bodies or lie just beneath the capsule as in the lymph nodes. Polynuclear leucocytes are occasionally quite numerous. Giant cells are usually rare but may be present in large numbers, the nuclei arranged peripherally. Phagocytic cells may or may not be numerous. Rarely we have found cells containing typical bacilli when stained with aniline-water gentian violet. The connective tissue changes are not as marked as in the nodes.

In the liver characteristic cell foci may be seen scattered diffusely through the tissue, especially at the border of the lobule in close relation to the blood vessels. The subcapsular location is also common in this organ. The foci may be composed of a few cells or occupy an area the size of two or more lobules. The liver cells are pushed aside or become

included and degenerate, the protoplasm staining a diffuse, eosin red. Focal necroses are common in some cases. Polynuclear leucocytes are present, and rarely a few isolated giant cells. Dilatation and proliferation of the bile ducts is noted. The lymphoid cells are in increased numbers in the periportal regions. Connective tissue appears about the larger areas.

In the pancreas the islands of Langerhans may be greatly increased in size, but no focal lesions have been seen. In one or two instances, extension from a neighboring lymph node has caused atrophy and fibrous tissue changes.

In the kidney infiltration of the perivascular space between the cortex and medulla is quite striking, consisting usually of lymphoid elements (Plate XXII., Fig. 3). Scattered through the cortex, and occasionally subcapsular, are more or less definite focal areas composed of groups of epithelioid and lymphoid cells, chiefly the latter. These infiltrate the interstitial tissue, compressing and destroying the convoluted tubules and glomeruli (Plate XXII., Fig. 4). The process may be very general and diffuse. Proliferation of both types of cell has been seen in the papillary extremity of the medulla. Mitosis is present to a moderate degree. Subcortical depressions may be present, due to foci which contain connective tissue elements.

A few small foci have been noted in the cortex of the adrenal, just beneath the capsule.

Sections of the uterus from the case described macroscopically showed infiltration of the muscle with typical epithelioid cells. These force their way between the fibers and form large nests of cells, the central portion being prone to degeneration and necrosis (Plate XXII., Fig. 5). Another case of uterine involvement was detected in sections only. No foci were seen in the ovaries.

Extensive proliferation of epithelioid cells and infiltration with lymphocytes have been seen within the stroma of the testicle and epididymis (Plate XXIII., Fig. 1); sometimes one element predominating, sometimes the other. Connective tissue may replace the secreting tubules in great part.

Occasionally necrotic foci are seen composed of dense nuclear débris, the tubules containing masses of polynuclear leucocytes.

Infiltration of lymphoid cells about the blood vessels of the heart muscle has been seen (Plate XXIII., Fig. 2).

Scattered through the parenchyma of the lungs are irregular groups of epithelioid and lymphoid cells. These may lie just beneath the pleura or about a blood vessel or bronchus. The infiltration occurs in the interstitial tissue causing a small tubercle-like focus. It presents a central portion of epithelioid cells and a periphery of lymphoid cells (Plate XXIII., Fig. 3). Larger areas are composed of a mixture of the two elements. Mitosis is present and characteristic intracellular bacilli are to be found after careful search (Plate XXIII., Fig. 4).

In one area of a salivary gland a diffuse lymphoid cell infiltration occurred (Plate XXIV., Figs. 1, 2), and once a small, round tubercle was seen, in which the polynuclear leucocytes were conspicuous.

The thymus gland showed a small focus of epithelioid cells in one case.

From a study of our various sections of diseased bone it would seem that the primary lesion lies in the marrow of the epiphysis. The marrow becomes very vascular and the number of giant cells increased. At various points small, round foci of epithelioid cells develop (Plate XXIV., Fig. 3). As these foci coalesce and extend, the marrow fat spaces disappear, and the normal marrow is replaced by connective tissue. Development of blood vessels is marked. Mitoses are numerous and the marrow cavity becomes densely cellular, the number of leucocytes being large.

Already with the first changes in the marrow, signs of proliferation appear in the bone. The periosteum becomes very cellular and new bone is rapidly formed in a bold network, the Haversian canals containing large blood vessels (Plate XXV., Figs. 1, 2, 3). The osteoblasts are very numerous. Beyond the limits of active bone proliferation there is a diffuse and extensive connective tissue formation with

atrophy of the muscles. Internally the absorption of bone may be rapid.

The diseased vertebræ and ribs demonstrate the above process in its various stages. As the involvement of bone extends, the contents of the marrow cavity become more disintegrated and occasionally rupture occurs, and a yellowish, granular material is discharged, containing a large excess of leucocytes.

In the extremities proliferation of connective tissue occurs about the joints. The marrow of the epiphysis may be markedly diseased, and that in the diaphysis, separated by the epiphysial line, quite normal. The periosteum of the diaphysis may be very cellular and active, causing some new bone formation. The articular surfaces of the joint remain smooth.

The sclera, choroid, and lacrimal gland of the diseased eyes showed areas of infiltration, chiefly of lymphoid elements. Some typical tubercles were seen and also intracellular bacilli. Sections of cord, etc., examined to date have shown no lesions. In the one case of paraplegia the dorsal median vessel of the cord presented an infiltration of its wall (Plate XXVI., Fig. 1). An infiltration of a nerve ganglion adjacent to the adrenal was seen (Plate XXVI., Fig. 2).

No microscopic lesion of the skeletal muscles has been seen. Characteristic foci have been found, however, in the associated connective tissue and also in the adipose tissue.

Hypersensitiveness.— In order to learn whether the prolonged disease of the guinea-pig renders it hypersensitive to the toxins of the abortion bacillus, as is the case with the tuberculous guinea-pig, we have injected a suspension made in the following manner: A series of old agar slant cultures were scraped and the growth added to old bouillon cultures of various strains, forming an opaque, light brown fluid. This was allowed to freeze and thaw in the ice-box for about two weeks. It was then shaken for two hours on three successive days and finally it was heated on a water-bar

at 60° C. for half an hour and then centrifugalized, the clear fluid being used to inject.

Two cubic centimeters of this fluid injected subcutaneously caused death in a markedly diseased guinea-pig in seventeen hours, and even as small an amount as one-half cubic centimeter caused death in twenty-seven hours. Coryza and dyspnea were noted. If moderately diseased, the animal did not succumb until the fifth day after injection and in those mild or chronic cases in which the lesions seemed to be slight or quiescent only a local swelling occurred at the point of inoculation. This swelling which never ulcerated was composed of a soft, granular, yellow material, composed chiefly of mononuclear cells. In only one case was B. abortus cultivable from this material.

In the fatal cases the fever rose to 105° and 106° F. and became subnormal before death. The controls always had about a degree of fever for a week or ten days. No local lesion developed. The lesions seen post-mortem in an acute case were those of an intoxication: subcutaneous edema and hemorrhages; free fluid in the body cavities; congestion of the viscera, especially of spleen, liver, pancreas, and kidneys; hemorrhages into the stomach wall; liver and lymph nodes. In sections of the wall of the local lesion thus produced by the toxin there is a delicate layer of connective tissue externally, and internal to this, groups of characteristic epithelioid cells and marked proliferation of new blood vessels. Lymphoid cells are numerous and leucocytes in moderate numbers. Giant cells are rarely present. The central portion of such a lesion is composed of numerous, large phagocytic cells and débris.

SUMMARY. — Bacillus abortus may be said to cause lesions in guinea-pigs of a practically constant and most remarkable character. These usually appear between the third and sixth week (within ten days as determined in sections by the microscope), the acute changes extending over a period of ten to twenty weeks, after which reparative processes appear. The disease is accompanied by fever and tends toward final

recovery, though the animal may die from rupture of the spleen, emaciation, and exhaustion.

All the tissues of the body may be attacked with the exception of the muscles. This universality and frequency is best seen in sections under the microscope, as only the far advanced lesions are recognized by the unaided eye. In the tissues involved and in the histological changes produced, the disease closely resembles tuberculosis. The lesions have a predilection for the perivascular and subcapsular regions of the various organs. Injections cause at first a profound disturbance of the circulation in certain organs, notably the spleen, which becomes enormously engorged. Intra-abdominal inoculations are frequently followed by adhesions about the spleen. The localization of the disease in the testicle as well as in other organs, even when B. abortus is inoculated subcutaneously, is quite remarkable. The proliferation of bone although not common is extraordinary when present.

It was not our intention to test the ability of B. abortus to produce abortion when introduced into healthy pregnant animals. However, in one case the animal was pregnant and received one cubic centimeter of a forty-eight-hour bouillon culture (Strain No. III.) subcutaneously. Two weeks later she aborted. Cultures of the spleen of the embryo remained sterile. This animal died five weeks later as the result of a distended and ruptured spleen.

