A STUDY OF THE PATHOGENIC PROPERTIES OF BACILLUS PROTEUS.*

By W. P. LARSON, M.D., and E. T. BELL, M.D.

(From the Department of Bacteriology and Pathology of the University of Minnesota, Minneapolis.)

Plates 65 to 67.

It is probable that no microörganism is encountered more frequently by those engaged in routine bacteriological work than some member of the *proteus* family, and yet there are few that at present attract less attention than these common bacteria. In fact, our standard text-books on bacteriology and pathology devote little or no space to these organisms.

The term *proteus* means a variety of form. Undoubtedly, many organisms have been classified as *proteus* bacilli which really do not belong to this class. Today the term *proteus* embraces such a large group of bacteria as to have no well defined limits.

GENERAL CHARACTERS OF PROTEUS BACILLI.

Hauser (1) described three types of *proteus* bacilli, classified according to their action upon gelatin. *Bacillus proteus vulgaris* is a rapid liquefier, liquefaction appearing in cultures from six to eight hours old. *Proteus mirabilis* liquefies more slowly. Both of these types, according to Hauser, form zoöglea in gelatin cultures. *Proteus zenkeri* neither liquefies gelatin nor forms zoöglea in this medium. These organisms are aerobic bacteria, growing readily on all culture media.

It is not the purpose of the present study to attempt to clarify the somewhat confused classification of the *proteus* group of organisms. We shall limit ourselves to a brief description of those with which we have experimented.

The *proteus* bacilli are Gram-negative, actively motile organisms, whose morphological characters resemble those of the colon typhoid

* Aided by a grant from the Research Fund of the University of Minnesota. Read before the Association of American Pathologists and Bacteriologists, Toronto, April, 1914. Received for publication, February 18, 1915.

group. The Gram-positive, spore-forming organisms described by some writers, we believe, should not be classified with the *proteus* family.

The strains with which we have experimented showed beginning liquefaction within twenty-four hours. Liquefaction has never been observed earlier than twelve hours. Milk was curdled readily, and the coagulum redissolved in about ninety-six hours. Loeffler's blood serum was usually liquefied. When cultivated on the various sugar media, the *proteus* resembles the paratyphoid bacilli. Dextrose and maltose are fermented with acid and gas formation. Saccharose is unaffected by most strains. Lactose, as a rule, is not fermented.

Jordan (2) found that the ratio of hydrogen to carbon dioxide formed in dextrose media is characteristic of the *proteus*. According to this author the amount of hydrogen formed always exceeds that of carbon dioxide; and this fact may be used as an aid in identification. Indol is formed in very perceptible proportions.

Widely divergent opinions prevail relative to the pathogenic properties of the *proteus* organisms. Many authors regard them as obligatory saprophytes. There is ample evidence in the literature, however, to show that they may at times become pathogenic.

In reviewing the literature in an attempt to determine precisely with what diseased conditions the *proteus* has been observed, one is handicapped by the imperfect descriptions of the organisms termed *proteus*. In fact, in some cases only the morphology of the germ is discussed, and it appears that a few of the earlier writers have felt justified in classing any pleomorphic bacillus as a *proteus*.

Foà and Bonome (3) recovered an organism which they believed to be the *proteus* from a case of volvulus in the year 1889. About a year later Krogius (4) reported a case of cystitis caused by the *proteus*. In the same year Schnitzler (5) reported a series of nine cases of cystitis due to the same organism. Jaeger (6) studied a series of ten cases of Weil's disease, some of which came to autopsy, in infections which occurred in the garrison at Ulm. He concluded that Weil's disease was a specific infectious disease due to *Bacillus proteus*.

In this country a *proteus* infection was described as early as 1892 by Flexner (7), who isolated *proteus vulgaris* from a case of peritonitis which had terminated fatally. Since then it has been found in a multitude of diseased conditions, such as pyelonephritis, prostatitis, enteritis, abscesses of various localities, caries of the bone, pleuritis, meningitis, arthritis, osteomyelitis, and gangrene. Not only man but some of the domestic and even the cold-blooded animals are subject to *proteus* infections. Jensen (8) has repeatedly found the *proteus* to

be the cause of diarrhea of calves. Jaeger described an epidemic of fowls in which the *proteus* likewise was found to be the infectious agent. He isolated this organism from chickens, geese, and ducks which had succumbed to the infection. In the year 1897 Wyss (9) studied an epidemic among the fish in Lake Zurich, which was, he believed, caused by *proteus vulgaris*. He recovered the organism from thirteen carcasses.

SOURCES OF PATHOGENIC PROTEUS STRAINS.

The first *proteus* strain with which we experimented was isolated from a laparotomy wound in a case which subsequently terminated fatally. The *proteus* was repeatedly isolated from this patient in pure culture. The second strain was isolated from a severe eye infection following a cataract operation. A third strain was isolated from an infection of the finger, believed to have been contracted at an autopsy. A fourth was isolated from the heart's blood of a case which had died from peritonitis following a gunshot wound of the intestines. The fifth strain was from a case of gangrene of the lung.

These organisms all proved to be pathogenic for rabbits. In our preliminary report (10) we have called attention briefly to some of the histological changes produced by *Bacillus proteus*.

Below we give the details of the experiments conducted during the past three years.

