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A COMPARATIVE STUDY OF THE BIOLOGICAL CHARACTERS AND PATHOGENESIS OF BACILLUS X (STERNBERG), BACILLUS ICTEROIDES (SANARELLI), AND THE HOG-CHOLERA BACILLUS (SALMON AND SMITH).

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The observations which here follow were begun under the direction of Surgeon-General Sternberg for the purpose of critically comparing both the cultural characters and pathogenic action of his Bacillus X, derived from yellow-fever cadavers, with those of Bacillus icteroides (Sanarelli), which had been recently announced as the specific agent of yellow fever.[1]

During this investigation our attention was attracted to the remarkable cultural resemblances of Bacillus icteroides and the hog-cholera bacillus, which led us to take up a new line of comparative experiments with these bacilli.

In a preliminary note, published in the *Medical News*, April 29, 1899, we have briefly called attention to some of the cultural resemblances of these bacilli and the similarity of the lesions produced in the guinea-pig, rabbit and dog by Bacillus icteroides to those found in these animals after inoculation with the hog-cholera bacillus. We also noted the marked agglutinative reaction which the serum of an animal immunized with Bacillus icteroides exerted toward the hog-cholera bacillus; and we recorded the fact that the former bacillus, when fed to the domestic pig, would bring about an acute fatal infection in which the principal lesion was found in the large intestine.

As the result of the comparative study which we had made at that time of Bacillus X (Sternberg) and Bacillus icteroides (Sanarelli), we expressed the opinion that while the former belonged to the colon group, the latter should be considered as a variety of the hog-cholera bacillus, and hence as a secondary invader in yellow fever.

In this report we propose to give more at length the observations upon which these conclusions are based.

I.

BACILLUS X.

Since the cultural characters of Bacillus X are in striking contrast to those of Bacillus icteroides and the hog-cholera bacillus, we have

[1] Sanarelli. A lecture on yellow fever, with a description of the Bacillus icteroides. *British Medical Journal*, 1897, lii, p. 7.

concluded to give this bacillus separate consideration. The difference in its pathogenic action, as manifested toward the smaller animals, is an additional reason for adopting this course.

The culture of this bacillus which we have used in our comparative experiments was received by Dr. Sternberg from Dr. E. H. Wilson, of the Hoagland Laboratory, Brooklyn, New York, where it had been kept as a stock culture for about four years. The original culture had been isolated by Sternberg,[2] during the summer of 1889, in the city of Havana, from yellow-fever cadavers—Cases Nos. 18 and 28. It had not been isolated by direct culture from the cadaver, but from the serous effusion beneath the skin and from the livers of the guinea-pigs which had died from the subcutaneous inoculation of a small quantity of the blood and crushed liver parenchyma of cases of yellow fever autopsied soon after death. When placed in our hands, Bacillus X had been cultivated on artificial media for about seven years.

Characters of Bacillus X.—*Morphology.*—As originally described by Sternberg, Bacillus X "resembles the colon bacillus in form, but is somewhat larger, from 2 to 4 μ in length by 0.8 to 1 μ in diameter; sometimes occurs in pairs; may grow out into short filaments, not common." It is stated that "in recent gelatine cultures it is often so short an oval in form that it might be mistaken for a micrococcus." The morphological characters of Bacillus X as observed in our cultures agree with this description. The bacillus is decolorized by Gram's method.

Motility.—Bacillus X exhibited active movements in cultures made directly from the yellow-fever cadavers. This motility was not constant and was not observed in cultures which were brought from Cuba to the United States. Sternberg states: "These movements were usually not observed in all the bacilli in a field under observation, but one and another would start from a quiescent condition on an active and erratic course, sometimes spinning actively upon its axis, and again shooting across the field as if propelled by a flagellum." It was noted that cultures passed through the guinea-pig were more apt to be motile.

We have not observed motility in this bacillus. For the purpose of reviving its active movements we have repeatedly passed it through guinea-pigs, but without success as regards the restoration of motility. When stained by suitable methods, however, such as Pittfield's, flagellate forms, few in number, may be seen. The number of flagella varies from 1 to 6.

[2] Report on the Etiology and Prevention of Yellow Fever. Washington, 1890, pp. 187–200.

Bouillon.—Bacillus X grows freely in this medium, which becomes heavily clouded without the formation of a surface pellicle. After 48 to 72 hours a small, somewhat dense deposit is found at the bottom of the tube. The bacillus grows rapidly in bouillon to which glucose, lactose or saccharose has been added, with the appearance of numerous small gas bubbles at the surface of the liquid.

A disagreeable odor, resembling that of the colon bacillus, is given off by all cultures of Bacillus X.

Gelatine.—In stick-cultures, the growth resembles that of the colon bacillus. The surface growth may consist of a soft milk-white layer, or may be quite thin and dry, with very irregular outline. The tendency of the growth is to overspread the surface of the medium. Abundant development occurs all along the line of puncture and presents a yellowish color by transmitted light. Delicate tufted outgrowths frequently appear along the line of puncture.

In gelatine plates, the deep colonies, after 24 hours at 20° C., may readily be detected with the eye as minute white points. Under the low power they appear as round, finely granular masses, of a pale yellow color. Older deep colonies become opaque, change their color to a dark brown and often show a tendency to become lobulated. Surface colonies after 24 hours are of a pale or faintly yellow color, finely granular throughout, with irregular margin. Some of these colonies may show a delicate veining. After 48 hours, surface colonies are 2 to 3 mm. in diameter, and of a pearl-gray color by reflected light. Under the microscope they show dense granular, brown centres, surrounded by a lighter colored portion that is distinctly veined. The central part of the colony shades off gradually into a pale, spreading, irregular margin. In the smallest surface colonies, the peripheral portion is broader, paler and slightly indented, while the central part has a yellowish tinge and is delicately veined, giving a striking resemblance to young colonies of the colon bacillus. Old surface colonies, by reflected light, are glistening, raised, of a milk-white color and irregular outline. Under the low power they show a dark-brown, granular centre surrounded by a colorless, irregular margin.

Acid Potato-Gelatine.—The bacillus grows freely as a dense, grayish-white, shining layer, slightly raised above the surface.

Acid Potato-Gelatine Containing 1 per cent KI.—Develops readily on this medium.

Agar-Agar.—In stroke cultures the growth appears as a moist, soft, grayish-white, raised layer which, while spreading to some extent on either side of the line of inoculation, does not show a tendency to over-

spread the medium. The margins are frequently thin and notched. This growth on agar closely resembles that of the colon bacillus.

On agar plates, the deep colonies are round, oval or whetstone-shaped, of a brownish color, finely granular and sometimes show a tendency to lobulation.

At thermostat temperature, after 24 hours, the surface colonies are from 2 to 4 mm. in diameter and by reflected light of a grayish, or milk-white color. Under the low power they are finely granular, of a brownish-yellow color and often show a distinct veining; the margin is irregular in outline. These spreading colonies resemble those of the colon or typhoid bacillus.

Potato.—Grows rapidly on potato; at first, as a thin, moist, slightly brownish or yellowish-brown layer, with tendency to overspread the surface of the medium; later, the growth while remaining quite moist, becomes more raised and presents a dirty-grayish, or grayish-yellow color; no gas bubbles are to be seen at any time.

Milk.—Grows well in this medium, which is generally coagulated at the end of 2 to 6 days at thermostat temperatures. Litmus milk is decolorized and slightly reddened at the end of 24 hours. At the expiration of 48 hours coagulation has begun, and is generally complete after 3 to 6 days. We have observed that cultures which have recently been passed through animals have, as a rule, brought about the coagulation of milk more slowly.

Fermentation Tube; Glucose Bouillon 1 per cent.—Grows rapidly in glucose bouillon, producing prompt and marked fermentation. At the end of 24 hours, the amount of gas present in the closed branch of the tube varies from 4.5 to 6 cm. There is but little increase in the quantity at the expiration of 48 and 72 hours. Reaction strongly acid.

Lactose, 1 per cent.—This medium is also promptly fermented. At the end of 24 hours the quantity of gas equals 4.5 to 5 cm. This is only slightly increased after 48 to 72 hours. Reaction acid.

Saccharose, 1 per cent.—This medium is at first slowly fermented. The quantity of gas at the end of 24 hours does not exceed 0.5 cm. During the second day the fermentation is more rapid, amounting to about 3 cm. at the end of 48 hours. This may be slightly increased at the end of 72 hours. Reaction acid.

Indol Reaction.—In Dunham's solution Bacillus X produces indol. This reaction is not seen after the addition of sulphuric acid alone, but requires the addition of a nitrite in order to bring about the change in color, which is less intense than is usually seen with the colon bacillus.

Pathogenesis.—Our experiments have been confined to guinea-pigs, rabbits and dogs.

Guinea-pigs. (*a*) *Subcutaneous Inoculation.*—Sternberg found that this bacillus was pathogenic for guinea-pigs when injected into the cavity of the abdomen in doses of 2 to 3 cc. of a culture in agua coco, but that the subcutaneous injection of 0.5 to 1 cc. gave negative results in 11 out of 13 guinea-pigs inoculated. Two died within 24 hours.

The fact that the animals were not kept under observation longer than one week will probably account for the failure to record a larger number of deaths from the subcutaneous injection of Bacillus X, since we have found that this bacillus when injected subcutaneously in quantities varying from 1 cc. to 5 cc. of a 24-hour bouillon culture, is almost always pathogenic for guinea-pigs.

The animals for a few days following the injection may appear quiet and refuse food, which is frequently the case; or they may exhibit no symptom of illness. Although the guinea-pigs do not appear to be sick, carefully recorded observations show that there is a steady loss of weight. The period within which the animals die, however, is a very uncertain one, varying from 1 to 7 weeks. Exceptionally, a guinea-pig has survived the subcutaneous injection of 1 to 2 cc. of Bacillus X.

We give brief protocols of some of our experiments:

Exp. I.—July 16, 1897. Guinea-pig No. 315; weight 300 grammes. Injected subcutaneously with 2 cc. of a 24-hour bouillon culture of Bacillus X (original). Death after 7 days. Site of inoculation marked by necrosis of skin, $2\frac{1}{2} \times 1\frac{1}{2}$ inches. Subcutaneous tissues moist and injected. Inguinal lymph glands enlarged and pale. Liver congested, markings indistinct. Spleen small, pale. Kidneys swollen and injected. Adrenals enlarged and hyperæmic. Mucous membrane of small intestine congested. Cultures from blood, liver and urine positive, a few colonies of Bacillus X being obtained from each of these sources. Cultures from bile, spleen and kidney negative.

Exp. IV.—August 9, 1897. Guinea-pig No. 348; weight 377 grammes. Injected subcutaneously with 4 cc. of a 24-hour bouillon culture from liver of dog No. 347. Death after 37 days. Animal emaciated. Axillary and inguinal glands enlarged. Parietal peritoneum injected. Liver congested. Spleen normal. Kidneys and adrenals enlarged and hyperæmic. Lungs deeply congested. Stomach contains a small quantity of mucoid, blood-stained material; mucous membrane generally injected. Mucous membrane of small intestine deeply congested throughout. Large intestine and appendix normal. Cultures from blood, spleen, liver, kidney, bile and urine all negative.

After repeated passage through the peritoneal cavity of guinea-pigs, the virulence of the original culture of this bacillus was perceptibly in-

creased, so that death most often occurred within 8 to 9 days after the subcutaneous injection of 1 cc. of a 24-hour bouillon culture. In order, however, to bring about a fatal result in less time it was necessary to inject a larger quantity of this more virulent culture.

When death has occurred within 3 to 7 days, Bacillus X has been recovered from the site of inoculation in large numbers, and in fewer numbers from the blood and internal organs as well as from the urine and bile. From guinea-pigs that have survived longer than 8 days we have failed to recover this bacillus in culture, except in one instance where death occurred on the 26th day after injection. In this case cultures from the liver and bile only were positive.

(*b*) *Intraperitoneal Inoculation.*—We have found Bacillus X to possess more marked pathogenic action for guinea-pigs when injected into the peritoneal cavity. While the original Hoagland culture, injected by this method in doses of 1 to 5 cc., did not produce death until after considerable intervals, cultures recently obtained from animals sufficed to bring about a fatal result at shorter periods. As a rule, death occurs within 24 hours in medium-sized guinea-pigs injected intraperitoneally with 1 cc. of the more virulent cultures. The animal is profoundly affected within a few hours after the injection, remains quiet, and dies at intervals varying from 10 to 24 hours. At autopsy there are found serofibrinous peritonitis and marked congestion of the abdominal viscera.

Exceptionally, this quantity of virulent culture has failed to produce death in guinea-pigs weighing 500 grammes until after a period of 3 or more weeks.

From animals that have resisted the peritoneal injection longer than 8 days, we have generally failed to obtain the bacillus in culture. From those dying within shorter intervals, and especially within 24 hours, the organism has been recovered in pure culture from the blood, liver, spleen, kidney, bile, urine and abdominal cavity. The number of colonies has been more abundant from the abdominal cavity than from other sources; hence, although the blood and internal organs may be invaded by Bacillus X in animals that are overwhelmed with large doses, our experience has shown that where life is more prolonged, the number of bacilli undergoes rapid reduction in the abdominal cavity as well as in the blood and organs.

Our observations have led us to believe, therefore, that Bacillus X does not multiply to any considerable extent in the bodies of inoculated guinea-pigs, but that death is brought about sooner or later by the action of its toxic products. To this we shall again recur.

Rabbits. Sternberg observed that Bacillus X, when injected into the

cavity of the abdomen in doses of 1 to 10 cc., was quite pathogenic for rabbits, the animal frequently dying within a few hours. This applied to

TABLE I.

RABBITS INOCULATED WITH BACILLUS X.

No.	Weight in grammes.	Method.	Quantity.	Result.	Lesions.	Cultures.
1	642	Subcutaneous.	2 cc. 24-hour culture.	Death after 3 days.	Abscess at site of inoculation; lymph glands enlarged; liver engorged; spleen slightly enlarged; kidneys congested; adrenals normal; mucosa of upper part of small intestine congested.	Wound: numerous colonies. Spleen and urine: few colonies. Blood, liver, bile, and kidney negative.
2	1040	Subcutaneous.	5 cc. 24-hour culture.	Death after 3 days.	Same lesions as noted above.	Cultures from wound and urine positive; other sources negative.
3	600	Abdominal cavity.	5 cc. 24-hour culture.	Death after 25 hours.	General serofibrinous peritonitis; punctate hæmorrhages over small and large intestines; liver engorged; spleen small; kidneys congested; thymus gland much enlarged.	Cultures positive.
4	525	Abdominal cavity.	2 cc. 24-hour culture.	Death after 11 days.	Animal emaciated; no peritonitis; liver congested; spleen small; intestine normal.	Cultures negative.
5	527	Intravenous.	3 cc. 24-hour culture.	Death after 7 days.	Rabbit emaciated; liver and kidneys congested; spleen small; increased fluid contents in small intestine, its mucosa slightly injected.	Cultures negative.

cultures recently isolated from yellow-fever cadavers. Of 27 animals inoculated, only 3 recovered. Subcutaneous and intravenous inoculations with small doses (2 to 4 minims) of a fluid culture were negative.

One cc. of an agua coco culture, subcutaneously, was fatal at the end of 30 hours. He concludes that the negative results obtained by subcutaneous and intravenous inoculations show that Bacillus X does not induce septicæmia in the rabbit, and the fatal result of intraperitoneal injections is due rather to its toxic products than to invasion of the blood. Direct examination of the blood of rabbits which had succumbed within a few hours, showed very small numbers of the bacilli. No enlargement of the spleen was found.

We have found this bacillus pathogenic for rabbits in quantities of 1 to 5 cc., whether injected subcutaneously, into the cavity of the abdomen, or intravenously.

The results of the inoculation of rabbits with this bacillus are shown in Table I.