As this paper was being written our attention was called to an article by Schroeder[16] on "Milk as a carrier of infection." He made the observation several years ago that a considerable percentage of guinea-pigs inoculated with milk to test the presence of the tubercle bacillus suffered from a curious chronic disease resembling tuberculosis in its gross pathological appearances. He was able to transfer this disease from animal to animal but was unable to isolate the organism. More recently he has observed a cow whose milk caused the same disease. With "specially modified culture media" he obtained a growth of a non-acid fast, very small, comparatively short bacillus. This organism is Gram-positive. Inoculations proved it to be the etiological factor.

When writing, he had under observation six cows giving milk containing this bacillus, although drawn so carefully as to exclude all external

contamination. Five per cent of milk samples tested contained this bacillus and ten per cent of a herd of one hundred and fifty animals showed the presence of the bacillus in their milk, although all the cattle appeared healthy. One cow at autopsy showed no udder disease and only slight liver lesions not yet studied.

The disease in guinea-pigs is characterized by a very large spleen; large, edematous lymph nodes with areas of degeneration; a swollen liver with numerous necrotic areas; and in males, by a more or less complete breaking down of the testicles. Two very unusual symptoms, paralysis and a peculiar joint disease, have occasionally occurred. The latter he believes due to a micrococcus which was isolated and which may be harmless when not associated with the bacillus. He thinks the disease has been overlooked, owing to the organism not growing readily on ordinary media, and the fact that macroscopic lesions may not appear in guinea-pigs until after six or more weeks. According to the present system of milk inspection this organism would not be recognized and would not be included in the present method of determining the number of bacteria. The thermal death point of this organism was 60° C. for fifteen minutes.

This disease corresponds so closely to the one that we have observed that it is most likely due to the same or a closely related organism.

Circular 198, Bureau of Animal Industry, issued March 2, 1912, by the U.S. Department of Agriculture, identifies the above bacillus as B. abortus, it having been demonstrated to be Gram-negative.

THE INOCULATION DISEASE IN OTHER ANIMALS.

Mice: White and gray mice have been inoculated intra-abdominally or at the root of the tail in thirty cases. The material inoculated has been one-half to one cubic centimeter of a twenty-four to forty-eight-hour bouillon culture; a turbid suspension of an agar slant, or the crushed spleen of an infected mouse. Many of the animals died from complications, frequently without apparent cause. Of the animals living nine weeks or more, there were nineteen, and of these thirteen, or sixty-eight per cent, showed distinct macroscopic lesions.

An enlarged spleen was common to all these cases and occurred also in a few of the other cases even after a period of three to four weeks. The organ was of normal shape but swollen, tense, and of darker color; weight from three-tenths to seven-tenths gram. The lymph nodes were enlarged in very few instances and presented no focal lesions. Of the other organs the lungs were apparently diseased in a few

cases. Irregular pneumonic areas, sometimes of a light grayish color, were noted. Occasional indefinite light areas were seen on the kidney surface, but not much importance was attached to these as infection with coccidium klossiella muris was common. In one of the mice having the disease six months a whitish area was present on the margin of a liver lobe and both epididymi were much enlarged.

Microscopically the spleen showed moderate distention of the sinuses, but not as marked as in the case of guinea-pigs. No epithelioid-like foci were seen. A diffuse hyperplasia was apparent from the many mitoses. In the lymph nodes this hyperplasia was also prominent. The liver presented many sub-endothelial roundish or elongated masses of lymphoid cells. Other small round foci occurred in the parenchyma. The whitish area on the liver margin proved to be an infarction of degenerated liver cells. The lesion cutting off these cells was of a chronic fibrous character containing numerous newly formed blood vessels and a rare giant cell. The character of the cells strongly suggested B. abortus as the cause. The foci in the lung were composed chiefly of lymphoid cells and occasionally groups of larger embryonic connective tissue cells. The kidneys, even when free from Klossiella muris, contained numerous foci of lymphoid and plasma cells scattered through the cortex, usually perivascular. The diseased epididymi were the seat of an extensive infiltration of lymphoid elements invading the interstitial tissue and rarely the tubules themselves. By the blocking of a duct a large cyst had been formed which was full of phagocytic cells, spermatozoa, and débris. In the adipose tissue adjacent was a focus of round cells. In another section a similar focus contained two giant cells (Plate XXVI., Fig. 3). From serial sections this focus was seen to be in close proximity to a blood vessel.

It is highly probably that B. abortus produces lesions in mice. With small doses (.5 cubic centimeter of a twenty-four-hour bouillon culture) they may occur after a period of a few weeks. Constant disease may be expected with somewhat larger doses. The method of inoculation is immaterial.

After a longer period (three to six months) diffuse lesions of a chronic inflammatory character ensue and become extensive.

Rabbits: Ten rabbits have been inoculated with B. abortus. The injections have varied in amount from one cubic centimeter of a twenty-four-hour bouillon culture to a turbid suspension of a forty-eight-hour growth on slanted agar. Intravenous inoculations were made in addition to the routes already employed. In some instances repeated inoculations were made. A subcutaneous injection causes a local lesion, sometimes measuring two to three centimeters in diameter or there may be only a moderate indurated area along the course of the needle. No ulceration results and the mass may become partially absorbed and slightly encapsulated. On section it contains a yellow, granular material resembling that found in like lesions in guinea-pigs. The animal appears in good health at all times and gains weight readily after an initial loss of fifty to two hundred grams. Fever is not present.

The animals autopsied have not given any evidence of disease although cultures of the organism may be readily obtained from the spleen after a period of ten weeks or more. The spleen may be slightly enlarged.

Microscopically the organs appear normal. Sections of the subcutaneous swellings resemble those in guinea-pigs resulting from toxin inoculation. Around a necrotic center of phagocytic cells and débris is a layer of large epithelioid cells and a few leucocytes. New blood vessels are present. The connective tissue is in slight amount. In an animal living six months which received three injections a month apart, a suspicious round cell focus was seen in the papilla of the kidney.

Cattle: A heifer was inoculated intravenously with five cubic centimeters of a forty-eight-hour bouillon culture but remained well.

Monkeys, rats, and pigeons have been injected without showing any signs or symptoms up to the present.

CULTIVATION OF B. ABORTUS. — In our work, as in former investigations, the chief problem has been to obtain successful cultures from tissues. Of the methods enumerated we have followed that of Nowak with slight modifications. A small bit of the washed cotyledon is removed with sterile forceps and transferred to a slant of ordinary beef-infusion-peptone agar. This is ground and broken up with a flat, platinum wire against the side of the tube, and the surface of the medium is smeared with it. A loop of the condensation water is then transferred from this tube to a second, and from this to a third tube. In the case of guinea-pigs a bit of the liver, spleen, or kidney is transferred to agar as described above. Inoculations by stabbing the organs with a platinum wire have given satisfactory results, but when the organisms are relatively few they may be missed by this method.

From experience we find that the spleen and lymph nodes contain the most organisms. Then come the bone marrow, liver, kidney, and lung, in order. A cube of lung tissue, three to four millimeters in size, may only give rise to two to four colonies. For this reason we have used the spleen regularly.

The tubes inoculated from the cotyledon are incubated at 37° C. in the ordinary way for twenty-four hours and then any colonies marked which have appeared. The tubes are then placed in an ordinary quart fruit jar having a screw metal top and rubber washer. A slant of ordinary agar is inoculated with B. subtilis and placed in this jar with the other tubes. The jar is sealed by screwing down the cap firmly and it is then placed in the incubator at 37° C. for two or more days. By this time small, characteristic colonies have appeared on the surface of the media in addition to those already noted. By this method we have been able to

isolate B. abortus in pure culture from original tissues which we have had at our disposal, and in practically all the cases of guinea-pigs suffering from this disease. We have succeeded in isolating the organism from the latter after a period of thirty-seven weeks, but in these chronic cases one to eight colonies developed instead of thirty or more as is common when cultures are made after a period of ten to twenty weeks. More recently we have found that by merely sealing the culture tube with sealing wax, and incubating it longer in some cases, we have secured satisfactory results.

The action of B. subtilis was studied in some detail in an endeavor to find out under what conditions B. abortus grows most readily. For this work we used a normal salt solution suspension of the original culture. This assured the presence of B. abortus and controls demonstrated that growth by ordinary incubation was practically negative. It was found that an excess of actively growing B. subtilis prohibits the growth of B. abortus. One square centimeter of fresh growth of B. subtilis to seventy-five cubic centimeters of space in the jar gives a profuse growth in forty-eight hours; 1 to 125 gives a moderate growth. Even one square centimeter to seven hundred cubic centimeters of space gives a distinct growth, the control remaining sterile. The age of the bacillus subtilis culture shows a diminishing influence up to the fourth or fifth day, after which it fails to stimulate growth. The failure evidently coincides with the period of sporulation. The manner in which B. subtilis and B. abortus tubes are corked is immaterial, provided the diffusion of gases is not interfered with. The bacillus subtilis tube may be open; the cotton plug may be loosely adjusted or dipped in paraffine as is customary with solid media in this laboratory, or a tin-foil covering may be also added, without checking the profuseness of the growth. As a routine the paraffine-dipped cotton plug was left in place.

The effect of using a sealed glass jar alone without the culture of B. subtilis was next investigated. Undoubtedly in some instances this is an advantage. The temperature in such a jar rises more slowly in the incubator naturally, and

therefore the tubes do not reach the incubator temperature until some time after those placed in with them but not in jars. The atmosphere of the jar remains at a more uniform degree of temperature and moisture unless the incubator is opened but rarely. It was possible for us to obtain original cultures in some instances by means of the sealed jar alone, the controls remaining sterile. This method cannot be depended upon and in several instances the tubes failed to develop.

We next tested the advisability of growing B. subtilis and B. abortus together in a test-tube sealed with sealing wax. Apparently there was not enough oxygen present for our strain of B. subtilis to grow appreciably under these conditions and neither organism developed. When, however, the seal was broken, B. subtilis promptly multiplied profusely.

The following simple procedure was also found effective. One tube of B. subtilis and the first transfer from a B. abortus culture were connected with a piece of webbed rubber tubing fifteen to twenty centimeters long and with an internal diameter of thirteen millimeters, by adjusting the rubber tube tightly over the ends of the plugged tubes, thus forming an inverted U. B. abortus grew as profusely as by the method of Nowak.

If the action of B. subtilis is merely one of reducing the oxygen tension in the jar, other organisms should act in a similar manner, although perhaps not in the same degree. Several kinds of bacteria were therefore tested, using B. subtilis as a control, among them B. coli, B. megatherium, a staphylococcus and an undescribed bacillus of guinea-pig pneumonia. It was found that B. coli, B. megatherium, and the staphylococcus gave results similar to B. subtilis. The pneumonia organism, although growing as profusely as the others, was able to stimulate B. abortus only in a slight degree, for the growth was just visible. Similar tubes incubated in a sealed jar alone remained sterile. When forty-eight hours old these various cultures exerted no further favorable influence. None of these organisms seemed to give

a more favorable growth than B. subtilis, and we have therefore continued our work with the latter.

It was furthermore evident from our tests that different strains of B. abortus required different degrees of stimulation. Culture II., a and b, developed on blood serum without any aid. Some strains could be grown in a sealed jar; others by slightly more stimulation, as with the pneumonia organism, and still others required B. subtilis. Whether still other strains require still more favorable environment remains to be tested.

As the action of the above organisms seems to be one of diminishing the amount of oxygen, we attempted to produce similar results by the use of alkaline pyrogallol. This was employed in glass jars and in Buchner tubes in varying amounts. With the former we attempted to produce partial anaërobic conditions. In the latter we varied the amount to include various degrees of anaërobiosis; *i.e.*, a series of tubes were inoculated in the same manner with B. abortus (first transfer) known to grow readily with B. subtilis; the dry cotton plugs were pushed in about two centimeters and dry pyrogallol in amounts ranging from .001 to .5 gram were added, and then one cubic centimeter of a ten per cent solution of sodium hydrate, and the tube quickly sealed with a rubber stopper. Prolonged incubation gave no growth save in the control tube, in which it was profuse in seventy-two hours. After four or five days one of the other tubes was uncorked and placed in a jar under the influence of B. subtilis, and B. abortus developed readily. These experiments show the need of very carefully adjusting the tension of oxygen.

In our experiments we used ordinary agar media, but we also tested agar made from extract of meat in place of the meat infusion, and also agar made plus glycerine. Apparently meat infusion agar is more favorable for growth but, with a stimulant like B. subtilis, growth on the other two is as profuse. We have made various experiments with ordinary agar by adding various quantities (drops) of dextrose or sodium carbonate or both, and also by growing B. coli on

the medium for twenty-four hours and reslanting it before inoculation. No growth has occurred as a result of these changes in the media.

Other conditions of growth were ascertained after the second or third transfer, when the organism grew moderately without the use of B. subtilis under ordinary conditions. In one or two instances we obtained growth from the first transfer however.

Fermentation tubes of ordinary bouillon inoculated with B. abortus, after an incubation of two to seven days, showed marked clouding of the bulb with a sharp line of demarcation at the junction of the branch. In other words, the organism gave pronounced evidence of being a strict aërobe from the first.

It was found that if ordinary bouillon tubes were inoculated and placed under the influence of B. subtilis the bacilli multiplied much less readily than on agar slants. This retardation could be overcome in some measure by using a broader surface of medium, as for example, a thin layer in an Erlenmeyer flask.

Plate cultures of B. abortus after several days' incubation showed minute colonies on the surface and just beneath. With prolonged incubation other colonies developed somewhat deeper in the medium. These latter were much smaller than those at or near the surface and remained so.

Shake agar cultures gave a profuse surface growth, but in no instance did it extend beyond two or three millimeters beneath the surface. A similar growth was obtained in the agar-gelatine-serum of Bang when a readily growing organism was inoculated. No zonal growth was noted, only the surface and immediate substratum becoming cloudy. When this medium was inoculated directly from an original source no growth occurred. When, however, the tube was placed with B. subtilis in a sealed chamber, burr-like, white colonies developed throughout the medium seven to ten days after those grown on slanted agar had appeared. There was no zonal character to the growth and the deeper (4 centimeters)

colonies were the smaller. Deep agar inoculations treated in a like manner remained sterile.

At the time we attempted to grow B. subtilis and B. abortus in a sealed tube we also placed B. abortus alone in a sealed culture tube and were somewhat surprised to find a profuse growth after a rather prolonged incubation period (8–17 days). This method has been used extensively since and in every instance has proved as satisfactory as with B. subtilis. In some instances the results have been exactly the same. In others, the colonies have not developed for several days after the growth had appeared with B. subtilis, and the colonies remained smaller, but it was of interest to note that occasionally many more colonies developed on the sealed tube than on the one associated with B. subtilis (Plate XXVII., Fig. 1). As might be conjectured, the size of the sealed culture tube, as well as the number of organisms transferred, bears a distinct relation to the result. Ordinarily we have used tubes thirteen and one-half centimeters long and eight millimeters in diameter. Sealing the tubes with sealing wax or paraffine gives equally good results. Tubes supplied with small rubber stoppers are, however, more easily manipulated and as effective.

It is probable that strains will be found to vary more or less in their relation to oxygen and to the kinds of culture media usually employed. This is shown by our own experience with the six strains thus far studied. For instance, Culture II., a and b, we were able to isolate without B. subtilis directly from guinea-pigs inoculated with the original material. In this instance, the tissues of two guinea-pigs were broken up as described, and spread over slants of ordinary agar containing one-half to one cubic centimeter of defibrinated guinea-pigs' blood. After a period of some four days, pure cultures were obtained (Plate XXVII., Fig. 2). These two sub-strains have been kept distinct and their cultural characteristics studied.

Culture I. was received from Professor MacNeal on a slant of blood agar. A goodly amount was transferred to an ordinary agar slant and, although the growth was very feeble

at the end of twenty-four hours, in four days it was quite abundant, and no further difficulty was experienced in cultivating it on ordinary agar in air.

Culture IV. from Professor Bang was contained in capillary tubes of serum bouillon and also on serum agar. The serum bouillon culture was transferred to ordinary agar slants and also to agar containing a small amount of guinea-pigs' defibrinated blood. These tubes were then exposed to the action of B. subtilis, the pneumonia organism, and in a sealed jar by themselves. Upon the plain agar slants no colonies developed, but upon the blood agar with the action of B. subtilis a profuse growth was obtained in forty-eight hours, and with the pneumonia organism in seventy-two hours; the tubes shut up in the jar and those in the chamber of the incubator remained sterile. (Professor Bang in a letter stated that the bacilli grew in the old manner (specific sub-stratum), but that he had also found the bacilli grew on the surface at times.)

It will thus be seen that of these two cultures sent to us and grown artificially for some time, one grew very readily on our media, while the other required not only the action of B. subtilis but also the presence of blood in the media. This is the one instance where we have found the presence of blood necessary. It is quite probable that the manner in which they had been cultivated before coming to us was responsible for the differences noted.