The intervals within which rabbits have died after the subcutaneous inoculation of Bacillus X have varied considerably. In some cases the animals have survived from 16 to 43 days. In a few experiments the only effect has been the local formation of pus at the site of inoculation. The result of cultures would indicate that this bacillus, when inoculated subcutaneously in rabbits, shows little tendency to invade the blood. While large quantities (5 cc.) injected into the abdominal cavity generally cause a fatal peritonitis and death within 24 hours, smaller quantities (1 to 2 cc.) do not bring about a fatal result until after a considerable interval (1 to 3 weeks). Cultures from animals that have survived longer than one week have generally proved sterile. In a few young rabbits inoculated intravenously, death has occurred within 36 to 43 hours with positive cultures from blood and organs; while in older animals a fatal result has generally been brought about after the lapse of from 12 to 20 days with negative cultures from all sources. In all chronic cases emaciation has been a prominent feature.

The results of the inoculation of rabbits with Bacillus X indicate that, as with the guinea-pig, this organism does not multiply to any extent in the blood, but that death is brought about by toxæmia. This conclusion is supported by the results obtained by the inoculation of rabbits and guinea-pigs with filtrates from cultures of Bacillus X grown for a period of 40 days at thermostat temperature. The results are briefly recorded in Table II.

Aside from a small quantity of blood-stained serum in the abdominal cavity, no post-mortem lesion was noted. These experiments serve to show that death may be rapidly produced in guinea-pigs and rabbits by the toxic substances contained in old filtered cultures of Bacillus X.

Dogs.—Our experiments with these animals have been confined to the

intravenous injection of Bacillus X, and were made for the purpose of comparing the results with those obtained by the intravenous inoculation of Bacillus icteroides in dogs. We have inoculated by this method 11 dogs, of which number 6 have died and 5 recovered. We submit protocols of a few of these experiments.

TABLE II.

GUINEA-PIGS AND RABBITS INJECTED WITH FILTRATES FROM CULTURES OF BACILLUS X.

Animal.	Weight. in grammes.	Quantity.	Method.	Result.	Cultures.
Guinea-pig.	276	5 cc.	Abdominal cavity.	Death after 7 hours.	Sterile.
Guinea-pig.	263	5 cc.	Abdominal cavity.	Death after 12 hours.	Sterile.
Guinea-pig.	638	5 cc.	Subcutaneous.	Death after 15 days.	Sterile.
Guinea-pig.	642	5 cc.	Subcutaneous.	Recovery.	
Rabbit.	485	10 cc.	Abdominal cavity.	Death after 10 hours.	Sterile.
Rabbit.	580	15 cc.	Abdominal cavity.	Death after 35 hours.	Sterile.
Rabbit.	840	20 cc.	Abdominal cavity.	Recovery.	

Exp. I.—July 16, 1897, 11.30 A. M. Dog No. 318; weight 13 lbs. Inoculated into ear vein with 10 cc. of a 24-hour 1 per cent glucose bouillon culture of Bacillus X (original). When returned to its cage, the dog appeared dejected and sick. 1 P. M., animal drowsy; vomited freely at this hour. 2.30 P. M., again vomited a frothy mucous fluid. July 17, 9 A. M., dog quiet, rectal temperature 101° F. During the day became brighter and took its food. Recovered.

Exp. II.—July 23, 1897, 1.20 P. M. Young dog No. 327; weight 13 lbs. Injected into the ear-vein with 5 cc. of a 24-hour glucose bouillon culture of Bacillus X from blood of rabbit No. 314. Lively, active dog. At 1.35 P. M. animal appears quite sick, is lying on its side; does not respond to the voice. 2.05 P. M., vomits freely partly digested food, followed by fluid action from the bowels with tenesmus. 2.23 P. M., small, brown, watery stool; this repeated after a short interval with marked tenesmus. 2.51 P. M. and 3.05 P. M., vomits with much effort a small quantity of grayish frothy fluid mixed with mucus. 3.30 P. M., again vomits. 4 P. M., dog lies on its side with extremities extended; temperature at this hour 104.1° F. Temperature previous to injection 101° F. Death 6 P. M., 28½ hours after the inoculation.

Autopsy.—Thorax: Thymus gland large, dark red in color, with few

small hæmorrhagic areas on surface. Mediastinal glands swollen, of a dark-red color. Both layers of pericardium injected. Small hæmorrhagic area over right auricle, which is distended with blood; left auricle empty. Right ventricle distended; left ventricle contracted; numerous hæmorrhagic areas beneath endocardium in this ventricle; valves normal. Myocardium pale red. Both lungs congested; lower lobe of right lung œdematous. On cut section, reddish serous fluid exudes freely. Small hæmorrhagic areas under the visceral pleura over the lower lobe right lung.

Abdomen: Omentum injected. Numerous small hæmorrhages under serous coat of small intestine. Liver mottled, pale and red; light areas most extensive; outlines of lobules distinct; central veins appear injected; peripheries of lobules of pale, yellowish color. Spleen enlarged, dark red, firm. Adrenals, small, pale. Gall-bladder moderately full of dark brownish bile. Kidneys enlarged; cortex swollen, pale; pyramids injected. Stomach contains small quantity of bile-stained fluid; mucous membrane over greater curvature dark red in color; injection uniform. Small intestine, duodenum and upper part of jejunum contain considerable quantity of soft, black, tarry material; mucous membrane pale throughout. Peyer's patches not swollen. Beginning at the ileocæcal valve and extending to anus the rugæ of mucous membrane of large intestine are the seat of marked hæmorrhages which extend into the submucosa. Small quantity of fluid blood in the large intestine. Bladder contracted; contains about 4 cc. of albuminous urine.

Cultures from the blood, liver and spleen give numerous colonies of Bacillus X; urine and bile negative.

Exp. III.—August 6, 1897. Dog, weight 10 lbs. Injected at 2.45 P. M. with 13 cc. of a 72-hour 2 per cent lactose bouillon culture of Bacillus X from rabbit No. 338. 3.05 P. M., animal restless; vomits partially digested food with much retching; 3.15 again vomits, followed by watery stool mixed with mucus; considerable tenesmus; 4 P. M., rectal temperature 96.4° F. Before the injection, temperature 101° F. Found dead the following morning at 8 o'clock, less than 18 hours after inoculation.

Autopsy.—Thorax: Lungs slightly injected. Several small hæmorrhagic areas beneath pleural surface of upper lobe of right lung. Subendocardial hæmorrhages in the left ventricle.

Abdomen: Liver of a pale, grayish color; cut surface dry, markings indistinct. Spleen slightly enlarged, dark red, soft. Kidneys swollen; on cut section injected throughout. Stomach contains about 200 cc. of fluid blood, mucous membrane dark red throughout. Much fluid

blood in both small and large intestine. Mucous membrane of small intestine swollen, dark red in color. Less injection in the large intestine. Bladder contracted, empty.

Cultures from the blood, liver, spleen and urine positive; bile and kidney negative.

In another experiment with a young dog, weight 7½ lbs., injected into the ear-vein with 3 cc. of a 24-hour bouillon culture from dog 347, death occurred after 11 hours, with intense hæmorrhagic gastro-enteritis.

Four of our dogs have died from a single injection of Bacillus X, one after a second injection and one after repeated injections.

The clinical picture has been the same in all of the dogs injected, namely, vomiting, increased action of the bowels with rectal tenesmus and marked prostration. No after effects have been observed in dogs that have recovered, the animals appearing to regain their appetite and strength within two or three days.

Lesions. (*a*) *Macroscopic.*—We have already sufficiently indicated, in the foregoing protocols, the gross lesions to be seen at autopsy in animals inoculated with Bacillus X. Briefly recapitulating these, we may say that in guinea-pigs the lesions are injection of the subcutaneous vessels at the site of inoculation, or sometimes local abscess or sloughing of the skin; enlargement of the inguinal and axillary lymph-glands; injection of the parietal peritoneum and of the mucous membrane of the stomach and small intestine; congestion of the lungs, and, as a rule, engorgement of the liver and kidneys. Hyperæmic swelling of the adrenals has always been present. The spleen is generally small, pale and firm, although occasionally slightly enlarged and dark in color.

In the rabbit, aside from local abscess-formation at the site of inoculation and swelling of the lymph-glands, the most prominent and constant post-mortem finding has been congestion and swelling of the mucous membrane of the small intestine, accompanied by increase in the fluid contents of the small bowel, and sometimes with areas of hæmorrhage beneath its serous coat.

In animals dying within a short interval following the inoculation, the liver and kidneys have been congested, while the spleen has been small or only slightly enlarged. Enlargement of the thymus gland has occasionally been noted.

In both guinea-pigs and rabbits, intraperitoneal inoculation has usually been followed by a serofibrinous peritonitis.

In dogs the hæmorrhagic lesions have been much more pronounced than in the rabbit or guinea-pig. These have consisted of numerous small hæmorrhages beneath the endocardium, under the visceral layer

of the pleura and scattered over the surface of the small intestine. In addition, intense engorgement of the mucous membrane of the stomach and small intestine, with frank hæmorrhage into the lumen of these viscera has occurred in several cases. In one instance this congestion with hæmorrhage into the submucosa was confined almost entirely to the large intestine. Hæmorrhagic areas involving the vesical mucous membrane have also been noted in dogs. The liver has been pale, or mottled yellow and red, while the kidneys have been swollen and injected. The spleen has been slightly enlarged and soft, or small and firm; the adrenals pale and small.

(*b*) *Microscopic.*—For the purpose of microscopic examination, tissues have been hardened in absolute alcohol, 5 per cent formalin, Orth's fluid and Flemming's osmic solution. Also fresh frozen sections of the several organs have been examined in normal salt solution for the purpose of detecting any fatty change.

As the result of careful microscopic examination, there are few lesions to record in the guinea-pig and rabbit. No changes from the normal have been found in the spleen. Sections of the lymph-glands and of the adrenals in guinea-pigs show marked dilatation of the blood-vessels of both cortex and medullary portion. No finer lesions have been discovered in these structures. The same engorgement of the lymph-nodes has been observed in the rabbit, whereas the adrenals have shown no change. As in other acute intoxications, moderate granular and fatty degenerations have been seen in the hepatic cells, but in this organ the most prominent feature has consisted in engorgement of the intralobular capillaries. In some instances this dilatation of the capillaries has been so great as to lead to compression and narrowing of intervening rows of cells. Circumscribed hæmorrhages in the liver have also been observed. Small foci of coagulative necrosis were found in the liver of one rabbit that died on the 20th day after intravenous injection. These areas of necrotic cells were small, few in number, and situated within the lobules. No changes in the kidney have been observed save dilatation of the blood-vessels and cloudy swelling of the secreting epithelium in acute cases.

In dogs that have died within a short time after intravenous injection (11 to 13 hours) no lesions were observed in sections of the liver other than granular degeneration of the cells, but in animals that have died after longer intervals (28½ hours to 3½ days), in addition to some fatty degeneration there is present extensive coagulative necrosis of the hepatic cells. The distribution of the necrotic areas, as in other acute infections, is quite variable. While some of these are situated about the

central vein, involving part or the entire circumference of the latter, other foci are located within the lobules. At times an entire lobule is included in the necrosis; again, parts of adjacent lobules. Within these areas the liver-cells stain brightly with eosin and appear as swollen, quite refractive bodies, some of them containing minute fat-drops. No nucleus can be seen in many of these cells, or it may appear as a pale shadow, or much contracted and staining deeply with hæmatoxylin. In some of the cells the nucleus has undergone fragmentation. Within the capillaries of the affected area, a few small cells with round, normally staining nuclei are to be seen. The number of leucocytes within the capillaries does not appear to be increased, and there is an entire absence of any invasion of the necrotic foci by these cells. Necroses of single cells were also observed in sections of the dog's liver. The changes in the kidney were limited to parenchymatous degeneration of the secreting epithelium. The gross lesion in the intestine of the dog consisted of swelling and intense injection of the mucous membrane with hæmorrhages. The microscopical changes were confined to a rather free desquamation of the epithelium covering the villi and that lining Lieberkühn's follicles, and marked dilatation of the blood-vessels of the mucosa and submucosa. Small hæmorrhages into the superficial layer of the submucosa were also observed.

Referring to the gross lesions found in guinea-pigs and rabbits that have died after inoculation with Bacillus X, it will be seen that these agree with the appearances found by Escherich,[3] Emmerich,[4] Blachstein,[5] and other observers who have inoculated these animals with bacilli belonging to the colon group. The varying length of time during which the animals have lived after receiving the inoculation and the negative result of cultures in those that have survived for considerable periods, also agree in the main with our results.

We have not been able to find in the literature any reference to the intravenous injection of dogs with a member of the colon group. Emmerich, however, observed in dogs that were injected subcutaneously with considerable quantities of his Bacillus Neapolitanus, repeated vomiting, profuse diarrhœa and prostration. Death occurred in one of three thus inoculated, with ulceration of the mucous membrane of the small intestine and enlargement of the solitary follicles.

For the purpose of comparative experiment, we have inoculated one

[3] Arbeiten aus dem pathologischen Institut zu München, p. 68. Stuttgart, 1886.

[4] Untersuchungen über die Pilze der Cholera asiatica. *Arch. f. Hygiene*, 1885, iii, p. 313.

[5] *Bulletin of the Johns Hopkins Hospital*, 1891, ii, p. 96.

dog intravenously with 5 cc. of a 4-hour plain bouillon culture of Bacillus coli communis, recently isolated from the abdominal cavity of a patient who had died from general peritonitis. The clinical symptoms observed in this dog corresponded to those seen in dogs injected with Bacillus X, namely, repeated vomiting, increased action of the bowels with rectal tenesmus, prostration and rise of temperature. This animal, although apparently quite sick for two days, made a good recovery.

That Bacillus X, after cultivation on artificial media for about seven years was still virulent for the smaller animals, appeared to us as hardly to be expected in a member of the colon group. It seems, however, that this retention of virulence has been shown by certain colon bacteria which have been kept in cultivation for even longer periods. Thus Novy[6] found that 1 cc. of a 24-hour bouillon culture of Emmerich's bacillus which had been cultivated on artificial media for a period of 10 years would bring about death within 18 hours when injected into the abdominal cavity of the guinea-pig.

Recalling the important biological characters of Bacillus X, viz., the slight motility observed in recently isolated cultures; the appearance of colonies in gelatine; the coagulation of milk; the fermentation of glucose, lactose and saccharose; the production of indol and its decolorization by Gram's method, we think that these, together with the results obtained from animal experimentation, are sufficiently distinctive to warrant us in placing this bacillus in the colon group.

II.

BACILLUS ICTEROIDES AND THE HOG-CHOLERA BACILLUS.

The culture of Bacillus icteroides with which we have made the majority of our experimental observations, was obtained by Dr. Sternberg from the laboratory of Professor Roux, in Paris. When received by us, it bore the label of the Laboratory of Hygiene of the University of Montevideo. This culture we have transplanted from time to time on agar-agar, and have labeled it "Bacillus icteroides, original."

We have also received, through the courtesy of Dr. A. Agramonte, Acting Assistant Surgeon, U. S. Army, two cultures of Bacillus icteroides, one of which was isolated from the cadaver of Private Patrick Smith, 8th U. S. Infantry, who died in Havana, and concerning the

[6] The Etiology of Yellow Fever. *Medical News*, 1898, lxxiii, p. 330.

diagnosis of whose case there was much uncertainty on the part of his medical attendants; the other, from a yellow-fever cadaver at Santiago, Cuba. These latter cultures we have designated "Bacillus icteroides, Havana," and "Bacillus icteroides, Santiago," respectively.

The cultures of the hog-cholera bacillus with which we have made comparative observations, were obtained from the Bureau of Animal Industry, Washington, D. C., through the courtesy of Dr. E. A. de Schweinitz, and from the Pathological Laboratory of the Johns Hopkins University, through the kindness of Dr. Harvey Cushing. These cultures we have designated "Hog-cholera No. 1," and "Hog-cholera No. 2," respectively.

Comparison of the Characters of Bacillus Icteroides and the Hog-cholera Bacillus.

Each of these organisms is a facultative anaërobic bacillus which decolorizes by Gram's method, and does not liquefy gelatine.