We have also made experiments to find the quickest and most certain way of educating this organism to grow aërobically. Apparently it makes no great difference whether the organism be passed through a series of rapid transfers under the influence of B. subtilis or whether the first colonies developing unaided furnish the source for subcultures. The action of B. subtilis is beautifully shown in the following manner. If we smear the surface of an agar slant from a culture which requires the aid of B. subtilis to develop, and expose it to the action of B. subtilis for forty-eight hours, a profuse film of growth covers the entire surface. If a similar tube is allowed to incubate unaided it often remains sterile,

or one to four or five colonies only develop from the extremely profuse inoculation. These do not grow readily and may not appear for four to six days, but once started they continue to grow even at room temperature.

In the first transfers growth is rare, but in the second and third a few colonies usually develop unaided. After five to seven transfers the organism has reached a stage which is nearly constant, and a loop of fresh culture develops readily on an agar slant in forty-eight hours.

It is an interesting fact that after the organism has adapted itself to ordinary aërobic conditions it does not lose this characteristic by further passage through guinea-pigs. Some of our guinea-pigs so inoculated have lived fifteen to twenty weeks, and yet upon making cultures from the spleen B. abortus developed even at room temperature (Plate XXVII., Figs. 3, 4).

Some notes on the morphology and cultural characters of B. abortus. — The following statements apply to all the strains studied, as no differences have been noted. The bacillus has been described as being .6 to 3 μ long and .5 μ broad. Bang himself stated that the longest organisms were the length of tubercle bacilli. Our measurements of the bacillus are .6 to .8 μ long and .5 to .6 μ broad. There is some variation in length which in some individuals may be equalled by the diameter. This type suggests a coccus. Some individuals may be 1 to 1.5 μ long, especially in bouillon cultures, but this is rare. The diameter is fairly constant.

In smears from cultures the bacilli lie separately or in twos, end to end, and rarely six in a chain. When in small clumps no definite arrangement is noted.

There is no motility, although Brownian motion may be very pronounced.

The bacillus is readily stained by all the ordinary dyes, perhaps most distinctly with dilute carbol-fuchsin. The stains used were carbol-fuchsin (concentrated and diluted); aqueous methylene blue; Loffler's methylene blue; and aniline water gentian violet. The organism stains uniformly

throughout, although irregularities (some portions staining deeply and some feebly) have been noted by Preisz and Holth. The ends are rounded but occasionally the corners are not as deeply stained, giving the bacillus an ovoid appearance. Carbol-fuchsin seems to accentuate the diameter; gentian violet, the length. The organism is Gram-negative and is readily decolorized by weak acids after having been steamed in carbol-fuchsin. No capsule has been demonstrated and no spores are formed. In old cultures degenerated and involution forms may be seen. Priesz speaks of branched forms.

Cultural characters: The following characteristics appear common to all of the six different strains studied. Of these four were from Massachusetts, one from Illinois, and one from Denmark.

On agar, the colonies have been described as transparent dewdrops with dentated edges. Preisz described them as having a bluish-white luster. Later they became yellowish-brown and McFadyean and Stockman laid considerable stress on the "rusty brown" color. Under the microscope MacNeal noticed a few coarse granules near the center of the colony.

We observed numerous small colonies on agar plates after forty-eight hours' incubation. The surface colonies appear as convex droplets of rounded, sharply circumscribed outline, about one to one and one-half millimeters in diameter, very glistening, *i.e.*, reflecting light markedly, and of a semi-opaque whitish color, suggesting droplets of mucus. They have an iridescent, mother-of-pearl sheen. Under the low power of the microscope the surface colonies have a very fine stippled appearance, brownish by transmitted light, darkest at the center. The deep colonies are much smaller than those on the surface and are of wedge or lemon shape. With the hand lens they are seen to have a lighter margin or are faintly banded with lighter zones.

The surface colonies develop slowly but persistently, until in plates containing only a few organisms it is not rare to have a colony five millimeters in diameter, of a whitish

opaque appearance. In place of the stippling, droplets as if of air or water are seen, very numerous and just external to the central portion of the colony. The deep colonies grow slightly and become darker brown. The plates, sown with many bacteria, develop in two weeks a fine ground-glass appearance due to the innumerable minute colonies; again those farthest from the surface are the smallest. Those which have burst the bottom film of agar spread out on the glass of the Petri dish in a thin, almost colorless growth five or more millimeters in diameter. They appear much lighter than the surface colonies macroscopically. If incubation is continued four weeks or more the principal change is one of density only. In the larger surface colonies the opaque points in the colony enlarge until readily seen with the hand lens, and extend into the glistening, mother-of-pearl peripheral zone.

On the agar slant after twenty-four hours a delicate, fine, granular film may be just visible on the inoculated surface. The margin is sharply defined and no extension occurs. After another twenty-four hours the film is much heavier, appearing as a translucent growth of characteristic luster. The border is well defined, steep, and slightly elevated, until the central growth increases, giving the appearance of a glistening, moist plaque. The water of condensation is moderately cloudy, the growth rarely extending out on the surface. An abundant, whitish sediment slowly forms. The growth increases in density for about a week, after which it remains stationary. When a few organisms only are distributed over the surface, the growth is slower, their presence not being recognized until after forty-eight to seventy-two hours. Development continues, however, for two to four weeks even at room temperature.

This slow growth, continuing until the colony is a relatively large one, seems characteristic. It has also been noted by us that when a few organisms are transferred to an agar slant, there is considerable variation in the size of the colonies, and after several days some of them may be twice the diameter of others. If these tubes be kept for several

weeks at room temperature, occasionally small daughter colonies appear on the glistening surface of an older colony, in some cases in such numbers as to give it a granular appearance. This is also seen on potato cultures.

When these strains were first isolated, and for some months afterwards, no changes in the medum were noted, but after having been cultivated artificially for a year it was seen that crystals develop in the agar which are probably due to the increasing alkalinity of the medium.

In an agar puncture the surface growth appears in forty-eight hours as a convex, opaque, whitish, glistening colony, three millimeters in diameter, sharply circumscribed. The growth along the stab extends one centimeter from the surface, and consists of fine, round colonies clustered together. After five days the surface growth may have extended to five millimeters in diameter but is not as convex. There is slight further extension along the needle-track, the deeper colonies being too small to be distinguished individually with the hand lens. After two or more weeks the surface growth dries out, presenting radiating lines and concentric depressions. Often the remaining surface of the medium is covered by a light secondary growth due to the moisture present which has inoculated the remaining surface. The average length of a stab growth remains at about one centimeter; in one instance (II. a) it extended to two and two-tenths centimeters.

If a tube of agar be inoculated while fluid and then cooled promptly, colonies appear on the surface within twenty-four hours. No zone of growth develops in the agar even after a period of two or three weeks. There is slight growth just beneath the surface, but in no instance has this been at a depth of more than three millimeters.

Cultures made on glycerine agar, and extract-of-meat agar, have not shown any appreciable variation from the above picture. Agar made from meat extract seems slightly less favorable than from meat infusion.

On blood agar prepared with defibrinated blood of horses, rabbits, or guinea-pigs there was no increase in the profuseness

of growth. After four to five days the inoculated surface presents a thick, grayish, greasy film, sharply bounded. The glass wall of the tube opposite the surface of the medium takes on a semi-opaque, smoky, greenish color which extends slowly to a height of three centimeters from the water of condensation, after several weeks of incubation.

On Loeffler's serum the bacillus grows readily but not as profusely as on agar. A delicate, circumscribed film with raised margin appears after twenty-four hours. The surface is finely granular and slowly becomes glistening with additional growth. In general, the appearances resemble those on agar.

McFadyean and Stockman obtained no growth on gelatine either at room temperature or at 37° C. Other investigators obtained a growth after four to six weeks.

Owing to the lowered temperature (22° C.) at which gelatine is incubated, growth on this medium is exceedingly slow, and on gelatine plates only after ten days may colonies be recognized with the microscope. They may not be readily seen with the naked eye until after three weeks, appearing as opaque, whitish droplets on the surface. With the hand lens they are very glistening and porcelain-like. Under the microscope other smaller colonies may be made out in the medium. The smallest appear as round, sharply circumscribed bodies with a delicate colorless stippling, the interior having a brownish tinge (with growth). The largest surface colonies have a brownish color, deepest at the center. As the colony develops, other small, blackish dots appear, forming almost a tracery in the brownish stippling. This blackish pigment is usually more concentrated at the center or may be uniformly distributed throughout. In some, distinct zones are seen, *i.e.*, a light margin of moderate width; a dark zone of similar width; a narrow light zone, and then a wide dark center. Besides the single colonies of distinct outline others occur, built up of fresh additions of growth or as the result of two organisms developing side by side. There are all variations from a round colony with a double rim at one point to slight outgrowths heaped up. After a

period of three weeks more, the plates sown with many organisms present a light, feathery cloud in which are numerous distinct colonies (surface), some, one millimeter in diameter. With a hand lens this feathery cloud may be resolved into colonies. The growth is chiefly at or near the surface, the deeper the colony the smaller it is.