Morphology.—According to Sanarelli,[7] B. icteroides appears as short rods, with rounded extremities, generally united in pairs, from 2 to 4 μ in length and, as a rule, twice as long as broad. Salmon and Smith[8] describe the hog-cholera bacillus as consisting of short rods, round at the ends, chiefly in pairs, measuring from 1.2 to 1.8 μ in length by 0.6 μ in breadth. The size of each of these bacilli, however, varies much according to the particular medium on which it is grown. In cultures on potato, much longer and thicker forms are shown by both.

Motility.—Both of these bacilli show very active movements.

Sanarelli gives the number of flagella for B. icteroides as 4 to 8. Moore,[9] who carefully studied the flagella of the hog-cholera bacillus, from a large number of counts places the average number of flagella as 3.3; the majority of the flagellate forms showed 3 to 6 flagella; some as many as 8 to 11 flagella.

Bouillon.—We have noticed no difference in the growths of B. icteroides and of the hog-cholera bacillus in flesh-peptone bouillon. This medium is only moderately clouded by both of these bacilli. No surface pellicle, as a rule, is to be seen. In old bouillon cultures (2 to 3 weeks), an appreciable deposit may be observed at the bottom of the tubes.

In bouillon to which glucose or lactose has been added, there is a freer growth of both of these bacilli, with the formation of fine gas bubbles at the surface of the glucose bouillon.

[7] Etiologia e patogenesi della febbre gialla. *Il Policlinico*, 1897, iv –M., p. 397.

[8] Hog-cholera. Bureau of Animal Industry, Washington, 1889, p. 64.

[9] Wilder Quarter-century Book, p. 339. Ithaca, 1893.

We have not detected any definite odor in cultures of these bacilli.

In old bouillon cultures of B. icteroides and of the hog-cholera bacillus, many long rods are to be seen; some of these are swollen at one or both ends or in the middle (involution forms).

Gelatine.—In stick cultures of B. icteroides there is a slow growth along the entire line of puncture which appears as a delicate white line. The surface growth is seen as a thin, transparent layer, which shows little tendency to spread. This applies to cultures of B. icteroides, original, and B. icteroides, Havana.

This growth in gelatine characterizes also the hog-cholera bacillus, except that the surface growth is somewhat thicker, more irregular in outline, and shows more tendency to spread. In this respect, B. icteroides, Santiago, agrees with the hog-cholera bacillus.

In gelatine plates, after 24 hours at 20° C., the colonies of B. icteroides are invisible to the naked eye. As seen under the low power, they are round, colorless and finely granular. In crowded plates, some of the deep colonies without increasing much in size, become opaque and dark in color, appearing as round, almost black masses; others show a slight radial striation; still others a brownish tinge. Frequently the deep colonies present a dark centre surrounded by a lighter peripheral zone. Surface colonies show little tendency to spread. Under the low power, they are generally circular in outline, with sharply defined, smooth margin, although the latter may be irregular and indented. They present, as a rule, a central nucleus, surrounded by a colorless, granular zone which extends quite to the margin of the colony. In older colonies (4 to 10 days), the margin frequently becomes clearer and quite refractive, while the central part takes on a slight yellowish tinge.

The colony with central nucleus surrounded by a colorless, granular zone, with or without narrow, clear margin, may be taken as the typical surface colony of B. icteroides.

Surface colonies which are less often seen show a delicate radial or undulating striation extending from the central portion toward the periphery of the colony, constituting the so-called atypical colony. Sometimes this striation is made up of very numerous dark lines radiating from the centre to the periphery of the colony and giving the latter an appearance totally unlike the ordinary surface colony. Later these colonies may lose their striation entirely and show no distinctive markings. To the naked eye, well-developed surface colonies, by reflected light, are round, sharply defined, raised and glistening, and have been aptly compared to droplets of boiled starch or mucus. They present a delicate bluish translucence.

Both the deep and surface colonies of the hog-cholera bacillus in gelatine plates show the closest resemblance to those of B. icteroides, the rate of growth of B. icteroides, original, B. icteroides, Havana, and of hog-cholera No. 1 has been the same; that is, colonies are invisible to the eye after 4 hours at 20° C., whereas the colonies of B. icteroides, Santiago, and of hog-cholera No. 2, can just be distinguished after this interval as very minute white points. Under the low power, deep colonies of the hog-cholera bacillus are round, colorless and finely granular, or they may show a slightly brown color. With age, some of these colonies in the depth of the gelatine take on a dark color, and sometimes become quite black. We do not think that attention has heretofore been called to this darkening of deep colonies of the hog-cholera bacillus in gelatine. Surface colonies, as a rule, are smooth in outline, though they may be irregular and present the appearances already described for typical colonies of B. icteroides.

Colonies with undulating striation are also occasionally seen. We have not observed, however, in gelatine plates of the hog-cholera bacillus, those atypical surface colonies with dark, radial striation [10] such as we have noted for B. icteroides. With this exception, we have observed no differences in colonies of these bacilli in gelatine plates.

Acid Potato Gelatine (natural acidity).—In stab and slant cultures there is a feeble development, after several days, all along the line of puncture or stroke. This applies to our several cultures of B. icteroides and the hog-cholera bacillus.

Acid Potato Gelatine with 1 per cent KI.—A very scant development also occurs in this medium with B. icteroides and the hog-cholera bacillus. The acidity of the medium used was such that 2 cc. of a decinormal sodium hydroxide solution were required to render 10 cc. neutral to litmus.

Agar-agar.—In stroke cultures on agar-slants grown at 35° to 37° C., B. icteroides forms a thin, moist, grayish-white layer. At 20° to 22° C., this growth is somewhat thicker and more convex.

The growth of the hog-cholera bacillus on agar-slants is quite similar to that of B. icteroides.

The Sanarelli bacillus recently obtained from cases of yellow fever, when grown as isolated colonies on agar-slants, first at 37° C., for 12 to

[10] Since the above was written we have received from the Bureau of Animal Industry a culture of the hog-cholera bacillus recently isolated during an epidemic of hog-cholera at Fremont, Nebraska, which gives in gelatine plates atypical surface colonies with radial striation which cannot be distinguished from colonies of Bacillus icteroides.

24 hours, and afterwards at 20° to 28° C., will, according to its discoverer, show characteristic colonies which serve to distinguish it from all other bacteria. Under these conditions the colonies show two distinct zones, a central, flat, transparent area surrounded by a thick, prominent opaque zone, giving to the whole colony the appearance of a drop of sealing-wax.[11] As the colonies grow older, the external opaque zone becomes more transparent, and nearly disappears, while the central part remains as an opaque body embedded in it. In cultures, however, that have been repeatedly passed through animals, these characteristic colonies are less often seen.[12]

Novy[13] states that this growth of the Sanarelli bacillus is always to be seen in isolated colonies grown on agar-slants, first at 39° for 20 to 24 hours, and afterward at 16° C. According to the same author, it is important that these temperatures should be observed, and that the medium should be distinctly alkaline.

In our experience, this characteristic appearance of colonies of B. icteroides on agar-slants grown as suggested by Sanarelli has not been constant. In our earlier experiments it was present in about 30 per cent of the cultures taken from the organs of inoculated guinea-pigs, rabbits and dogs; that is to say, a few isolated colonies in a tube presented this typical appearance. In our later cultures we have entirely failed to observe it. This failure to obtain the characteristic colony on agar-slants has also been noted by P. Foa[14] and by de Lacerda and Ramos.[15]

The appearance of the thin central area, surrounded by a thicker opaque peripheral zone, is not, however, peculiar to B. icteroides, since we have occasionally observed the same appearance in isolated colonies of hog-cholera No. 1 grown on agar-slants, first at thermostat, and afterward at room temperatures. We have not observed any disappearance of the external thicker zone in colonies of the hog-cholera bacillus. Theobald Smith[16] has already described and illustrated the peculiar appearance of concentric zones in isolated surface colonies of the hog-cholera bacillus grown in gelatine Esmarch rolls. These he considered "very likely due to changes of temperature in the laboratory, alternately retarding and augmenting the growth."

[11] *Policlinico*, pp. 428 and 429.

[12] *Ibid*, p. 431.

[13] *Medical News*, 1898, lxxiii, p. 329.

[14] Sul bacillo itterode (Sanarelli). *Giornale d. r. Accad. di med. di Torino*, 1898, 4. s., xlvi, pp. 57 and 113.

[15] Le bacille ictéroïde et sa toxine. *Arch. de méd. expér.*, 1899, xi, p. 378.

[16] Hog-cholera. Bureau of Animal Industry, p. 192 and Plate xi, Fig. 2. Washington, 1889.

As usually seen, isolated colonies on agar-slants of B. icteroides and of the hog-cholera bacillus, after 24 hours at 37° C., appear as thin, slightly convex or flattened discs, with smooth margins, and by reflected light present a waxy appearance. They measure 2 to 3 mm. in diameter and possess, as a rule, a faint bluish translucence.

Potato.—According to Sanarelli, B. icteroides appears as a moist, invisible growth on this medium. Our experience has shown that the appearance of the growth on potato is quite variable. When first transferred by us to potato, B. icteroides (original), grew as a moist, thin, brownish layer, with a tendency to spread over the surface of the medium. We have since had occasion to observe that upon different potatoes it may grow as a colorless, moist layer, or as a faint yellowish layer, or that it may show a decided brownish color.

The growth of the hog-cholera bacillus on potato in our hands has also shown decided differences on different potatoes. While, as a rule, it has presented itself as a thin, moist, yellowish or brownish layer, it has frequently appeared as a moist, invisible growth. On parallel potato cultures which we have recently made, our cultures of B. icteroides and those of the hog-cholera bacillus have all shown the same thin, moist, yellowish growth.

Plain Blood Serum.—B. icteroides, original, and B. icteroides, Havana, show only slight development on this medium. The growth is closely limited to the needle stroke and appears as a delicate, slightly raised, almost colorless layer. At the bottom of the stroke, near the surface of the fluid, there may occur a slight expansion in the growth, which is raised and of a dull grayish-white color. B. icteroides, Santiago, shows a much freer development all along the line of inoculation, and appears as a slightly raised, somewhat glistening grayish-white layer.

The appearance of the growth of hog-cholera Nos. 1 and 2 is quite similar to that of B. icteroides, Santiago, there being a free development all along the line of stroke.

On *Loeffler's blood serum* the same relative difference in development takes places as already indicated above for plain blood serum; that is to say, while the growth of B. icteroides, original, and of icteroides, Havana, is exceedingly limited, spreading but little along the line of stroke, that of icteroides, Santiago, and hog-cholera Nos. 1 and 2, is quite free all along the line of stroke, appearing as a transparent, flat, or as a slightly elevated grayish-white layer.

Isolated colonies of B. icteroides, original, and B. icteroides, Havana, on plain and glucose blood serum, appear as small, flat, transparent or slightly grayish colonies which do not exceed 0.5 to 1 mm. in diameter, while those of B. icteroides, Santiago, and hog-cholera bacillus Nos. 1

and 2 are larger, more elevated, of grayish-white color and measure from 1 to 3 mm. in diameter.

This relative difference in the growth of B. icteroides and the hog-cholera bacillus, as shown above, has been quite constant in our cultures on blood serum.

Milk.—This medium is not coagulated by either of these bacilli. The reaction of the milk becomes slightly acid at first, afterwards changing to neutral and later becoming strongly alkaline. The change to neutral and alkaline proceeds a little more rapidly with the bacillus of hog-cholera than with B. icteroides, original, and B. icteroides, Havana.

With B. icteroides, Santiago, the change from acid to alkaline corresponds with our cultures of the hog-cholera bacillus.

With our several cultures of Sanarelli's bacillus and the hog-cholera bacillus, the milk assumes a distinct opalescent appearance after 10 to 14 days at thermostat temperature. Later the medium becomes partly translucent.

Litmus milk is slightly decolorized by both organisms, taking on a somewhat muddy appearance. Later the original blue becomes restored and gradually deepened to an indigo blue.

Fermentation Tube.—The action of B. icteroides and of the hog-cholera bacillus upon the three sugars is the same. Both of these bacilli produce prompt and marked fermentation of glucose. In peptone bouillon containing 1 per cent glucose, there is a prompt appearance of gas during the first day, which is rapidly increased in amount during the second 24 hours, and reaches its maximum on the third or fourth day. The reaction of the bouillon becomes strongly acid. We have seen no appreciable difference in the quantity of gas produced by our cultures of B. icteroides and of the hog-cholera bacillus. The volume of gas represents about one-third of the closed branch of the fermentation tube. In composition this gas consists of CO_2, 1 part; H, 2 parts.

No gas appears as the result of multiplication of either of these bacilli in peptone bouillon containing lactose or saccharose, provided means have been used to exclude all trace of muscle sugar. The reaction of the medium becomes distinctly alkaline.

Indol.—Tested by Kitasato's method, our cultures of B. icteroides and the hog-cholera bacillus give a faint indol reaction.

After discussing the general characters of the hog-cholera group of bacteria, Smith[17] says:

[17] Additional Investigations concerning Infectious Swine Diseases, by Theobald Smith and Veranus A. Moore. Bureau of Animal Industry, Bulletin No. 6, p. 27, Washington, 1894.

"If we attempt to sum up those characters which are to circumscribe the hog-cholera group of bacteria, we are at once confronted by the scarcity of common characters. Pathogenesis, though of great importance from the standpoint of pathology, is probably the last character acquired, and evidently the most variable and most readily lost. If we base the unity of this group on morphological and biological characters, we are likewise met by variations in size, absence of motility, variations in the appearance of the colonies. There are, however, certain underlying characters, as expressed by the behavior of these bacteria in bouillon containing dextrose, saccharose and lactose, which I think will serve as a very important group-character, differentiating such group sharply from the colon group. I would therefore suggest that, for the present, all bacteria whose size approximates that of this group, which do not liquefy gelatine, and whose fermentative properties are the same as those described for this group, should be arranged under it."

A comparison of the cultural characters already given indicates clearly that Bacillus icteroides should be placed in the hog-cholera group of bacteria.

Comparative Pathogenesis.

Both of the bacilli under consideration possess a considerable range of pathogenesis for animals. The hog-cholera bacillus is pathogenic, in varying degree, for mice, guinea-pigs, rabbits, pigeons, dogs and hogs. The lesions occurring in several of these animals are more or less characteristic and have been fully described by various observers. It will be of interest to compare the appearances found in the same animals when inoculated with B. icteroides.

Our observations with the latter bacillus have been generally confined to the inoculation of guinea-pigs, rabbits, pigeons, dogs and hogs. More recently, for comparative purposes, we have inoculated a few white mice subcutaneously with B. icteroides and have also fed the same animals with fluid cultures (0.1 cc.) of this bacillus. While the subcutaneous inoculation of mice is fatal after 2 to 4 days, we have observed that where infection has taken place through the digestive tract, death occurs with considerable regularity after about one week. The duration of the infection, and the gross lesions (enlargement of the spleen, areas of necrosis in the liver, congestion of the kidneys and of the mucosa of the small bowel) correspond closely with those already

recorded by Smith for mice inoculated with cultures of the hog-cholera bacillus.

Guinea-pigs and Rabbits.—These animals are quite susceptible to infection with B. icteroides or the hog-cholera bacillus, whether the culture is introduced beneath the skin, into the peritoneal cavity, into the trachea or intravenously. Infection may also be brought about by feeding moderate quantities of a fluid culture of either bacillus. As regards the degree of susceptibility, the rabbit has been found to succumb to smaller doses of both of these bacilli than the guinea-pig.

Smith[18] succeeded in producing death in rabbits by inoculating them with $\frac{1}{4000000}$ cc. of a bouillon culture of the hog-cholera bacillus. We have found $\frac{1}{1000000}$ cc. of a 24-hour bouillon culture of B. icteroides sufficient to kill rabbits. Of two animals inoculated subcutaneously with this quantity, one died after 11, and the other after 12 days, with the usual lesions at autopsy.

In reference to the infection of guinea-pigs with B. icteroides, Sanarelli states that while the duration varies with the mode of inoculation, the disease follows a cyclical course which is not influenced, as a rule, by the quantity of culture inoculated subcutaneously. By the latter method guinea-pigs die on an average in from 5 to 8 days, the majority on the 7th. Exceptionally, they may die after 48 hours, or survive until 15 to 30 days. In further demonstration of this cyclical course of the disease, Sanarelli has killed inoculated guinea-pigs every 12 hours from the time of inoculation and has carefully studied cultures from the blood and organs, with the result that while a few colonies can be obtained from the spleen and liver after 12 to 24 hours, these organs are sterile from the 2d to the 5th day. On the 6th day, however, there is a sudden general invasion of the blood and organs by B. icteroides which is followed by the death of the animal on the 7th day.

While Bruschettini[19] has confirmed Sanarelli's experience that the duration of the disease in guinea-pigs is not influenced by the quantity of the virus injected, provided the cultures are obtained from the guinea-pig or rabbit, he finds that cultures obtained from the blood of the dog will kill medium-sized guinea-pigs in 2 to 3 days, and sometimes within 24 hours.

We are not able to verify Sanarelli's statement that the dose injected subcutaneously, whether 0.1 cc. or 5 cc., has no influence on the duration of the malady. His statement appears to hold good provided the quantity does not exceed 0.5 cc. Under these circumstances, guinea-pigs

[18] Hog-cholera, p. 71.

[19] *Gazz. d. ospedali*, May, 1899, No. 64.

inoculated by us have died, as a rule, after 6 to 8 days; exceptionally, however, after 3 to 4 days. When the quantity injected was larger (1 cc. to 3 cc.), death has generally occurred at much shorter intervals, varying from 17 hours to 4 days. Exceptionally, guinea-pigs have survived doses of 3 cc. for a period of 7 days.

As to the sterility of cultures taken from the 2d to the 5th day after inoculation of guinea-pigs with B. icteroides, we can confirm the statement of Sanarelli; but we have obtained the same general results with guinea-pigs infected subcutaneously with small quantities of the hog-cholera bacillus; that is to say, while a few colonies may be obtained from the liver or spleen during the first 24 hours following the inoculation, cultures thereafter remain sterile until the day preceding the death of the animal—generally until the 5th to the 6th day after the injection. Since this behavior of the hog-cholera bacillus within the bodies of guinea-pigs corresponds with that recorded by Sanarelli for B. icteroides, it does not seem to us proper that any analogy should be drawn between this so-called cyclical course of the disease in the guinea-pig and the course of yellow fever in the human being. It would rather seem to indicate that after the introduction of a certain quantity of B. icteroides or the hog-cholera bacillus into the body of the guinea-pig, and the destruction of the few organisms that primarily invade the blood, an interval varying from 3 to 5 days is required before the natural resistance of the animal is overcome through the absorption of the toxic products of the micro-organisms multiplying at the point of injection, and that when this resistance is destroyed, the bacilli rapidly invade the blood and organs. In more susceptible guinea-pigs, the primary invasion is followed by rapid multiplication of the bacilli and death from septicæmia after 48 hours.

Turning now to the lesions produced in guinea-pigs and rabbits by the hog-cholera bacillus, these are found to be fairly constant and characteristic, namely, slight purulent infiltration at the site of inoculation, enlargement of the spleen and the presence of focal necroses in the liver. Fatty degeneration of the heart muscle is common. The duration of the disease depends upon the quantity and virulence of the culture inoculated, and varies from 4 to 12 days, the majority of the animals dying at about the end of one week.

In order to illustrate the lesions produced in these animals with B. icteroides, we submit protocols of some of our earlier experiments:

Exp. I.—Guinea-pig No. 456; weight 495 gm.; November 9, 1897, inoculated subcutaneously with 1 cc. of a 20-hour lactose bouillon culture of B. icteroides from blood of dog 443. Colonies from this dog on

agar-slant show characteristic "wax-seal" appearance. Death November 13, 1897, after 4 days. Weight after death, 318 gm.

Slight amount of purulent exudate at site of injection. Lymph-glands swollen and pale. Visceral peritoneum injected. No fluid in abdominal cavity. Small intestine of a rose-pink color. Spleen much swollen, soft, deep reddish-brown in color. Liver pale, numerous punctiform areas, yellowish-white in color, beneath its capsule and on section. Kidneys pale. Adrenals enlarged and congested. Upper lobe of right lung injected.

Cultures from blood, spleen, liver, kidney and urine give numerous colonies of B. icteroides.

Exp. III.—Guinea-pig No. 458; weight 680 gm.; November 9, 1897, 12.30 P. M., received under the skin 2 cc. of a 24-hour bouillon culture of B. icteroides from blood of dog No. 443. Death at 6.30 A. M., November 10, 1897, after 18 hours.

Considerable œdema at site of injection. Lymph-glands swollen. Parietal peritoneum injected. Spleen firm and small. Liver swollen and dark. Kidneys congested. Lungs normal.

Cultures from liver and spleen show a few colonies; other sources negative.

Exp. V.—Guinea-pig No. 461; weight 412 gm.; November 16, 1897, inoculated subcutaneously with 0.5 cc. glucose bouillon culture of B. icteroides from liver of guinea-pig 459. Death November 23, 1897, after 7 days. Weight after death, 268 gm.

No lesion at site of injection. Inguinal glands swollen and pale. Abdominal cavity contains about 2 cc. turbid fluid. Surface of liver and spleen covered with a thin grayish exudate. Spleen much swollen. Liver shows numerous small necroses, round, oblong, and of irregular shape; these are to be seen on the upper and under surface and on section. Kidneys swollen, congested throughout. Adrenals swollen and injected. Bladder filled with albuminous urine. Both lungs congested; pleural surfaces injected; small quantity of clear serum in pleural cavities.

Cultures from blood negative; from spleen, liver, bile, abdominal cavity, kidney and urine, positive. Colonies particularly numerous from spleen and abdominal cavity.

Exp. XI.—Guinea-pig No. 475; weight 420 gm.; November 27, 1897, received subcutaneously 0.3 cc. of a 24-hour lactose bouillon culture of B. icteroides from liver of dog 443. Death December 2, 1897, after 4 days and 20 hours.

Slight purulent exudate at site of injection. Inguinal and axillary glands swollen and pale. Both layers of peritoneum injected. Spleen

large and dark in color. Kidneys and adrenals swollen and much congested. Liver contains a small number of necroses. Lungs normal.

Cultures positive from blood, organs, bile and urine; very numerous colonies from spleen and bile.

Exp. I.—Rabbit; weight 2000 gm.; injected subcutaneously with 0.2 cc. of B. icteroides, original. Death after 7 days.

Lymph-glands enlarged. Splenic tumor. Liver large, congested and contains many small focal necroses. Kidneys swollen; on section, injected throughout.

Cultures positive from blood and organs.

Exp. II.—Rabbit; weight 1545 gm.; injected subcutaneously with 0.2 cc. of B. icteroides, original. Death after 8 days.

Some caseous purulent exudate at site of injection. Spleen swollen and dark. Liver normal. Kidneys congested. Lungs normal.

Cultures positive from blood and organs.

Exp. III.—Rabbit; weight 1707 gm.; received subcutaneously 0.2 cc. of a 24-hour culture of B. icteroides, original. Death after 8 days.

Splenic tumor. Numerous necroses in liver. A few hæmorrhagic areas scattered over duodenum. Injection of mucous membrane of upper part of small intestine. Small ecchymoses over the surface of both lungs.

Cultures from blood and organs positive.

It is seen from the foregoing protocols that the most prominent gross lesions in guinea-pigs and rabbits inoculated with B. icteroides are acute splenic tumor and multiple focal necroses in the liver. The latter have been quite constantly met in guinea-pigs when death has occurred after the 4th to the 6th day. In rabbits these necroses were sometimes absent. We have observed these necroses in animals inoculated with B. icteroides, original, and B. icteroides, Havana. Sanarelli does not record this lesion in guinea-pigs and rabbits, but states that the liver is always congested, except that, when the guinea-pig dies after many days, the liver presents a pale, gray, nutmeg appearance, and is evidently degenerated.

These necroses so frequently met by us, have been also observed by other investigators. De Lacerda and Ramos[20] record the finding of scattered yellow points (“*des points jaunes disséminés*”) in the liver of one rabbit out of four inoculated with B. icteroides. They say nothing about the microscopic examination of these yellow points. Domenico della Rovere[21] records as the result of his infection of guinea-pigs with B. icteroides, “as a new finding in the liver, foci of small cells situated in the midst of the lobules; the protoplasm of the liver cells appears very granular, the nuclei discolored and feebly stained, as well as the nuclei

[20] *Arch. de méd. expér.*, 1899, xi, p. 390.

[21] Sul bacillo icteroide (Sanarelli). *Riforma medica*, 1898, xiv, pt. 3, p. 98.

of the cells lining the biliary canals and capillaries." Della Rovere also observed like foci of small cells situated within the hepatic lobules of rabbits inoculated with B. icteroides. Agramonte [22] found these necroses in the livers of guinea-pigs inoculated with the culture of B. icteroides isolated by him from yellow-fever cadavers at Santiago, Cuba.

We will give briefly the results of *microscopic examination* of the several organs. For this purpose, tissues were hardened in 95 per cent alcohol, 5 per cent formalin, Orth's fluid and Flemming's osmic solution. The description applies to the tissues of both guinea-pigs and rabbits.

Frozen sections of the fresh organs were examined in normal salt solution for the detection of any fatty change. Fatty degeneration of the heart muscle was generally present. The gross change in the spleen, lymph-glands and adrenals appeared to be due to the large increase of the blood supply of these structures. No degenerative changes were observed except in the case of one guinea-pig that died on the 17th day after inoculation with B. icteroides, Havana. Here extensive foci were found in both the follicles and splenic pulp. The cells in these areas had been converted into a granular detritus, in the midst of which abundant minute nuclear fragments were to be seen. A moderate number of polymorphonuclear leucocytes were invading these areas from the margin.

In the kidney, both fatty and parenchymatous degenerations of the tubules of the labyrinth were observed; the former was slight, the latter more marked. The general blood supply of the organ was increased, as shown in the marked dilatation of the glomerular capillaries and of the intertubular vessels. An amorphous exudate and a few red corpuscles were frequently found within Bowman's capsules. Sometimes there was apparent loss of the glomerular epithelium; no interstitial changes were present. The lesions in the kidney did not differ from those seen in other acute experimental infections.

The liver presents, in a majority of the cases, more marked changes. In addition to the congestion which is generally present, there is sometimes seen slight fatty metamorphosis. This was never marked on microscopic examination of frozen sections. although the color of the organ at autopsy at times seemed to indicate this change. In the majority of cases, in both guinea-pigs and rabbits, there was an increase in the number of polymorphonuclear leucocytes within the hepatic capillaries. In some instances the capillaries were so crowded and distended with these leucocytes as to constitute veritable thrombi. There did not ap-

[22] The Bacillus icteroides (Sanarelli) and Bacillus X (Sternberg). By Geo. M. Sternberg. *Centralbl. f. Bakteriologie*, 1899, xxv, p. 659.

pear to be any relation between these collections of leucocytes and the areas of necrosis, the latter presenting the most striking feature to be seen in the liver (Fig. 1, Plate XIX). These foci varied much in size. Sometimes only a few cells were affected, but generally a considerable part of the lobule was involved. At times an entire lobule and adjacent parts of several lobules had undergone the necrotic change. Various stages in this process of coagulative necrosis were seen. Areas were observed in which the protoplasm of the cells stained brightly with eosin and appeared more granular than usual, while the nuclei remained visible, although paler than normal. In other areas, the nuclei of the more centrally placed cells had quite disappeared, while those of the peripheral cells had only suffered a slight alteration in their capacity to take nuclear stains. In still other areas, the cells had become hyaline and refractive, and the nuclei had entirely disappeared without leaving behind any nuclear detritus within the cells. The number of leucocytes present in these areas varied considerably. At times none were to be seen; but, as a rule, many were found collected about the margin, or were contained within the capillaries of the outer zone of the area. Again, they had penetrated in large numbers to the centre and had there undergone fragmentation. In animals that died after considerable periods (9 to 17 days), foci were observed in which the liver cells had completely disappeared and in whose place there were to be seen, in addition to a few polymorphonuclear leucocytes, many cells with large epithelioid nuclei, and fewer cells with small, round, deeply staining nuclei.

The foregoing description applies equally, in our experience, to the livers of rabbits and guinea-pigs inoculated with the hog-cholera bacillus.

Dogs.—The clinical picture brought about in dogs by the intravenous injection of B. icteroides is strikingly reproduced by the inoculation of these animals with the hog-cholera bacillus. In the course of our experiments we have inoculated eight dogs with B. icteroides. Of these 5 have died and 3 recovered. We give protocols of some of the fatal cases.

Exp. I.—Dog No. 443; weight 10½ lbs. September 24, 1897, 1.45 P. M., inoculated intravenously with 5 cc. of a 24-hour lactose bouillon culture of B. icteroides, original. Temperature before inoculation 102° F. Within 15 minutes, frothy, tenacious saliva was observed about the mouth; animal very restless. 2.20 P. M., loose action with tenesmus, followed by vomiting of a frothy, gray fluid; the act of vomiting was several times repeated with much retching. 2.45 P. M., slimy stool mixed with blood. 4.20 P. M., temperature 105.2°.

September 25, 9 A. M., temperature 104°; 3 P. M., temperature 105.8°. Animal has had several black, tarry-looking stools; remains quiet and refuses food. This elevation of temperature continued until October 1, when it became subnormal, falling to 98° F. at 1.40 P. M., October 2, and to 93.4° F. at 10.20 A. M., October 3. Death at 6 P. M., October 3, after 9 days.

Autopsy at 2.30 P. M., October 4, the body in the meanwhile having been kept in ice-chest. Subcutaneous tissues dry. Inguinal lymph-glands swollen and injected. Stomach and intestine pale. A few hæmorrhagic areas scattered over surface of ileum. Mesenteric glands swollen and moderately congested. No fluid in abdominal cavity. Spleen firm, slightly enlarged. Liver of a generally pale color; outlines of lobules distinct. Kidneys swollen, pale; small, distinct hæmorrhagic points beneath capsule; on section, cortex is thickened and cloudy in appearance; pyramids slightly injected. Bladder contracted; contains a small amount of albuminous urine. Mucous membrane of the stomach and intestine pale. Lungs normal. Right cardiac cavities distended with fluid blood.

Cultures from blood, spleen, liver, bile, urine and kidney give colonies of B. icteroides.

Microscopic examination of frozen sections shows moderate fatty degeneration of the liver, which is confined to the peripheral part of the lobules. Hardened sections show, in addition, small areas of coagulative necrosis.

Exp. II.—Dog No. 465; weight 16½ lbs. November 16, 1897, at 3 P. M., injected intravenously with 5 cc. of a 24-hour bouillon culture of B. icteroides, original. Temperature before inoculation 102.6° F. 3.27 P. M. vomited food, followed by micturition and evacuation of bowel with tenesmus. Acts of vomiting and defecation repeated several times during the next 2 hours; animal considerably prostrated. 4.10 P. M., temperature 102.6°. November 17, 9.40 A. M., temperature 103.4°; 4 P. M., 104°; animal refuses food; appears much dejected. Temperature remained elevated until November 22, at which time the dog's condition was much improved.

November 30, at 2 P. M., the animal was a second time injected with 5 cc. of a 24-hour culture of B. icteroides from the blood of dog 443. When returned to its cage, the dog was profoundly prostrated, and died 28 minutes later.

Autopsy 30 minutes after death. An abundance of subcutaneous fat. Spleen small and light-red in color. Liver dark and congested; blood flows freely from cut surface. Kidneys swollen, cortex pale, pyramids injected. Bladder firmly contracted. Mucous membrane of stomach

pale. Mucous membrane of small intestine deeply congested from the pylorus to the ileocæcal valve. Lungs normal.

Cultures from liver and spleen, positive; blood, bile, abdominal cavity, kidney and urine, negative.

Frozen sections of the liver and sections hardened in Flemming's solution show slight fatty degeneration.