In the gelatine stab, growth is slow and only after ten days to two weeks does a fine, white cloud appear along the puncture line with scattered colonies in the lower third. One or more small, opaque, convex, white colonies one to two millimeters in diameter appear on the surface. After seven weeks, growth still continues, although the medium is dried out. It may extend the whole length of the stab, and delicate, white, isolated colonies result. No liquefaction takes place.

On the agar-gelatine-serum of Bang, as was mentioned above, growth occurs only on the surface or immediately beneath it when a readily growing organism is inoculated. The fact that colonies are able to delevop in the depths when the tube is incubated under the influence of B. subtilis and not in deep agar similarly treated is evidence that this medium is more favorable in certain respects.

According to Preisz, bouillon cultures scarcely became cloudy. McFadyean and Stockman had difficulty in obtaining a culture in ordinary bouillon direct from natural material but were more successful when a transfer was made from an agar culture. The addition of one per cent of grape sugar rendered the growth more luxuriant. Nowak was able to grow the organism in ordinary alkaline bouillon, the upper portion of the medium remaining clear until the tube was agitated.

In ordinary bouillon, prepared with fresh beef or veal and with an acidity of .8 to 1.2 per cent of a normal solution, after a series of forty-eight-hour transfers, a loop of a two to four-day old bouillon culture transferred to fresh bouillon causes very slight clouding after twenty-four hours' incubation, and on agitating a slight amount of fine white, powdery sediment arises. This clouding increases whether the tube

is agitated or not, and after a week the medium becomes turbid, remaining so for about a month and then gradually clears. The sediment covers the concavity to a moderate degree. It changes from a powdery to a flaky consistency and later becomes tenacious and stringy. As a rule no membrane or film develops, but if the tube be incubated for two to four days and then set aside at room temperature a white, delicate film may appear on the surface. In bouillon of an alkalinity of three-tenths to one per cent of a normal solution the growth is not as profuse.

If potato is inoculated from a bouillon culture, visible growth may not appear for two or four days. The outlines are irregular since the bouillon spreads over the uneven surface. A more uniform streak is made by inoculating from an agar culture. After twenty-four hours a delicate, white, glistening moist streak develops which slowly assumes a yellowish and after several days a brownish hue. The growth becomes elevated, glistening, viscid to the touch, with sharp, steep borders. The color in many instances is a chocolate brown and resembles, as McFadyean and Stockman have stated, the appearance of glanders on potato. Different strains may give slightly different shades of color, but by further subcultures on the same or other potato media variations from tube to tube are found in any one strain. The same daughter colonies noted in the isolated agar colonies are occasionally present on the potato medium. The inoculated potato itself may turn dark after about a week's incubation.

In milk, on incubating the organism with pyrogallol, Preisz observed coagulation with the separation of whey, but other observers, although obtaining good growth, have observed no coagulation, McFadyean and Stockman stating that when it was present a streptococcus was also found. Nowak obtained slight acid production in litmus milk. With two of his strains grown with B. subtilis the milk became distinctly red in eight days.

The organism grows readily in milk but not as profusely as in bouillon. After a week or ten days the normal acidity

is found to be lessened usually to three-tenths per cent of a normal solution, when phenolphthalein is used as an indicator. Litmus milk remains blue during a period of four to six weeks after inoculation whether kept in a large chamber of the incubator or under the influence of B. subtilis. There is no precipitation or visible alteration in the milk.

The presence of indol and nitrites cannot be demonstrated.

The optimum temperature is conceded to be 37° C. Preisz obtained no growth at room temperature although Nowak did. McFadyean and Stockman used a temperature between 30° and 37° C., an original isolation not growing at the room temperature, but they observed that once the culture had started it would continue to grow at room temperature. We have found the organism grows best at 37° C., and slightly even at 20° C.

Bang was able to isolate the bacillus from mummified fetuses and from material kept seven months in the cold. McFadyean and Stockman found material infectious at seven months, but not after the lapse of a year, nor could the organism withstand drying three days under calcium chloride. Preisz was able to rejuvenate a culture with pyrogallol after seventy days, and Nowak succeeded with agar slants after months; in deep agar after two years. According to Preisz the organism withstands exposure to 50° C. for thirty minutes but is killed at 55° C. in three minutes. McFadyean and Stockman exposed dry smears in an oven for one hour at 55° C. and later obtained a growth, but not after an exposure of two hours. In a water bath the organism withstood 55° C. for ten minutes but not 59° C.

When actively growing cultures on agar slants were left exposed to light in the laboratory for three months and then fresh slants made with large amounts only a few colonies developed. One strain died out entirely under these conditions. Bouillon cultures under the same conditions for a period of four months gave a growth.

Smears made by placing a loopful of a forty-eight-hour bouillon culture on cover-slips and keeping them under a

bell glass showed the presence of living organisms for seventeen or more weeks depending on the strain, but the older films require a week for development.

To determine the thermal death point, tubes sixteen millimeters in diameter were employed containing exactly ten cubic centimeters of bouillon of an acidity of 1.5 per cent. A forty-eight-hour culture of the organism was made and after the temperature of the bouillon in the tubes had been raised in a water bath to 45° C. for fifteen minutes, one of these tubes was inoculated with three loopfuls (loop 1.5 millimeter internal diameter) of a forty-eight-hour culture. The tube was slightly agitated and after a further exposure of ten minutes cooled in a vessel of ice-water and then incubated for a week at 37° C. Other tubes were inoculated in a similar manner at higher temperatures. It was found that different strains have slightly different thermal death points. From several tests it appears that Strain No. I. is killed by a temperature of 56° C., the other strains at 59° C. for ten minutes. Briefly it may be said that the thermal death point of the B. abortus is 59° C. for ten minutes.

Fermented bouillon containing one per cent of dextrose, saccharose, and lactose was used to determine fermentation capacities. No action was noted either in the production of acid or gas. Alkali production reduced the one per cent acidity of the medium to nearly neutral, and in some instances it became one-tenth of one per cent alkaline (phenolphthalein) in ten days.

As has been mentioned there is no acid production either in milk or sugar bouillon. On the contrary the organism regularly produces alkali. Further tests were made with ordinary bouillon and alkaline (.3 per cent) bouillon, and incubating the tubes in the large chamber alone or with B. subtilis. No acid production was demonstrated in the case of the alkaline bouillon, the reaction becoming more alkaline. This was relatively less than in ordinary bouillon as the growth was visibly less.

The cultural characteristics of this organism are quite

constant when once its ability to grow on artificial media has been established. Among these characteristics may be mentioned the glistening iridescent colonies on agar and the variation in their size; the colonies on and the non-liquefaction of gelatine; the slow growth in bouillon; the conspicuous pigmented growth on potato; and the organism's inability to ferment dextrose, saccharose or lactose, or to produce acid.

[In concluding, I wish to acknowledge my indebtedness to Dr. Theobald Smith for suggestions and assistance in this work.]

BIBLIOGRAPHY.

1. Holth. Berl. tierärztl. Wchnschr., xxv, 1909, 686; Zeitschr. f. Infektionskrank. d. Haustiere, x, 1911, 207.
2. Lehnert. Säch. Veterinärber., 1878, 95.
3. Bräuer. Säch. Veterinärber., 1880, 72; Deutsch Zeitsch. f. Thiermed., xiv, 1895, 95.
4. Nocard. Recueil de Méd. Vét., 1886, 669.
5. Sand. Deutsch Zeitschr. f. Thiermed., xxi, 195.
6. Bang and Stribolt. Zeitschr. f. Thiermed., i, 1897, 241; Maanedsskrift f. Dyrlaeger., 1900.
7. Preisz. Centralbl. f. Bakt., 1 Abt. Orig., xxxiii, 1903, 190.
8. Nowak. Ann. de l'Inst. Pasteur, xxii, 1908, 541.
9. McFadyean and Stockman. Jour. Vet., 1909, 459.
10. Zwick. Centralbl. f. Bakt., 1 Abt. Ref., xlvii, 1910, 219.
11. MacNeal and Kerr. Jour. Inf. Dis., vii, 1910, 469.
12. Russell. Agri. Exp. Sta., Madison, Wis., Sept. 1, 1911.
12a. Larson. Jour. Inf. Dis., 1912, 178.
13. Wall. Maanedsskrift f. Dyrlaeger, xxi, 1910.
14. Grönsted. Maanedsskrift f. Dyrlaeger, 1909, 395.
15. Hesse. Centralbl. f. Bakt., 1 Abt. Ref., xlvii, 1910, 183.
16. Schroeder. Proceed. of Amer. Ass'n of Amer. Milk Com., Phila., 1911, 131.