Exp. III.—Young dog No. 504; weight 12¼ lbs. December 13, 1897, 11.20 A. M., injected intravenously with 2.5 cc. of a 24-hour glucose bouillon culture of B. icteroides from liver of dog 443. Temperature before injection 102.2° F. Within the next few hours following the injection there were repeated acts of vomiting and defecation with much tenesmus, followed by marked prostration. 4 P. M., temperature 105.4°.

December 14, 9.20 A. M., temperature 103.6°; 4 P. M., 106.2°; animal has frequent loose stools mixed with mucus and blood. The animal remained in this condition and died at 8 A. M., December 22, 1897. Weight after death 9¼ lbs.

Autopsy 3 hours after death. Lymph-glands much swollen and injected. No fluid in abdominal cavity. Spleen enlarged, soft, dark red in color. Liver paler in color than normal; outlines of lobules distinct; peripheries pale; central veins congested, surrounded by lighter areas; cut surface presents the same appearance. Kidneys swollen; cortex swollen, cloudy. Urinary bladder partly contracted, containing albuminous urine. Stomach contains several ounces of a dark green, brownish fluid; mucous membrane pale. The same pallor applies to the whole mucous membrane of small intestine. Some congestion of the rectum, which contains a small quantity of dark grumous-looking fluid. Lungs normal. Increased fluid in pericardial sac; numerous punctate hæmorrhages beneath visceral pericardium; all cavities of heart distended with dark fluid blood; many punctate hæmorrhages beneath endocardium of both ventricles; myocardium pale.

Cultures from blood, spleen, liver, bile, kidney and urine gave colonies of B. icteroides.

Frozen sections of the liver show marked fatty degeneration, the lobules being involved throughout; the hepatic cells are filled with fine and medium-sized oil drops; the cells lining the bile ducts also contain many fine oil drops. Frozen sections of the kidney show advanced cloudy swelling, with slight fatty change in the secreting epithelium.

In two other dogs injected intravenously with 5 cc. of a culture of B. icteroides, death occurred after 52 and 16 hours respectively, preceded by the same clinical symptoms. There was present intense hæmorrhagic gastro-enteritis, but no fatty change in the liver, although focal necroses were present in this organ.

For comparative purposes we have also inoculated dogs with the bacillus of hog-cholera. Of these 5 have died at various intervals following the inoculation, and 5 have recovered.

We submit protocols:

Exp. I.—Dog No. 595, weight 12½ lbs. May 16, 1898, 1.15 P. M., received intravenously 5 cc. of a 24-hour bouillon culture of the hog-cholera bacillus. Temperature before inoculation 102.6°. Within an hour animal appeared much affected. Vomited at 2.05 and 2.40 P. M. Frequent acts of micturition and defecation during the afternoon. The stools consisted of watery fluid mixed with mucus and blood. 5 P. M., temperature 103.2°. At this time animal is completely prostrated. Death at 7.30 A. M., May 17.

Abdominal cavity contains a small quantity of a blood-stained serum. Peritoneum injected throughout. Scattered points of hæmorrhage over large and small intestine. Spleen swollen and congested, firm. Liver dark in color and deeply congested. Bladder firmly contracted. Kidney swollen; cortex pale, pyramids injected. Stomach contains dark fluid blood; its mucous membrane is uniformly and deeply congested. Small intestine also contains fluid blood; its mucous membrane is deeply injected and hæmorrhagic from pylorus to ileocæcal valve. Thoracic organs normal.

Cultures from blood, liver and spleen positive.

Frozen sections show cloudy swelling but no fatty change in either liver or kidney.

Exp. V.—Dog No. 1060; weight 14 lbs. June 2, 1899, 2.40 P. M., received intravenously 5 cc. of a 24-hour bouillon culture of the hog-cholera bacillus. Temperature before inoculation 100° F. The symptoms as already described for the preceding animal appeared within an hour. Spells of vomiting, micturition and defecation were especially frequent in this animal. June 3, 9.30 A. M., temperature 101.8°; 4 P. M., 101.9°. Dog refuses food; appears to be very sick. June 4, 10.30 A. M., temperature 105.4°; 4 P. M., 105.8°. This elevated temperature continued until June 8, after which date fever subsided and animal gradually recovered its strength.

June 15, 1899, 3.40 P. M., again injected in the ear-vein with 5 cc. of a 48-hour culture of the hog-cholera bacillus, followed by death at the end of 45 minutes.

Autopsy 18 hours after death, body having been kept in an ice-box in the meanwhile. About 25 cc. of blood-stained serum in the abdominal cavity. Omentum and both layers of peritoneum congested. Spleen pale and flaccid, not enlarged. Liver firm, congested. Bladder contracted; its mucous membrane distinctly congested. Kidneys swollen

and much congested. Gastric mucosa intensely injected throughout, swollen and hæmorrhagic. Mucous membrane of small intestine is swollen and of a raspberry-jam color from pylorus to the ileocæcal valve. That of the large bowel is less congested. Lungs normal. All cavities of the heart distended with dark fluid blood; numerous subendocardial ecchymoses in left ventricle.

Cultures from the blood, sterile; from the liver, kidney and spleen, positive.

Frozen sections of the liver show moderate fatty degeneration of the hepatic cells.

Exp. IX.—Dog No. 1109; weight 15 lbs. This animal received intravenously three injections of the hog-cholera bacillus, as follows:

August 22, 1899, 8 cc., September 6, 1899, 25 cc., November 4, 1899, 25 cc. of a bouillon culture. After each injection there were the same clinical symptoms already described in previous dogs, followed by marked fever. Death November 18, 14 days after last injection.

Autopsy 6 hours after death. Spleen somewhat swollen, firm and moderately injected. Liver of paler color than normal; markings indistinct. Kidneys swollen, cortex thickened and cloudy. No hæmorrhagic lesions in stomach or intestinal canal. Bladder partly distended with albuminous urine. Lungs normal.

Cultures from blood, sterile; spleen, liver and kidneys, positive.

Frozen sections of the liver show fatty degeneration. Hardened sections show small areas of coagulative necrosis.

In two additional experiments with dogs, 5 cc. of a bouillon culture of hog-cholera bacillus injected intravenously, brought about death, in the one case after 10 hours, and in the other at the end of 21 days, the animal being extremely emaciated. In the former, hæmorrhagic lesions were marked in stomach and small intestine. In neither case was there any fatty degeneration of the hepatic cells.

In our experiments with dogs we have recovered B. icteroides or the hog-cholera bacillus respectively in pure culture. In no case of either series of experiments was there a mixed infection.

An examination of the foregoing protocols of dogs inoculated with the hog-cholera bacillus, will serve to demonstrate that the same clinical picture is seen in these animals as in those inoculated with Sanarelli's bacillus. We have constantly observed repeated vomiting, increased action of the bowels with tenesmus, frequent micturition, and pronounced prostration with fever. These symptoms have appeared within an hour or less after the injection, and in two instances were followed by death after 10 and 18 hours respectively. At autopsy, intense hæmorrhagic

gastro-enteritis was present as seen in dogs that died within a short interval after injection with B. icteroides.

Thus, in dogs inoculated with the hog-cholera bacillus, we have been able to reproduce a part of the clinical and anatomical picture which Sanarelli considers analogous to that seen in yellow fever in the human being. It is important to observe, however, that these symptoms and lesions in the dog are not peculiar to either B. icteroides or the hog-cholera bacillus, since we have fully reproduced them in dogs by the intravenous injection of a member of the colon group (Bacillus X, Sternberg). There are probably other bacteria that would bring about the same symptoms and lesions in dogs.

As regards the fatty degeneration of the liver, the second part of the anatomical picture upon which Sanarelli lays considerable importance, we have met with this in less degree in dogs injected with the hog-cholera bacillus. Although it was present in 2 out of 5 autopsies, the degree of fatty degeneration was not marked in any of these. On the other hand, in only 1 of 5 dogs in which a fatal infection was produced with B. icteroides was this change present in sufficient degree to constitute an important pathological lesion. In 2 other dogs injected with Sanarelli's bacillus, there was a moderate degree of fatty degeneration, just as there was in 2 of the dogs injected with the hog-cholera bacillus. Thus, in the young dog of the icteroides series in which fatty degeneration of the liver was a prominent feature, this did not reach that extent or degree seen in the liver of yellow fever. Moreover, there was a conspicuous absence of cellular necrosis, always so prominent a feature in the liver of the latter disease.

The percentage of cases in which we have observed fatty degeneration in the liver of dogs inoculated with B. icteroides (60 per cent), while slightly less than that observed by Sanarelli[23] (71.3 per cent), is in excess of that reported by de Lacerda and Ramos[24] (50 per cent). We have been unable to find detailed reports of observations by other workers.

In connection with the production of fatty degeneration of the liver of dogs injected with B. icteroides, we observe that P. Foa,[25] having produced in the liver of one dog inoculated with B. icteroides extreme steatosis in all respects, as he states, similar to that found in the liver of yellow fever, failed to encounter again this change in a series of dogs (the number is not stated), although these died after varying intervals and in a marasmic condition. We have already shown in a pre-

[23] *Policlinico*, pp. 445, 449.
[24] *Arch. de méd. expér.*, 1899, xi, p. 390.
[25] *Giornale d. r. Accad. di med. di Torino*, 1889, 4. s., xlvi, p. 115.

vious paper [26] that the hog-cholera bacillus, in larger doses several times repeated, may cause intense fatty degeneration of the dog's liver.

That B. icteroides may fail to bring about fatty degeneration even in the liver of human beings, is shown by the case of Private Patrick Smith, 8th Infantry, who died in Havana on the 9th day of his illness (Case No. 7 in Wasdin and Geddings' Report).[27] B. icteroides was isolated from the blood of this case 4 days before death by these observers, and again at autopsy by Agramonte.[28] The liver, however, neither at autopsy nor upon careful microscopic examination showed any trace of fatty degeneration, but only limited areas of necrosis, invaded by leucocytes (Fig. 2, Plate XIX). Although there was no intestinal ulceration present in this case, Agramonte isolated at autopsy also the typhoid bacillus, a culture of which we have been permitted to examine and verify. In this case, therefore, B. icteroides, which was present in the blood four days before death, did not produce that fatty degeneration of the liver which is so constant in yellow fever (Fig. 3, Plate XIX).

The focal necroses which we have observed in the livers of dogs inoculated with these two bacilli have been reported also by Foa [29] in the liver of a dog inoculated with Sanarelli's bacillus.

Pigeons.—Bruschettini [30] states that a small quantity of a virulent culture of B. icteroides when injected into the breast muscle of the pigeon causes a fatal infection. In his hands the injection of 2 cc. of the culture killed one pigeon after 12 days. By inoculating successive pigeons with blood obtained at autopsy, he was able to produce death in 4 or 5 days. Besides swelling and discoloration at the site of the inoculation, there was enlargement of the spleen and diffuse gastro-enteritis. Microscopically, the breast muscle was the seat of marked fatty degeneration.

We have injected a few pigeons with B. icteroides and found them tolerably resistant to this organism. Less than 3 cc. of a 24-hour bouillon culture injected into the pectoral muscle has not sufficed to bring about death. 3 cc. generally cause death after 5 days, but have occasionally failed to kill. We have found marked swelling at the point of injection, together with widespread necrosis of the breast muscle interspersed with areas of hæmorrhage. The spleen was enlarged, and the mucosa of the small intestine injected. Cultures were positive from the wound, blood and organs.

[26] *Medical News*, 1899, lxxv, p. 321.

[27] Report on the Cause of Yellow Fever. U. S. Marine Hospital Service, Washington, 1899.

[28] *Centralbl. f. Bacteriologie*, 1899, xxv, p. 661.

[29] *Op. cit.*, p. 115.

[30] *Op. cit.*, p. 698.

Microscopically, the muscle fibres are found to be much swollen and to have undergone complete necrosis.

The greater resistance shown by pigeons, as compared with the smaller laboratory animals, to infection with B. icteroides is also manifested toward the hog-cholera bacillus. The widespread necrosis at the site of inoculation is common to both of these bacilli.

Swine.—Our experiments with these animals have consisted in feeding them with pure cultures of B. icteroides, as well as with the viscera of infected pigs. We have also exposed young pigs to natural infection by confining them in boxes in which other swine had died after being fed with Sanarelli's bacillus.

Exp. I.—Young pig No. 919; weight 16¾ lbs. March 6, 1899, 1 P. M., was fed 25 cc. of a 24-hour plain bouillon culture of B. icteroides, original, which had passed through one guinea-pig. The culture was fed in a pint of milk. Rectal temperature prior to feeding, 102.4° F. The animal appeared ill on the following day, ate but little and persisted in lying down. Temperature, 9.30 A. M., 105.6°; 3 P. M., 105.6°. March 8, temperature 9 A. M., 104.2°; 3 P. M., 104°. Animal refuses food and shows weakness of the hind extremities. March 9, temperature 9.30 A. M., 102.6°; 3 P. M., 103.4°. March 10, temperature 9.30 A. M., 101.8°; 3 P. M., 102°. Slight diarrhœa appears on this date. March 11, temperature 9.30 A. M., 95.5°; 3.30 P. M., 102.8°. Slight diarrhœa continues. Animal has refused all food for 2 days. Death 6 P. M., March 11.

Autopsy 10.30 A. M., March 12, the pig having been kept on ice in the meanwhile. No lesions on lips or in mouth. Marked injection of subcutaneous tissues. Axillary glands swollen. Abdominal cavity contains about 20 cc. of blood-stained serum. Both layers of peritoneum congested. Omental vessels, as well as mesenteric, are much engorged. Mesenteric glands swollen and injected. Spleen swollen, soft, congested. Liver, attached to diaphragm by moderately firm adhesions, is of a dark color, and on cut surface shows a number of small, irregular, yellowish, bile-stained areas. Gall-bladder moderately distended with dark, greenish, thick bile; its mucous membrane deeply injected. Kidneys swollen, cortex pale, pyramids congested. Lungs normal; no fluid in pleural sacs. Stomach normal. In the lower two-thirds of the ileum there were a number of circumscribed areas of diphtheritic inflammation. The large intestine is the seat of extensive diphtheritic inflammation beginning a few inches below the ileocæcal valve; its mucous membrane congested, swollen and covered with an adherent fibrinous exudate. This condition extends about 18 inches down the gut. Below this point there

are numerous circumscribed superficial erosions until the rectum is reached, which shows no lesions.

Cultures from the blood, liver, spleen, kidney and mesenteric gland are positive.

Exp. II.—Pig No. 927; weight 14 lbs. March 12, 1899, was fed a portion of the viscera obtained from pig 919. Temperature before feeding was 103.5°, 12 M. March 15, animal shows decided symptoms of sickness. Temperature 9 A. M., 104.6°; 4 P. M., 106.4°. Refuses all food to-day. The fever, with lack of appetite, continued until March 18. From this time animal improved and fever subsided. Was killed March 31.

No fluid in abdominal cavity. Spleen slightly congested and firm. Liver normal in appearance. In the lower two feet of the ileum the mucosa is uniformly and deeply injected. The large intestine, beginning with the cæcum, contains a number of round or irregular healing ulcers. Some of the ulcers show a slightly raised margin and appear to be in various stages of cicatrization.

Cultures from blood, organs and mesenteric glands, negative.

As in these two experiments the animals were confined in a room where a dog had died a few weeks previously from the intravenous injection of the hog-cholera bacillus, we procured, as a matter of precaution, a second-story, well-isolated room, with cemented floor in which no animals had ever been inoculated. Four new wooden boxes were procured, and in each of these a young pig was placed on April 6, 1899. These animals had been purchased in open market and were fat and healthy in appearance.

As a result of feeding these pigs with cultures of B. icteroides, the animals promptly sickened and died from the 6th to the 12th day after inoculation. We give protocols of some of these experiments:

Exp. III.—Young pig No. 976; weight 15 lbs. April 8, 1899, 3 P. M., fed 25 cc. of a bouillon culture of B. icteroides, original, which had passed through one guinea-pig Temperature before inoculation, 102° F. April 10 there was diarrhœa with thin yellow stools of pea-soup consistency. Temperature 11 A. M., 106.2°; 4 P. M., 107°. April 14, fever and diarrhœa continue; animal shows distinct weakness in hind extremities, standing with back arched. Temperature 9 A. M., 101.5°; 4 P. M., 104°. From this date animal grew weaker, refused all food, and died 2.15 P. M. April 17, nine days after feeding. Weight after death 9½ lbs.

Autopsy one hour after death. No lesions in mouth or on lips. Some injection of subcutaneous tissues. Inguinal glands enlarged. No fluid in abdominal cavity. Small intestine generally congested. Spleen pale and firm, not swollen. Liver of dark-red color. Stomach contains

several somewhat curved linear ulcers with hæmorrhagic base. Small area of hæmorrhage beneath peritoneal coat of duodenum. A second similar area over surface of ileum. Mesenteric glands swollen. Mucosa of small intestine congested throughout and swollen. The cæcum and large intestine are the seat of marked diphtheritis with abundant yellowish-gray exudate which covers the mucous membrane as a lining; there are also distinct, irregular ulcers whose surfaces are covered with bile-stained necrotic material. These ulcers are also found in the rectum. Lungs normal.

Cultures from abdominal cavity, blood, bile, liver, kidney, urine and spleen, sterile; from mesenteric gland, positive.

Exp. IV.—Young pig No. 977; weight 12½ lbs. April 8, 1899, 3 P. M., fed 15 cc. of a 24-hour bouillon culture of B. icteroides, original, which had passed through one guinea-pig. Temperature before feeding, 103°. April 10, the animal eats but little; has thin, pea-soup-looking stools. Temperature 11 A. M., 103.5°; 4 P. M., 105°. This diarrhœa and fever continued, followed by increasing weakness and death on April 14, 1899, after 6 days.

At autopsy there are three ulcers on the mucous membrane of the upper lip. Two are very small and undergoing cicatrization. The third is nearly circular in outline, depressed, and with margins brightly injected. It measures 7 x 8 mm. A similar ulcer with necrotic centre is present on the lower lip. Inguinal glands swollen, pale. Parietal and visceral peritoneum generally injected. Spleen small. Liver injected. Kidneys show cloudy cortex. Mesenteric glands swollen and congested. Mucosa of stomach much congested over the greater curvature. Several small ulcers measuring 2 to 3 mm. in diameter are found in this region. Each ulcer bears a whitish superficial slough. Upon scraping this away, a small crater-like excavation is exposed. The mucosa of the small intestine is swollen and congested throughout. In the lower six feet of the ileum there are numerous small ulcers with superficial sloughs. There is also seen a Peyer's patch with thickened margins and excavated centre, the whole being covered with a thick, bile-stained necrotic material. For a distance of two feet above the ileocæcal valve, the entire surface of the mucous membrane has undergone necrosis. There is also diffuse necrosis of the mucous membrane of the cæcum and of the large bowel for a distance of about two feet (Fig. A). Below this point there are a number of discrete ulcers, the intervening mucous membrane being swollen and injected. These ulcers are less numerous in the rectum.

Cultures from the blood and spleen, negative; from liver and mesenteric glands, positive.

Exp. VI.—Young pig No. 979; weight 14 lbs. April 10, 1899, fed 15 cc. of a 24-hour bouillon culture of B. icteroides, original, passed through one guinea-pig. The same clinical picture was seen as in preceding experiments, viz., diarrhœa, fever beginning on April 12, and this followed by weakness, emaciation and loss of strength in hind legs.

FIG. A.

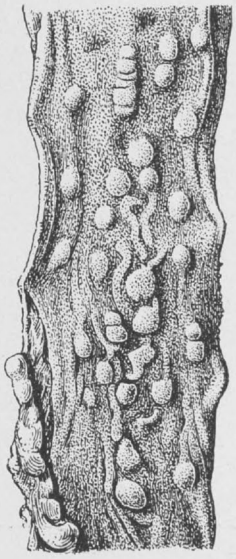

FIG. B.

FIG. A.—Colon of hog showing "cork-lining" appearance due to excessive thickening and necrosis of the mucous membrane. Death on 6th day after ingestion of 15 cc. of a bouillon culture of B. icteroides.

FIG. B.—Circumscribed nodular thickening in submucosa of large intestine of hog. Death on 12th day following ingestion of 15 cc. of a bouillon culture of B. icteroides.

Subnormal temperature observed on April 18 and continued till death, on April 22, 1899, after 12 days.

No lesions on lips or in the mouth. Inguinal and cervical glands swollen. Both layers of peritoneum injected. Mesenteric glands swollen. Spleen small and firm. Liver congested. There is a distinct grayish diphtheritic exudate upon the mucous membrane of the œsoph-

agus, which becomes more marked at the lower end. A circumscribed patch of diphtheritis is found near the cardiac end of the stomach and several small, shallow ulcers with hæmorrhagic bases along the lesser curvature. A few inches below the pyloric orifice there is a circular, raised, button-like mass, measuring 7 mm. in diameter by 3 mm. in thickness. Over this mass, the mucosa is intact. It projects slightly on the peritoneal surface. The mucosa in the lower ileum is swollen and congested. Small superficial erosions are found in this part of the gut. Two small ulcers are situated on the ileocæcal valve. The cæcum contains a few ulcers covered with a grayish, dirty-looking diphtheritic exudate. Areas of circumscribed diphtheritis are also present. The mucosa in the upper part of the bowel is swollen and congested. Commencing at a point about 12 inches below the cæcum, the bowel is studded with numerous grayish, firm, elevated nodular masses, varying

FIG. C.

FIG. C.—Section of one of the nodules of Fig. B, showing central necrosis. × 24.

from 4 to 7 mm. in diameter. Over most of these the mucosa appears to be intact, but a few show a central necrosis. These nodules are absent from the rectum. They appear to be situated in the submucosa and project slightly on the peritoneal surface of the bowel (see Figs. B and C).

Cultures from blood, spleen, liver and kidney, sterile; from mesenteric gland, positive.

It is important to note that in Experiments I, III, IV and V (the last protocol we have omitted), we obtained in pure culture a small actively motile bacillus, which upon being transferred to Loeffler's blood serum, showed the same sparse growth as has already been described for B. icteroides, original. The culture obtained in Experiment VI was accidentally destroyed before it could be further examined. In Experiment VII, however, (whose protocol we do not give), in addition to the acute lesions of the hæmorrhagic type of hog-cholera, there were present

on microscopical examination of sections of the colon, well-defined necrotic areas in the submucosa. Death occurred on the 11th day after feeding 15 cc. of a bouillon culture of B. icteroides, original. From the blood, spleen, liver, kidney and mesenteric gland, there was obtained, in pure culture, a bacillus which corresponded in morphological and biological characters to B. icteroides, original. One pig, Experiment VIII, resisted repeated feedings of B. icteroides, original.

In order to ascertain whether the domestic pig could be naturally infected with B. icteroides when placed in conditions favorable for acquiring the infection, the following experiment was made:

Exp. IX.—Young pig No. 995; weight 10 lbs. Lips and mouth free of lesions. April 25, 1899, was placed in the box in which pig 978 had died on April 17, 1899. Temperature before being placed in the box, 103°. The animal ate its food heartily and showed no sign of sickness nor fever until May 3, 1899, nine days after exposure. On this date its rectal temperature at 4 P. M. was 106.3°. From this time the pig appeared to be sick, ate but little and became emaciated and weak. Fever varying from 105° A. M. to 106.1° P. M., continued till May 9; there was no diarrhœa observed at any time. May 11, temperature subnormal. Death May 12, 1899, after 17 days.

Autopsy 6 hours after death. The skin of all of the extremities shows a bright flush. There are several small ulcers undergoing cicatrization on both lips. Lymph-glands moderately enlarged. Mesenteric vessels congested; mesenteric glands swollen; through the wall of the large intestine, numerous pale, circular, opaque areas are visible; these measure from 2 to 6 mm. in diameter. Spleen enlarged and dark in color. Liver congested. Kidneys swollen; cortex thickened and cloudy; pyramids injected. Near the cardiac orifice of the stomach there are several small roundish ulcers with raised grayish sloughs. The fundus of the stomach is deeply injected and of a raspberry-jam color. Mucosa of lower ileum swollen and hyperæmic. The cæcum is studded with small ulcers bearing yellowish or grayish sloughs. The valve is surrounded by similar ulcers which encroach upon its base. These ulcers are also present in the large bowel, together with numerous roundish areas of superficial necroses. These are also found in the rectum. Thoracic organs, normal.

Cultures from blood, liver, kidney, spleen and mesenteric gland, positive.

Of three other pigs exposed to natural infection, under like conditions, two died after 27 and 40 days, respectively, and one remained unaffected.

We have also succeeded in producing a fatal infection in two pigs by feeding the viscera of pigs that have recently died from acute infection with B. icteroides, death occurring in these cases after 15 and 16 days

respectively. The lesions in these various animals corresponded with those already described.

From the mesenteric glands of each of these pigs we have isolated in pure culture a bacillus which agreed in its sparse development on blood-serum with B. icteroides.

The foregoing experiments with the domestic pig were made with B. icteroides, original, which had been passed through the body of one guinea-pig.

In order to ascertain whether the cultures of this bacillus which had been obtained from other sources, would bring about a fatal infection in young swine, we made the following experiments:

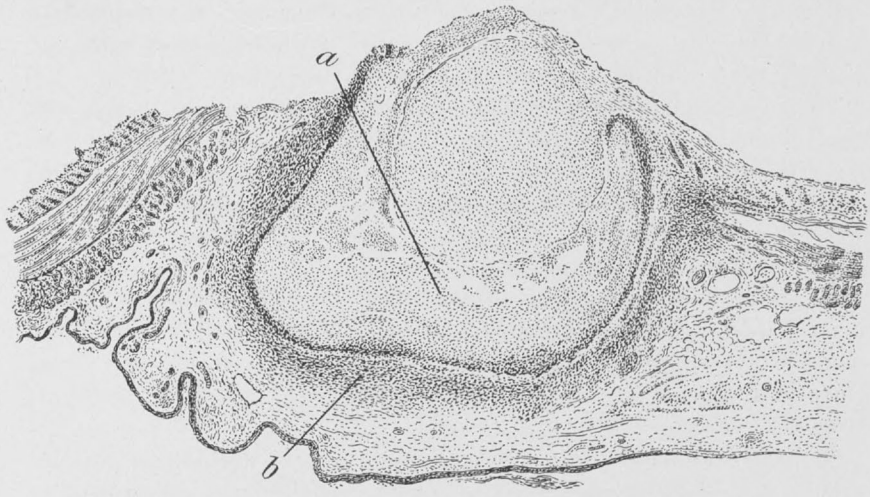

FIG. D.

FIG. D.—Section of a nodular mass implicating the several coats of the small intestine of a hog. Death on 42d day after being fed 25 cc. of a bouillon culture of B. icteroides. *a*, central necrosis; *b*, polynuclear leucocytes. ×24.

Exp. XV.—Young pig No. 1072; weight 13½ lbs. June 27, 1899, fed 25 cc. of a 24-hour bouillon culture of B. icteroides, Havana. The symptoms presented by this animal were similar to those already recorded, viz., diarrhœa, loss of appetite and gradual emaciation. There was no fever. The diarrhœa was of an intermittent character. Temperature became subnormal on July 23, and remained so until the date of death, August 11, 1899. Weight after death, 7½ lbs.

Autopsy 10 hours after death. Several healed erosions on lips. Both lungs injected and dotted with ecchymoses. Abdominal cavity contains about 5 cc. of slightly turbid serum. Spleen slightly enlarged, firm, capsule thickened. Liver enlarged and of pale color. Kidney shows

thickened and cloudy cortex; pyramids pale. Stomach normal. The mucosa of the upper ileum is swollen and injected and shows circumscribed patches covered with a thin grayish exudate. At a point about 9 feet below the pylorus there are two sharply circumscribed, round, nodular masses which appear to be situated in the submucosa. They are 8 mm. in diameter and project prominently toward the peritoneal surface. The mucosa is distinctly raised by these nodules, but appears to be intact. Scattered through the ileum, singly or in groups, there are 9 other neoplastic growths of like character. Some of these involve the entire thickness of the bowel and show on the mucous surface a distinct central necrosis (see Fig. D). The central necrosis in several of these button-like masses has reached the peritoneal surface and resulted in adhesions to the omentum. A few irregular, superficial ulcers are found in the cæcum and large intestine, including the rectum.

Cultures from the blood, sterile; from the abdominal cavity and bile, the colon bacillus; from the liver, in pure culture, a few colonies of an actively motile bacillus which grows sparsely and slowly on Loeffler's blood serum, and which corresponds in its cultural characters with B. icteroides.

Exp. XVI.—Young pig No. 1073; weight 14 lbs. June 27, 1899, fed 25 cc. of a 24-hour bouillon culture of B. icteroides, Santiago. Same symptoms as in preceding experiment. No fever. July 11, animal has lost flesh; eats but little and shows weakness in hind extremities. From July 14 the temperature was subnormal till death on July 31, 1899, 34 days after feeding.

Autopsy immediately after death. Both lungs dotted with a few discrete subpleural ecchymoses; otherwise normal. Spleen enlarged, firm. Liver of light, yellowish color; fatty. No lesions in stomach. The small intestine contains a number of the sharply defined nodular masses, such as have already been described in the preceding experiment. There are also areas of diphtheritis and ulceration in the cæcum and large intestine, including the rectum. Cultures sterile.

At this time our attention was called to the negative results obtained by Wasdin and Geddings[31] from feeding pigs with large quantities of a pure culture of B. icteroides in an experiment made at Delaware Breakwater. The conclusion arrived at by these observers—"that the domestic pig is incapable of infection by the Bacillus icteroides when introduced through the intestinal or digestive tract"—was so absolutely contradictory to the results obtained by us, that we proceeded once more

[31] Report on the Cause of Yellow Fever. Washington, 1899.

to repeat our observations, taking care that these should be guarded by every possible precaution.

With this object in view, we procured four young pigs from the same litter. On September 7, 1899, these pigs were placed in a separate building where hogs had never been kept. Their average weight at this time was 12¼ lbs., the smallest weighing 11, and the heaviest 13 lbs. They were retained under observation until September 26, at which date their average weight was 14¼ lbs. All appeared to be in excellent condition. On this date one of the pigs was killed and autopsied, with the result that its organs and digestive tract were found to be entirely free of lesions. Cultures negative. On the same day the three remaining pigs were sent to the Soldiers' Home, Washington, D. C., where, as far as we can ascertain, hogs have never been kept. One of the pigs, weight 14¼ lbs., was placed in a separate room to serve as a control. The remaining two pigs were fed cultures of B. icteroides, with the following result:

Exp. XVII.—Young pig No. 1128, weight 15½ lbs. No lesions about lips or mouth. September 27, 1899, fed 50 cc. of a 4-day bouillon culture of B. icteroides, original, which had recently been recovered from the blood of rabbit No. 1120. September 30, the animal has refused food and has had a slight diarrhœa. Drinks water freely. October 4, condition same; eats but little; weight 13 lbs., a loss of 2½ lbs. in one week. The control pig on this date has gained 1¼ lbs. Was again fed 200 cc. of the same culture. October 14, animal has slowly improved in condition; has more appetite; weighs 14 lbs., a gain of 1 lb. From this date the pig recovered rapidly. November 8, weight 16¾ lbs. Animal killed. No lesion found in any of the organs or digestive tract.

Cultures from organs and mesenteric gland negative.

That this animal was made sick by feeding with B. icteroides was shown by the loss of appetite, transient diarrhœa, and loss of weight during the first week.

It is of interest to note, also, as pointing to infection that the serum of this pig drawn on October 28 and November 8, in dilutions of 1 to 120, caused arrest of motility and good agglutination of B. icteroides at the end of one hour, whereas the serum of the control pig in dilution of 1 to 20 was entirely negative.