EXPLANATION OF PLATES.

(When not otherwise stated, the material was obtained from guinea-pigs.)

PLATE XXI.

FIG. 1. — Disease of ribs. Note bulging at the epiphyses; points about to discharge.

FIG. 2. — Disease of ribs. Upper rib normal, lower diseased.

FIG. 3. — Left anterior extremity. Irregular swelling and yellow points.

FIG. 4. — x 45 diameters. Inguinal lymph node showing marked general infiltration of the tissue with epithelioid cells. Lymphoid elements at the periphery.

FIG. 5. — x 420 diameters. Preceding figure magnified. Nests of epithelioid cells.

PLATE XXII.

FIG. 1. — x 30 diameters. Epithelioid elements extending beyond a lymph node into the surrounding fat tissue.

FIG. 2. — x 375 diameters. Preceding figure magnified. Note epithelioid cells with faintly staining protoplasm and large round or oval nuclei, containing a very small amount of peripherally arranged chromatin.

FIG. 3. — x 50 diameters. Kidney. Round cell infiltration about blood vessel.

FIG. 4. — x 130 diameters. Localized lesion in the cortex of the kidney.

FIG. 5. — x 20 diameters. Uterus. Nests of epithelioid cells between muscle bundles. Central necrosis.

PLATE XXIII.

FIG. 1. — x 6 diameters. Testicle. Areas of cell proliferation. Connective tissue formation. Area of necrosis.

FIG. 2. — x 120 diameters. Infiltration near blood vessel in heart muscle.

FIG. 3. — x 375 diameters. Lung. Tubercle formation.

FIG. 4. — x 1,500 diameters. Lung. Bacilli in epithelioid cells.

PLATE XXIV.

FIG. 1. — x 130 diameters. Salivary gland. Round cell infiltration.

FIG. 2. — x 315 diameters. Salivary gland. Tubercle formation. Note leucocytes present.

FIG. 3. — x 50 diameters. Cross section of rib. Small foci of disease in marrow.

PLATE XXV.

FIG. 1. — x 6 diameters. Cross section of ribs. Extensive proliferation of bone in the diseased ribs.

FIG. 2. — x 60 diameters. Fig. 1. Small rib on right magnified. Note beginning proliferation.

FIG. 3. — x 60 diameters. Section of diseased rib under same magnification as Fig. 2. Note absorption of the original rib as well as the marked proliferation of new bone. Atrophy of the neighboring muscle fibers.

PLATE XXVI.

FIG. 1. — x 130 diameters. Round cell infiltration in wall of dorsal blood vessel, lumbar cord.

FIG. 2. — x 250 diameters. Small, round cell focus in a nerve ganglion.

FIG. 3. — x 375 diameters. White Mouse IV. Cervical region. Focus in the connective tissue.

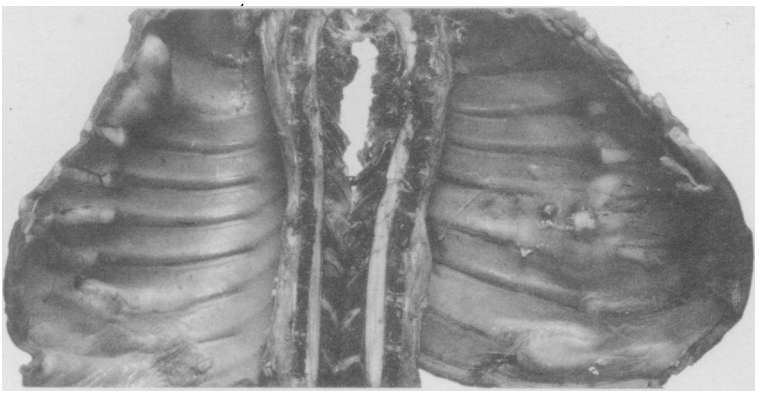
PLATE XXVII.

FIG. 1. — Cultures of an original isolation from splenic tissue. Guinea-pig received an intraabdominal inoculation of one cubic centimeter of a suspension of cotyledons, Cow No. 235 (Culture V.). Chloroformed after sixteen weeks. Agar slant on left incubated under the influence of B. subtilis; on right incubated in a sealed tube. Appearance after five days. An agar slant inoculated in a similar manner and incubated unsealed, remained sterile.

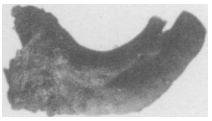
FIG. 2. — Culture of the original isolation of Strain No. IIa. on blood agar, without the aid of B. subtilis or sealing.

FIG. 3. — Guinea-pig inoculated with Culture IIb. growing readily aërobically. Animal chloroformed at the end of ten weeks. Each agar slant inoculated with a bit of spleen. Tube on the left incubated with B. subtilis. Tube in the center sealed and incubated. Tube on the right incubated unsealed. After seventy-two hours note the characteristic growth on each slant. The tube on the right shows the ability of B. abortus to retain its artificially acquired power to grow aërobically even after remaining in the animal body ten weeks.

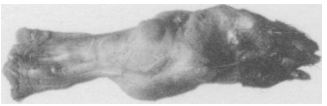
FIG. 4. — Culture obtained aërobically from a bit of spleen of an infected mouse. Animal inoculated with a readily growing culture (IV.) and chloroformed after two months.