Exp. XVIII.—Young pig No. 1130; weight 13½ lbs. No lesions about lips or mouth. September 27, 1899, fed 50 cc. of a 4-day bouillon culture of B. icteroides, original, received from Roux's laboratory, and which had been cultivated on agar for the period of two years without passage through any animal. September 30, animal has been sick since the day following the feeding. Has diarrhœa and has lost flesh. October 4, diarrhœal discharges continue; weakness in hind extremities; weight

9 lbs., a loss of 4½ lbs. in seven days. Was again fed 50 cc. of the same culture in milk, which it partially drank. Death 7 P. M., October 7, 1899, at the end of 10 days. Weight after death, 8½ lbs.

Autopsy at noon, October 8, 1899, the body having been kept in ice-box. Two circular ulcers with indurated margins and necrotic centres on inner surface of lower lip; one measures 2.5 mm., the other 5 mm. in diameter. Inguinal glands swollen. Lungs slightly injected throughout. The abdominal cavity contains 10 cc. of clear serum. Visceral peritoneum generally injected. Mesenteric glands swollen. Spleen moderately enlarged, soft, dark in color. Liver congested, firm, markings obscure. Kidneys swollen, pale; cortex cloudy. The œsophagus is congested throughout. The fundus of the stomach is also much congested. There are, in addition, near the cardiac opening, several elevated, sharply circumscribed patches of necrosis. The mucosa of the duodenum and of the lower ileum is swollen and deeply congested. The mucosa of the cæcum shows areas of necrosis. In the large bowel this necrosis becomes diffused and general, involving the entire surface of the gut for a distance of 18 inches. Below this point there are discrete ulcers covered with bile-stained exudate. These occur also in the rectum.

Cultures from the liver and mesenteric gland, positive. Small pieces of a mesenteric gland were also placed beneath the skin of a rabbit. The latter died on the 8th day. Cultures obtained from the rabbit, as well as those from the pig's liver and mesenteric gland by direct culture, give in pure culture an actively motile bacillus which grows sparingly on blood-serum.

The control pig was killed on November 9. Weight 17½ lbs. At autopsy no lesions whatever were found in the organs or digestive tract. Cultures negative.

We believe that the last-mentioned experiment will bear the most rigid scrutiny, and that it proves conclusively that B. icteroides, original, when fed to the domestic pig, will cause a fatal infection.

That an acute infection may be produced in swine not only by feeding pure cultures of B. icteroides, but that the disease may also be naturally acquired by these animals after exposure in infected pens is, we think, of especial importance when taken in connection with the comparison of the experimental lesions already obtained in other animals.

If we compare the lesions produced in the domestic pig infected with B. icteroides with those found in swine that have died of hog-cholera, it will be seen that these are practically the same, consisting of various necrotic, diphtheritic and ulcerative processes which, while affecting to a less extent the mouth, stomach and small intestine, have their chief seat, as a rule, in the large bowel.

According to Welch and Clement,[32] more characteristic lesions, but less often met in experimental cases, are the so-called "buttons," viz., certain elevated, circumscribed, round or oval areas of necrotic inflammation of firm consistence, which implicate the mucous and submucous coats, and sometimes all of the coats of the bowel. The foregoing experiments will show that we have succeeded in reproducing in swine infected with B. icteroides all of the acute lesions of the digestive tract such as are found in hogs dead of hog-cholera. As regards the more characteristic, button-like lesions of hog-cholera, we invite attention to Exp. XV, in which these circumscribed necrotic masses involving the several coats of the bowel were found in a pig that died on the 42d day after being fed a culture of B. icteroides, Havana. We also observed lesions of the same character, only in an earlier stage of development, in the large intestine of pig 979, Exp. VI, and pig 994, Exp. VII.

Although we did not employ controls in our earlier experiments, we consider it of especial importance that our pigs sickened so promptly after being fed cultures of B. icteroides, with such symptoms as loss of appetite, choleraic diarrhœa, fever, etc., and that the bacillus recovered by us from our several autopsies always showed the same cultural characters, viz., sparseness of growth on blood-serum, limited surface growth in gelatine stab cultures, and atypical radiating colonies in gelatine plates, such as we have constantly found with B. icteroides, original, and B. icteroides, Havana.

Without entering into a description of the microscopic lesions found in swine infected with B. icteroides, we may state that the changes in the intestine, liver and kidneys correspond closely with those seen in experimental hog-cholera. We have not observed hyaline thrombi in the glomerular capillaries, to which Welch and Clement have called attention in swine dead of hog-cholera and in animals inoculated with the hog-cholera bacillus.

Reciprocal Immunization.

Additional evidence pointing to the close affinity of B. icteroides and the hog-cholera bacillus may be found,—

(*a*) In the protective influence in guinea-pigs of sterilized cultures of B. icteroides against a fatal dose of the hog-cholera bacillus.

(*b*) In the protective influence in guinea-pigs of sterilized cultures of the hog-cholera bacillus against a fatal dose of B. icteroides.

[32] Remarks on Hog-cholera and Swine Plague. Proceedings of the Thirtieth Annual Convention of the United States Veterinary Medical Association and First Veterinary Congress of America, p. 206. Philadelphia, 1894.

(*c*) In the immunity produced in rabbits from a virulent culture of the hog-cholera bacillus by the injection of repeated doses of a living culture of B. icteroides of weak virulence.

(*d*) In the reciprocal agglutinative reactions of the sera of animals immunized with these bacilli.

(*e*) In the mutual reaction shown by the blood of yellow fever and hog-cholera upon B. icteroides and the hog-cholera bacillus.

Immunization of guinea-pigs from the hog-cholera bacillus with sterilized cultures of Bacillus icteroides.

In our first attempts to produce immunity in guinea-pigs, in which we used large doses (1 cc.) of a bouillon culture of B. icteroides, grown for 24 hours at 37° C., and afterwards sterilized for one hour in a water bath at 70° C., we found that our animals generally died in a much emaciated condition within about one week after receiving the first injection. We therefore substituted smaller doses (0.3 cc.), and observed that even with this quantity there was considerable loss of weight which was followed by death in some cases. It was for this reason that we postponed a second injection of the sterile culture until the guinea-pigs had begun to show a gain in weight. This usually caused the lapse of about 20 days between the several injections, as well as a considerable interval between the last immunizing dose and the injection of the virulent culture. Table III shows the results obtained.

It will be seen that of 12 pigs in which immunization was attempted, 2 died after 13 and 16 days following the first injection, and 2 within 3 and 6 days after the second injection of a sterile culture.

Of the 8 protected pigs that received 0.3 cc. of a virulent culture of the hog-cholera bacillus, one died on the 14th day, one on the 47th day and 6 recovered.

The two controls died on the 7th and 11th day respectively.

The result would appear to show that decided protection had been conferred against the hog-cholera bacillus by previous injections of sterile cultures of B. icteroides.

Immunization of guinea-pigs from B. icteroides with sterilized cultures of the hog-cholera bacillus.

We have also succeeded in conferring immunity upon guinea-pigs

from B. icteroides by previous injections of sterilized bouillon cultures of the hog-cholera bacillus. We submit the results in Table IV.

As shown in the table, 2 guinea-pigs died within 4 and 11 days after receiving the first injection of 0.3 cc. of a sterile culture of

TABLE III.

IMMUNIZATION OF GUINEA-PIGS FROM THE HOG CHOLERA BACILLUS, WITH STERILIZED BOUILLON CULTURES OF ICTEROIDES, ORIGINAL.

No.	Date of injection with sterilized culture of B. icteroides.	Weight in grammes.	Quantity of sterilized culture injected.	Date of injection with living culture of Hog-cholera Bacillus.	Weight in grammes.	Quantity of living culture injected.	Remarks.
	1899			1899			
1	May 9	415	0.3 cc.	.....	243	.....	Died 13 days after first injection. Cultures sterile.
2	May 9	320	0.3 "				
	" 31	275	0.3 "				
	June 19	256	1.0 "	July 1	243	0.3 cc.	Died July 15, 1899.
3	May 9	355	0.3 "				
	" 31	300	0.3 "				
	June 19	296	1.0 "	July 1	293	0.3 "	Recovered.
4	May 9	455	0.3 "				Died 6 days after second injection. Cultures sterile.
	" 31	395	0.3 "	.....	345	.....	
5	May 9	390	0.3 "				Died 3 days after second injection. Cultures sterile.
	" 31	370	0.3 "	.....	261	.....	
6	May 9	400	0.3 "				
	" 31	335	0.3 "				
	June 19	347	1.0 "	July 1	348	0.3 cc.	Recovered.
7	May 9	275	0.3 "	.....	210	.....	Died 16 days after first injection. Not autopsied.
8	May 9	290	0.3 "				
	" 31	220	0.3 "				
	June 19	232	1.0 "	July 1	193	0.3 cc.	Recovered.
9	May 9	370	0.3 "				
	" 31	300	0.3 "				
	June 19	277	1.0 "	July 1	276	0.3 "	Died August 17, 1899.
10	May 9	335	0.3 "				
	" 31	303	0.3 "				
	June 19	321	1.0 "	July 1	338	0.3 "	Recovered.
11	May 9	340	0.3 "				
	" 31	315	0.3 "				
	June 19	316	1.0 "	July 1	327	0.3 "	Recovered.
12	May 9	385	0 3 "				
	" 31	295	0.3 "				
	June 19	278	1.0 "	July 1	312	0.3 "	Recovered.
13	(Control)		.....	July 1	310	0.3 "	Died July 12, 1899.
14	(Control)		.....	July 1	257	0.3 "	Died July 8, 1899.

the hog-cholera bacillus. The remaining 10 animals and 3 controls then received on June 19, 0.2 cc. of a virulent culture of B. icteroides, with the result that only one control died on the 13th day.

TABLE IV.

IMMUNIZATION OF GUINEA-PIGS FROM B. ICTEROIDES, ORIGINAL, WITH STERILIZED BOUILLON CULTURES OF THE HOG-CHOLERA BACILLUS.

No.	Date of injection with sterilized culture of Hog-cholera Bacillus.	Weight in grammes.	Quantity of sterilized culture injected.	Date of injection with living culture of B. icteroides.	Weight in grammes.	Quantity of living culture injected.	Remarks.
	1899			1899			
1	May 19	740	0.3 cc.	June 19	760	0.2 cc.	
	" 31	783	1.0 "	July 6	645	0.3 "	Recovered.
2	" 19	795	0.3 "	June 19	860	0.2 "	
	" 31	885	1.0 "	July 6	815	0.3 "	Recovered.
3	" 19	682	0.3 "	June 19	735	0.2 "	
	" 31	740	1.0 "	July 6	732	0.3 "	Recovered.
4	" 19	665	0.3 "	June 19	740	0.2 "	
	" 31	730	1.0 "	July 6	672	0.3 "	Recovered.
5	" 19	700	0.3 "	June 19	755	0.2 "	
	" 31	770	1.0 "	July 6	792	0.3 "	Recovered.
6	" 19	665	0.3 "	June 19	687	0.2 "	
	" 31	715	1.0 "	July 6	670	0.3 "	Recovered.
7	" 19	668	0.3 "	.....	580	.....	Died 11 days after first injection. Cultures sterile.
8	" 19	545	0.3 "	.....	500	.....	Died 4 days after first injection. Cultures sterile.
9	" 19	732	0.3 "	June 19	705	0.2 cc.	
	" 31	692	1.0 "	July 6	700	0.3 "	Recovered.
10	" 19	610	0.3 "	June 19	615	0.2 "	
	" 31	645	1.0 "	July 6	614	0.3 "	Recovered.
11	" 19	727	0.3 "	June 19	672	0.2 "	
	" 31	790	1.0 "	July 6	730	0.3 "	Recovered.
12	" 19	642	0.3 "	June 19	698	0.2 "	
	" 31	662	1.0 "	July 6	687	0.3 "	Recovered.
13	(Control)		.....	June 19	457	0.2 "	
				July 6	375	0.3 "	Died Aug. 31, 1899.
14	(Control)		.....	June 19	695	0.2 "	
				July 6	555	0.3 "	Died July 9, 1899.
15	(Control)		.....	June 19	520	0.2 "	Died July 1, 1899.

On July 6, therefore, the remaining controls and the 10 protected guinea-pigs were again inoculated with 0.3 cc. of a virulent culture of B. icteroides. As the result of this second injection, one control

died on the 3d day and one on the 55th day following the inoculation, while the 10 immunized guinea-pigs recovered.

Immunization of rabbits from the hog-cholera bacillus with living cultures of B. icteroides.

The culture of B. icteroides, Havana, isolated from the cadaver of Private Patrick Smith, to whose case we have already made reference, when tested by us on guinea-pigs and rabbits, was found to be of decidedly weakened virulence as compared with the culture of this bacillus received from Roux's laboratory. We therefore selected 4 rabbits which had survived for a period of 26 days the subcutaneous inoculation of 0.3 cc. of a 24-hour culture of B. icteroides, Havana, and endeavored to heighten their immunity by repeated injections of

TABLE V.

IMMUNIZATION OF RABBITS FROM THE HOG-CHOLERA BACILLUS, WITH LIVING CULTURES OF ICTEROIDES, HAVANA.

No.	Date of injection with living culture of B. icteroides.	Weight in grammes.	Quantity of living culture of B. icteroides injected.	Date of injection with living culture of Hog-cholera Bacillus.	Weight in grammes.	Quantity of living culture of Hog-cholera Bacillus injected.	Remarks.
	1899			1899			
1	Aug. 18	2165	0.3 cc.				
	Sept. 13	....	0.5 "				
	Oct. 6	....	0.5 "				
	Oct. 26	....	1.0 "				Still living at end of
	Nov. 13	....	1.0 "	Dec. 11	2265	0.1 cc.	51 days.
2	Aug. 18	1520	0.3 "				
	Sept. 13	....	0.5 "				
	Oct. 6	....	0.5 "				
	Oct. 26	....	1.0 "				Still living at end of
	Nov. 13	....	1.0 "	Dec. 11	1615	0.1 cc.	51 days.
3	Aug. 18	1000	0.3 "				
	Sept. 13	....	0.5 "				
	Oct. 6	....	0.5 "				
	Oct. 26	....	1.0 "				Still living at end of
	Nov. 13	....	1.0 "	Dec. 11	1565	0.1 cc.	51 days.
4	Aug. 18	1050	0.3 "				
	Sept. 13	....	0.5 "				
	Oct. 6	....	0.5 "				
	Oct. 26	....	1.0 "				Still living at end of
	Nov. 13	....	1.0 "	Dec. 11	1487	0.1 cc.	51 days.
5	(Control)		.....	Dec. 11	1065	0.1 "	Died Dec. 17, 1899.
6	(Control)		.....	Dec. 11	1060	0.1 "	Died Dec. 15, 1899.

increasing doses of the living culture. Later we injected these rabbits and 2 controls with 0.1 cc. of a virulent culture of the hog-cholera bacillus. The results are recorded in Table V.

While the controls died on the 4th and 6th day after inoculation, the immunized rabbits were alive and apparently in good condition at the end of one month and 25 days, thus demonstrating that an animal so very susceptible as the rabbit to B. icteroides and the hog-cholera bacillus may be rendered immune from a fatal dose of the hog-cholera bacillus by repeated injections of a living culture of B. icteroides of weak virulence.

Reciprocal Agglutinative Reactions.

Reciprocal agglutinative reaction of the sera of animals immunized from B. icteroides and the hog-cholera bacillus.

Our first observation was made with the serum of a dog which had been partly immunized by the intravenous injection of increasing doses of B. icteroides. Serum obtained from this dog at the end of three months, tested in a dilution of 1 to 5,000, brought about prompt arrest of motility of the hog-cholera bacillus followed by agglutination at the end of one hour. The death of this dog prevented further tests of the serum.

A second observation made with a specimen of "anti-amaryllic" serum received from South America, showed that while B. icteroides was agglutinated in a dilution of 1 to 120,000, it required a dilution of 1 to 30,000 to bring about a like agglutination of the hog-cholera bacillus at the end of one hour.