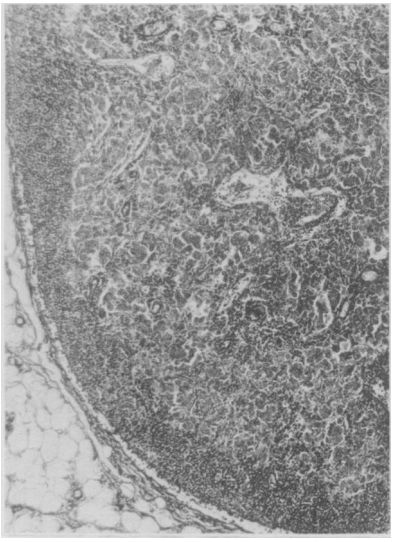
1



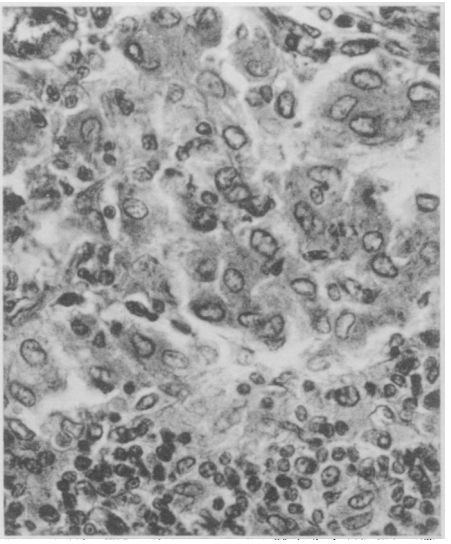
2



3

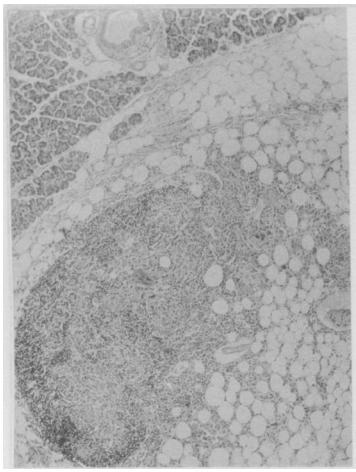


4

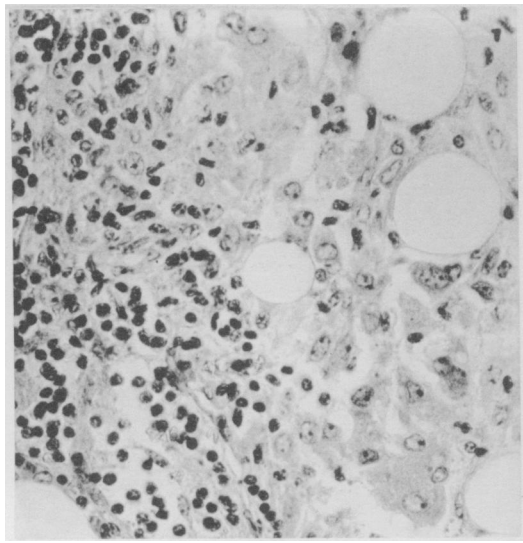


5

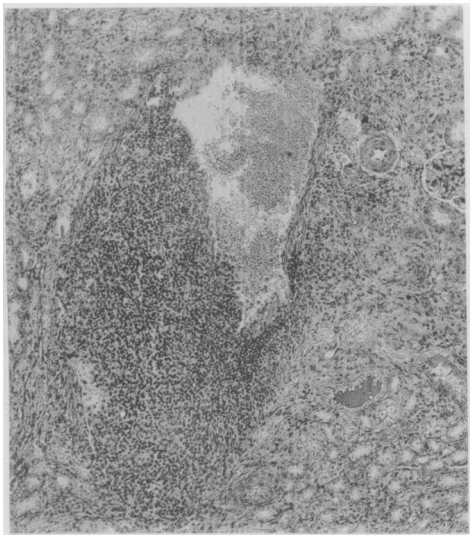
Fabyan B. Abortus



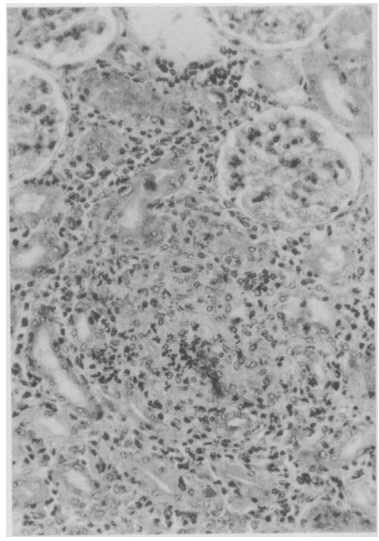
1



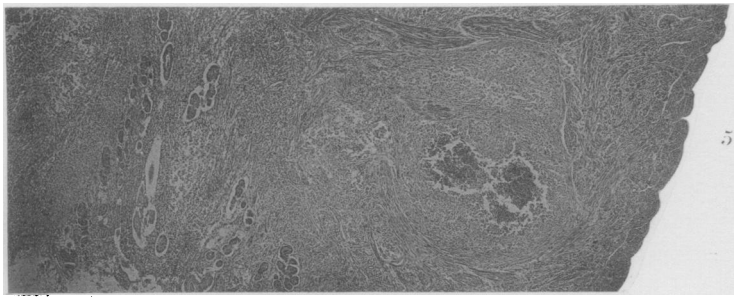
2



3



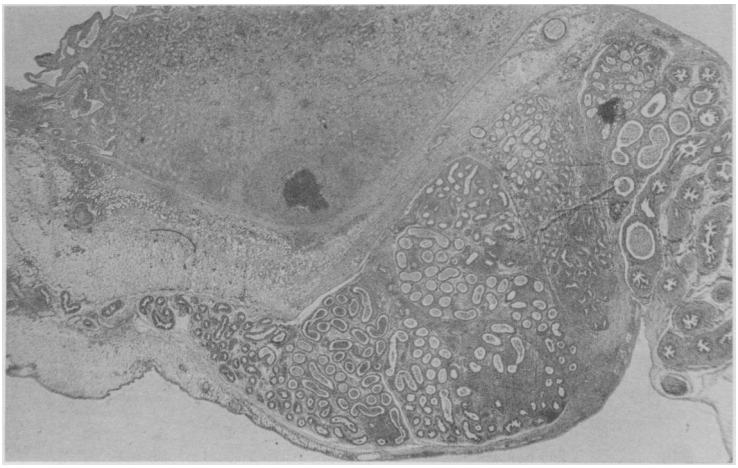
4



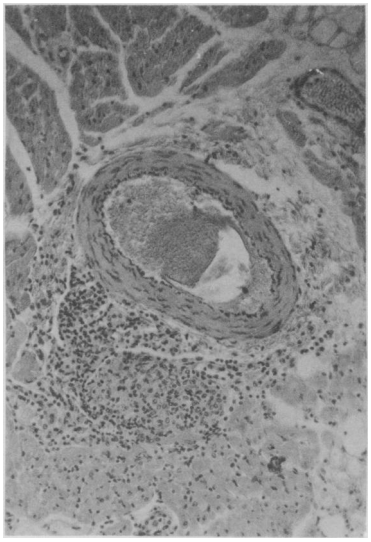
5

Fabyan

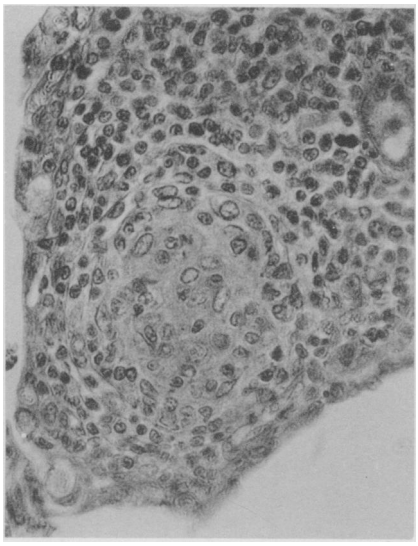
B. Abortus



1

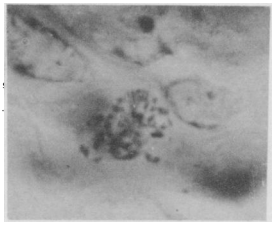


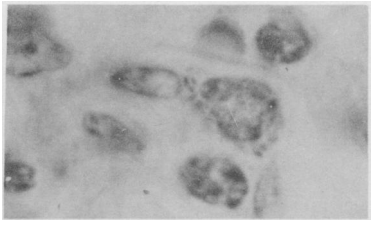
2



3

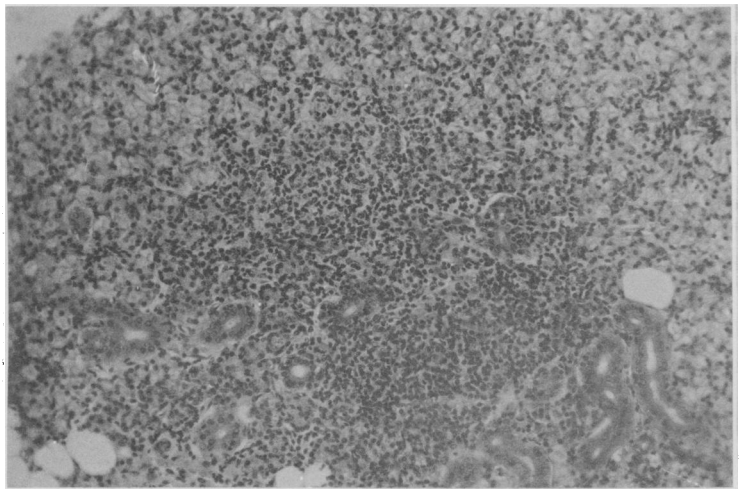
4



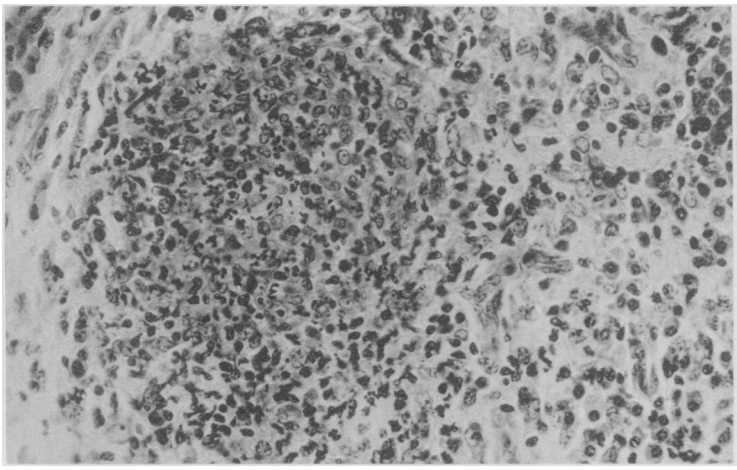


5

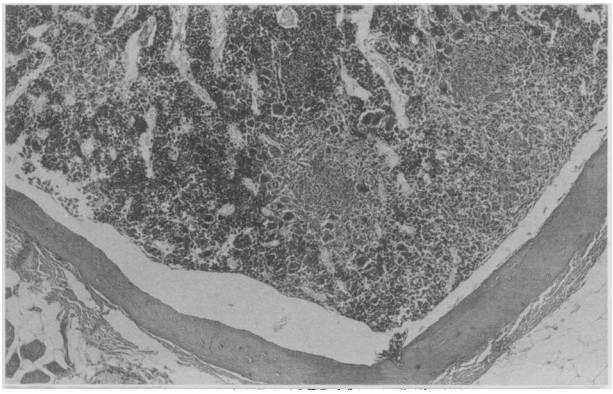
Fabyan B. Abortus



1

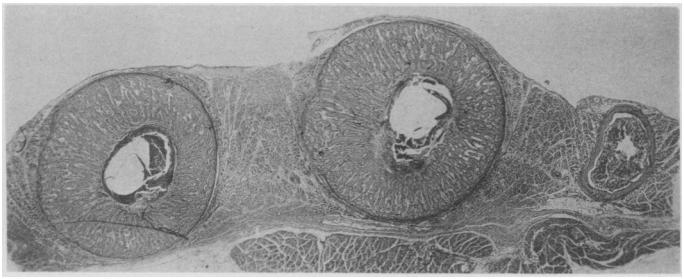


2

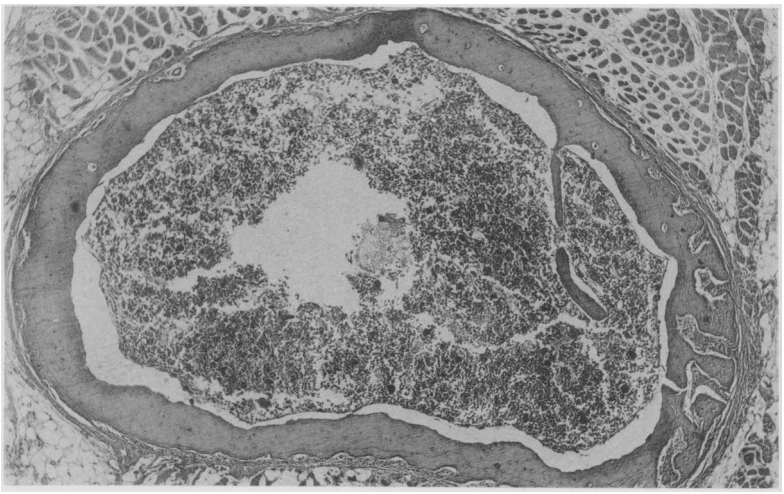


3

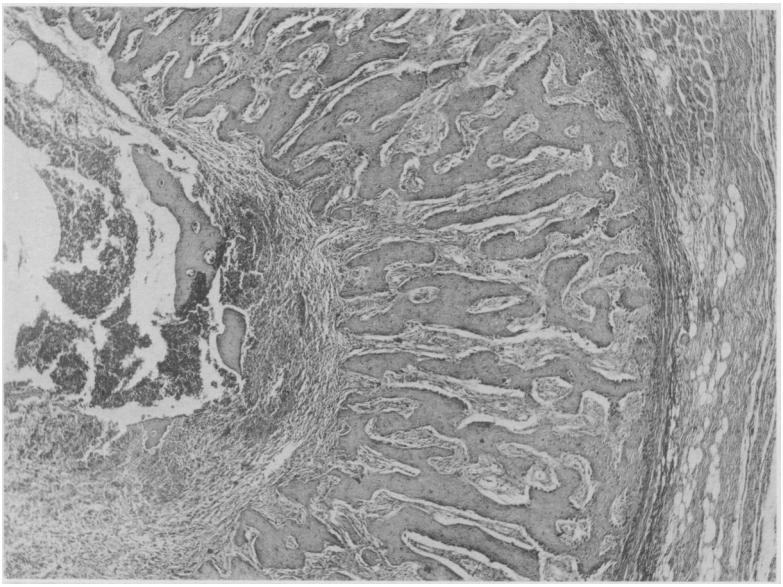
Fabyan B. Abortus



1

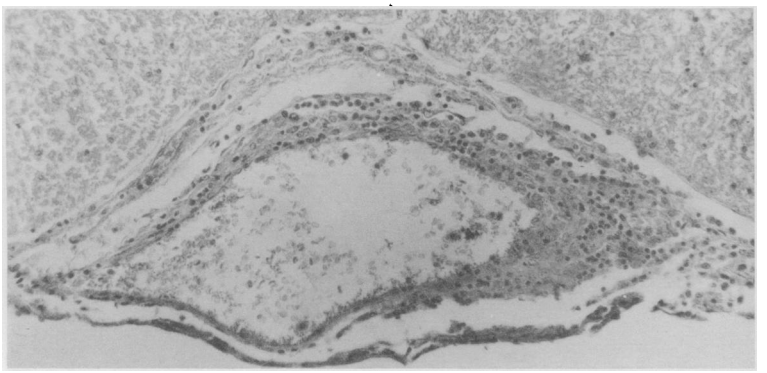


2

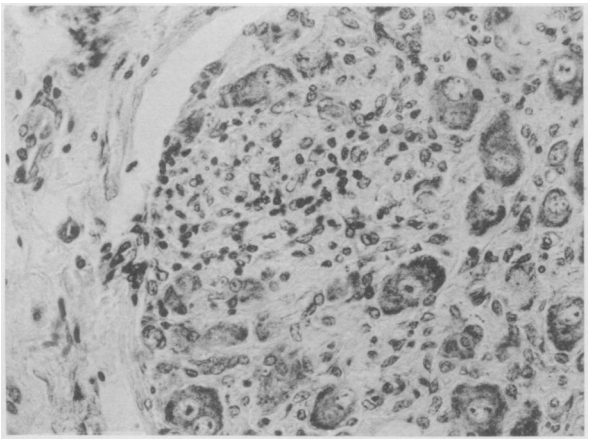


3

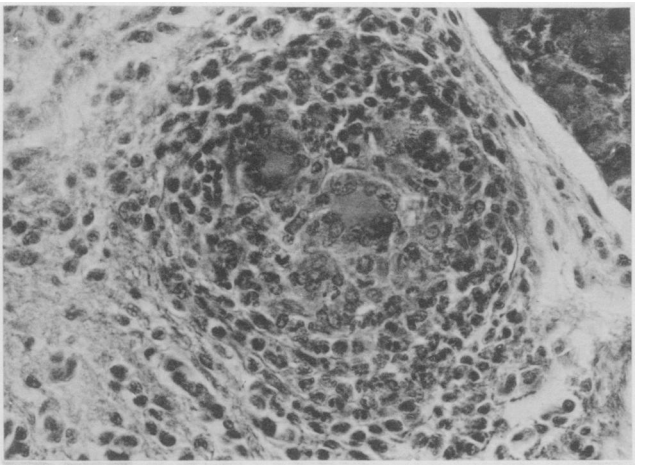
Fabyan B. Abortus



1

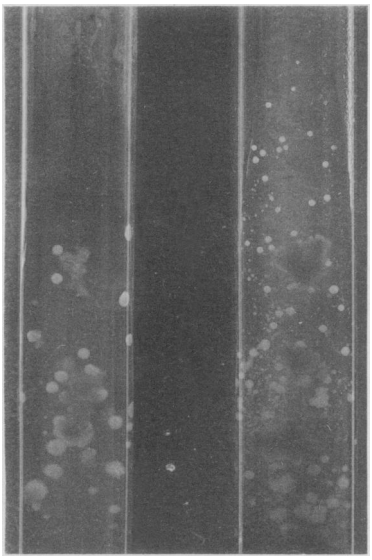


2

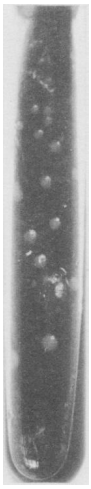


3

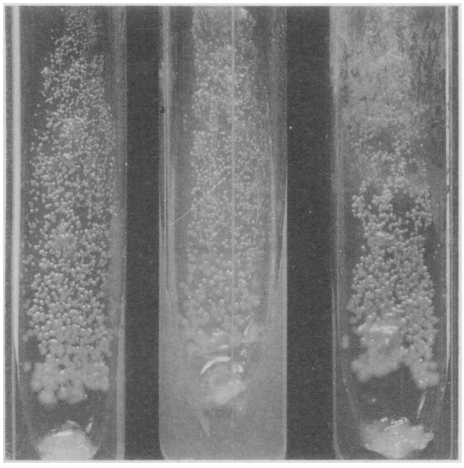
Fabyan B. Abortus



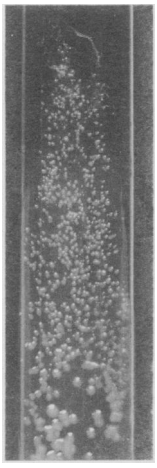
1



2



3



4

Fabyan B. Abortus