A third observation was made with the blood-serum of a dog which had been injected with gradually increasing doses of the bacillus of hog-cholera, during a period of three months. With this serum, the hog-cholera bacillus was agglutinated in a dilution of 1 to 2,000, and the Sanarelli bacillus in a dilution of 1 to 600, at the end of one hour.

These several sera, in a dilution of 1 to 20, were entirely negative in their reaction toward the typhoid bacillus and Bacillus coli communis.

Agglutinative reaction of the blood of yellow fever upon B. icteroides and the hog-cholera bacillus.

Sanarelli [33] has observed that the serum of the blood of yellow-fever cadavers produces in cultures *in vitro* of B. icteroides the phenomenon of agglutination, but that the intensity of this reaction is very variable. The serum obtained in one case on the 17th day of convalescence from yellow fever produced very slight agglutination.

Archinard and Woodson,[34] using the dried blood of yellow-fever patients, in the estimated dilution of 1 to 10 to 1 to 40, obtained in over 70 per cent of a series of 50 cases examined cessation of motion and agglutination, "the reaction being as characteristic as in typhoid fever cases." Accordingly they urge the practical value of the serum diagnosis of yellow fever. In a later paper,[35] these observers report that the reaction is present in over 80 per cent of cases of yellow fever or of recent convalescents.

Wasdin and Geddings [36] failed to confirm the observations of Archinard and Woodson. As the outcome of their experience with the test, using the blood of yellow fever patients and the blood of animals sick or dead of inoculation with B. icteroides, they state: "The results were most varying and bewildering and convince us that whatever may be the value of the reaction as a diagnostic point in enteric fever, it has little or none in yellow fever."

We have, through the kindness of Acting Assistant Surgeon A. Agramonte, U. S. Army, on duty in Havana, Cuba, received specimens of blood from a number of cases of yellow fever. These we have subjected to careful tests, using the method suggested by Wyatt Johnston which we have uniformly found satisfactory for testing the agglutinative reaction of the blood of typhoid fever. The dilution used by us was approximately 1 to 30. In addition to making duplicate tests of this blood with B. icteroides original, we have also tested its action on the hog-cholera bacillus and on Bacillus A (Archinard

[33] L'immunité et la sérothérapie contre la fièvre jaune. *Annales de l'Institut Pasteur*, 1897, xi, p. 753.

[34] The Serum Diagnosis of Yellow Fever. *New Orleans Medical and Surgical Journal*, 1898, l, p. 455.

[35] Bacteriological study in the etiology of yellow fever. *New York Medical Journal*, 1899, lxix, p. 109.

[36] *Op, cit.*, p. 78.

and Woodson),[37] since the latter bacillus responded to agglutination equally with the Sanarelli bacillus in the hands of these observers. We present the results in Table VI.

TABLE VI.

AGGLUTINATION TESTS WITH DRIED BLOOD FROM CASES OF YELLOW FEVER.—ESTIMATED DILUTION 1 TO 30.—TIME 4 HOURS.

No.	Case.	Day of illness.	B. icteroides, (Original).	Hog-cholera bacillus.	Bacillus "A" (Archinard & Woodson).
1	A. J. Bathon. (Doubtful case).....	8th	Negative.	Negative.	Negative.
2	Robert Stewart. (Undisputed case).....	9th	"	"	"
"	" "	12th	"	"	"
3	Wm. Kehrer. (Fatal case)	4th	"	"	"
4	Tom Buchanan. (Typical case)	5th	"	"	"
5	Mike Deveney. (Typical case)	5th	"	"	"
6	G. P. Thomas. (Typical case)	13th	"	"	"
7	J. G. Thatcher. (Typical case)	9th	"	"	"
8	George Woods. (Typical case)	7th	"	"	"
9	Lant Shears. (Typical case)	4th	"	"	"
10	Wm. Demuth. (Typical case)	4th	Positive at end of 1½ hours.	Positive at end of 1½ hours.	Partial arrest of motility with some agglutination.
11	B. Dadd. (Typical case)	5th	Negative.	Negative.	Negative.
12	Wm. Shaw. (Typical case)	12th	"	"	"
13	Fred Worrell. (Typical case)	Convalescent	"	"	"
14	Wm. J. Mooney. (Typical case—fatal)...	2d, 14 days before death.	"	Not sufficient blood for a fair test.	"
15	Jas. A. Hays. (Typical case—fatal)...	1st day after admission, 2 days before death.	"	Negative.	"
					"

[37] This actively motile bacillus (A) which was isolated by Archinard and Woodson in 32 of 39 autopsies of yellow-fever cases, (4 times in 5 cases from the blood taken at the elbow), differs from B. icteroides (Sanarelli) in its more rapid growth and tendency to spread in gelatine plates. The colonies are large, and of irregular outline. It does not coagulate milk nor bring about opalescence in this medium; it ferments glucose, but not lactose or saccharose; grows freely on blood serum and gives only a slight indol reaction. It belongs to the hog-cholera group and corresponds closely in its cultural characters with B. enteritidis (Gärtner). It is not a form of Bacillus coli communis as stated by Wasdin and Geddings (Report on the Cause of Yellow Fever, p. 16).

TABLE VI—*Continued.*

No.	Case.	Day of illness.	B. icteroides, (Original).	Hog-cholera bacillus.	Bacillus "A." (Archinard & Woodson).
16	Luis Colome. (Typical case—recovered)	6th	Negative.	Negative.	Negative.
17	J. J. Dougherty. (Typical case)	7th	"	"	"
18	Burton Fowler. (Typical case)	8th	"	"	"
"	" "	12th			
"	" "	15th	"	"	"
19	Anthony Wiedner. (Typical case)	5th	"	"	"
"	" "	12th			
20	Timothy Healy. (Typical case)	5th	"	"	"
"	" "	9th	"	"	"
21	Stephen Scanlan (Typical case)	6th	"	"	"
22	Wm. Harper	Convalescent	"	"	"
23	Jess Hilton	9th	"	"	"
24	Chas. Mitchell	7th	"	"	"
25	Max Thompson	9th	"	"	"
26	Dan. Coleman	9th	"	"	"
27	Chas. Rodgers	8th	"	"	"
"	" "	13th	"	"	"
28	Sigismund Fichman	6th	"	"	"
"	" "	9th	"	"	"
"	" "	12th	"	"	"
29	Feodora Fernandez	8th	"	"	"

It will be seen that only one of 29 (3.4 per cent) samples of yellow fever blood, taken on various days of the disease, or during convalescence, exhibited any agglutinative reaction towards B. icteroides; and, further, that this specimen of blood was also positive in its agglutinative effect upon the hog-cholera bacillus.

Our observations, therefore, while agreeing with those of Wasdin and Geddings, do not confirm the results obtained by Archinard and Woodson as to the agglutinative reaction of the blood of yellow fever upon B. icteroides.

Agglutinative reaction of the blood of hog-cholera upon the hog-cholera bacillus and B. icteroides.

We also submit in Table VII the results of tests made with fluid serum, dried blood and dried serum obtained, through the kind assistance of Dr. E. A. de Schweinitz, U. S. Department of Agriculture,

from hogs affected with hog-cholera. The material was obtained immediately after death from animals that were killed and upon post-mortem examination pronounced to be cases of hog-cholera by the veterinarian.

TABLE VII.

AGGLUTINATION TESTS WITH FLUID SERUM, DRIED BLOOD AND DRIED SERUM FROM HOGS SUPPOSED TO BE SUFFERING WITH HOG-CHOLERA.—TIME 2 TO 4 HOURS.

No.	Material used.	Hog-cholera bacillus No. 1.	B. icteroides, (Original).
1	Fluid Serum. Dilution 1 to 30.	Negative.	Negative.
2	" " " "	Positive.	Positive.
3	" " " "	"	"
4	" " " "	Motility arrested. No agglutination.	Motility arrested. No agglutination.
5	Dried Blood. Estimated dilution 1 to 30.	Negative.	Negative.
6	" " " " "	"	"
7	" " " " "	"	"
8	" " " " "	Positive.	Positive.
9	" " " " "	Negative.	Negative.
10	" " " " "	Motility arrested. No agglutination.	Motility arrested. No agglutination.
11	" " " " "	Negative.	Negative.
12	" " " " "	"	"
13	" " " " "	Positive.	Positive.
14	" " " " "	"	"
15	" " " " "	Negative.	Negative.
16	" " " " "	"	"
17	" " " " "	Impairment of motility with slight agglutination.	Impairment of motility with slight agglutination.
18	" " " " "	Negative.	Negative.
19	" " " " "	"	"
20	" " " " "	Impairment of motility with slight agglutination.	Impairment of motility with slight agglutination.
21	" " " " "	Negative.	Negative.
22	" " " " "	"	"
23	" " " " "	"	"
24	" " " " "	"	"
25	" " " " "	"	"
26	" " " " "	"	"
27	Dried Serum. Estimated dilution 1 to 30.	"	"
28	" " " " "	"	"
29	" " " " "	Positive.	Positive.
30	" " " " "	Negative.	Negative.
31	" " " " "	"	"
32	" " " " "	"	"

Thus, of 32 samples of serum or dried blood from cases pronounced to be hog-cholera, 6, or 18.75 per cent, gave a positive reaction, and 4, or 12 per cent, a partial reaction with both the hog-cholera bacillus and B. icteroides.

At what period of the disease these specimens of blood were taken, we have no positive means of determining. We think, however, that the results obtained with B. icteroides when taken in connection with the negative reaction shown by the blood of yellow fever are very suggestive.

CONCLUSIONS.

1. Bacillus X (Sternberg) belongs to the colon group.

2. Bacillus icteroides (Sanarelli) is a member of the hog-cholera group.

3. The various channels of infection, the duration of the disease and the gross and microscopical lesions in mice, guinea-pigs and rabbits are the same for Bacillus icteroides and the hog-cholera bacillus.

4. The clinical symptoms and the lesions observed in dogs inoculated intravenously with Bacillus icteroides, are reproduced in these animals by infection with the hog-cholera bacillus.

5. Bacillus icteroides when fed to the domestic pig causes fatal infection, accompanied by diphtheritic, necrotic and ulcerative lesions in the digestive tract, such as are seen in hogs when infected with the hog-cholera bacillus.

6. This disease may be acquired by exposing swine in pens already infected with Bacillus icteroides, or by feeding them with the viscera of infected pigs.

7. Guinea-pigs may be immunized with sterilized cultures of Bacillus icteroides from a fatal dose of the hog-cholera bacillus and *vice versa.*

8. Rabbits may be rendered immune by gradually increasing doses of a living culture of Bacillus icteroides of weak virulence from a fatal dose of a virulent culture of the hog-cholera bacillus

9. The sera of animals immunized with Bacillus icteroides and with the hog-cholera bacillus, respectively, show a marked reciprocal agglutinative reaction.

10. While the blood of yellow fever practically does not exercise an agglutinative reaction upon Bacillus icteroides, the blood of hog-cholera agglutinates this bacillus in a much more marked degree, thus pointing, we think, to the closer etiological relationship of this bacillus to hog-cholera than to yellow fever.[38]

DESCRIPTION OF PLATE XIX.

Fig. 1. Photomicrograph showing focal necrosis in the liver of a guinea-pig. Death on 6th day after subcutaneous inoculation with B. icteroides. See pp. 240–242. × 100.

Fig. 2. Photomicrograph of section of human liver showing focal necrosis invaded by leucocytes; no fatty degeneration. From case of Patrick Smith, 8th infantry. Death on 9th day of illness. B. icteroides isolated from blood 4 days before death. Typhoid bacillus isolated from spleen at autopsy. See p. 248. × 100.

Fig. 3. Photomicrograph of section of human liver in yellow fever, showing fatty degeneration. × 100.

[38] In a preliminary note on "The Etiology of Yellow Fever," by Reed, Carroll, Agramonte and Lazear (*Philadelphia Medical Journal*, Oct. 27, 1900), the authors state that they failed to find B. icteroides either in the blood during life of 21 patients in various stages of yellow fever or in cultures from the blood and organs at 11 autopsies of yellow-fever patients.

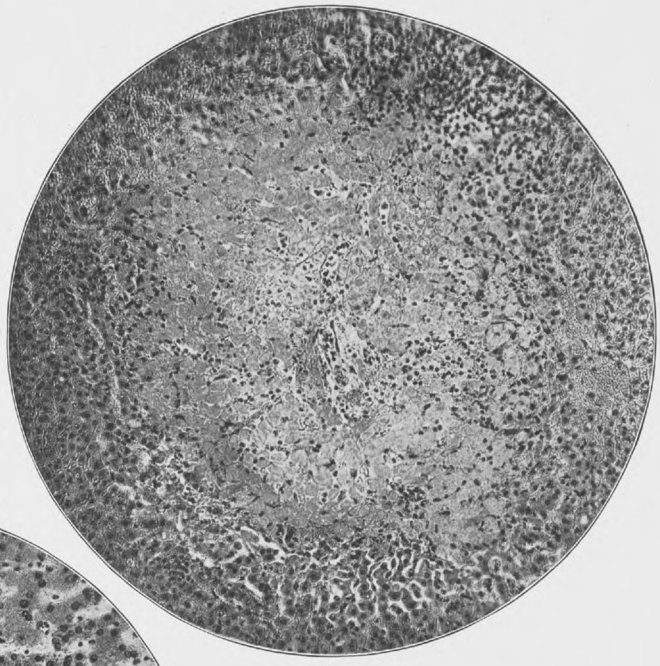

FIG. 1.

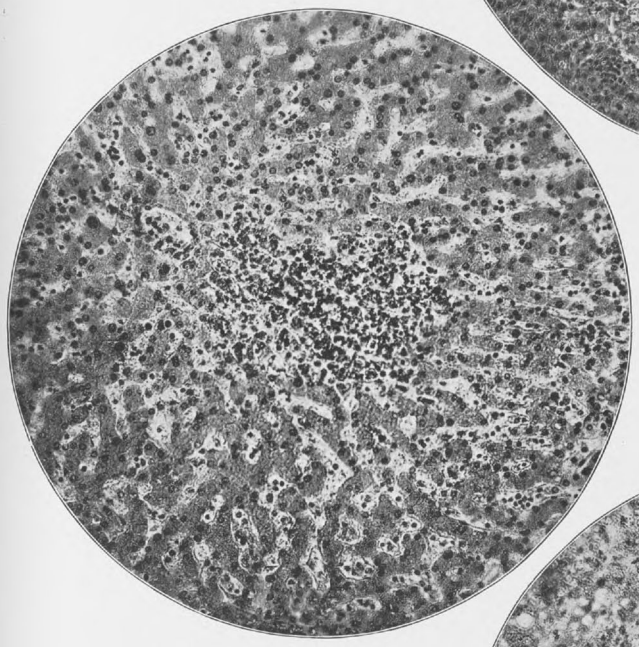

FIG. 2.

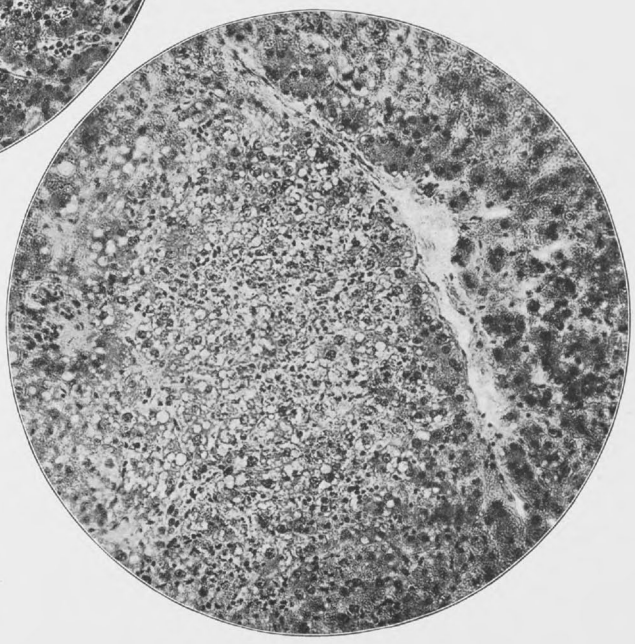

FIG. 3.