DESCRIPTION OF EXPERIMENTS.

Strain A.—Several strains of *proteus* bacilli have been used. Strain A was obtained from a suppurating laparotomy wound through which a tuberculous kidney had been removed. The following twenty-six experiments were made with this strain.

Experiment 1.[1]—Rabbit. Apr. 29, 1912. Inoculated intraperitoneally with one-fourth of a twenty-four hour slant agar culture obtained directly from the laparotomy wound. May 11. Death. Loss of weight, 25 per cent. At autopsy numerous soft, whitish nodules were found on the mesentery; also a few on the diaphragm and posterior abdominal wall. The lesions were in the peritoneum and subjacent tissue.

Microscopic Examination.—The nodules are composed mainly of a mass of polymorphonuclear leucocytes surrounded by a well developed wall of young connective tissue. There is necrosis in the central part of some of the abscesses. *Bacillus proteus* was recovered in pure culture from the lesions.

[1] These serial numbers are used for convenient reference. They do not always indicate the exact order in which the experiments were performed.

Experiment 2.—Rabbit. May 13, 1912. Inoculated intraperitoneally with a loopful of a twenty-four hour agar culture from experiment 1. May 30. Killed. Loss of weight, 11 per cent. At autopsy a number of small, whitish nodules were found on the omentum, mesentery, and liver.

Microscopic Examination.—The nodules are composed mainly of large connective tissue cells of the so called epithelioid type (figures 1 to 4). This is a typical proliferative inflammation. In histological structure it resembles an epithelioid cell tubercle. It will be described more fully below. One of the chief objects of our subsequent experiments with *B. proteus* was the study of this peculiar proliferative lesion.

Experiment 3.—Rabbit. May 20, 1912. Inoculated intravenously with 1 c.c. of a forty-eight hour broth culture from experiment 1. This broth culture was taken from an agar culture seven days old. May 27. Killed. Loss of weight, 10 per cent. At autopsy a large number of whitish nodules, some attaining a diameter of 5 mm., were seen in the liver. The lesions are scattered through the parenchyma of the organ. Similar nodules are found in both kidneys. The right lung contains large abscesses which have destroyed a considerable part of the organ.

Microscopic Examination.—The lesions are ordinary abscesses, consisting mainly of polymorphonuclear leucocytes. There is very little tendency toward encapsulation of the abscesses. *B. proteus* was obtained in pure culture from the lesions. This was the only instance in which we succeeded in producing macroscopic lesions by intravenous inoculation.

Experiment 4.—Rabbit. May 20, 1912. Inoculated intraperitoneally with 1 c.c. of the same culture used in experiment 3. May 25. Killed. Loss of weight, 19 per cent. At autopsy the omentum, mesentery, and the abdominal surface of the diaphragm were studded with small whitish nodules.

Microscopic Examination.—The nodules are composed of large connective tissue cells of epithelioid type, among which are a variable number of large and small mononuclear leucocytes. In some nodules the leucocytes predominate. No polymorphonuclear leucocytes are present. The lesions are, therefore, partly of proliferative and partly of exudative character. *B. proteus* was recovered in pure culture.

It will be noted that the original culture produced abscesses only (experiment 1). Cultures from experiment 1, injected intraperitoneally, produced proliferative lesions in experiment 2, and mixed exudative and proliferative lesions in experiment 4. When injected intravenously, the culture from experiment 1 produced abscesses (experiment 3).

Experiment 5.—Rabbit. June 7, 1912. Inoculated intraperitoneally with one-half of a twenty-four hour agar culture, taken from an agar culture which had stood in the laboratory since Apr. 16. No symptoms of infection appeared. June 13. Killed. Increase in weight, 1 per cent. No lesions were found at autopsy.

Experiment 6.—Rat. June 11, 1912. Inoculated intraperitoneally with 3 c.c.

of an eighteen hour broth culture, taken from the agar culture which had stood in the laboratory since Apr. 16. No symptoms of infection developed. June 17. Killed. Loss of weight, 10 per cent. No lesions were found at autopsy.

Experiment 7.—Rabbit. June 13, 1912. Inoculated intravenously with the same culture used in experiment 5. June 19. Killed. Loss of weight, 2 per cent. No lesions were found at autopsy.

These three experiments (5, 6, and 7) show that strain A had lost its virulence entirely by being kept in the laboratory for about two months.

Experiment 8.—Rat. June 11, 1912. 3 c.c. of an eighteen hour broth culture from experiment 4 were injected into the peritoneal cavity. Symptoms of infection developed and gradually became more severe. June 17. Animal moribund. Killed. Loss of weight, 29 per cent. At autopsy yellowish white nodules were found on the omentum, diaphragm, and liver.

Microscopic Examination.—Not made, but *B. proteus* was recovered in pure culture from the lesions.

Experiment 9.—Guinea pig. June 13, 1912. Inoculated intraperitoneally with 2 c.c. of a twenty-four hour broth culture from experiment 4, taken May 25. June 19. Killed. Loss of weight, 17 per cent. Large whitish nodules were found in the omentum at autopsy.

Microscopic Examination.—The nodules consist of epithelioid cells among which are a considerable number of polymorphonuclear leucocytes. The lesions represent a mixture of the exudative and the proliferative types of inflammatory reaction. They resemble the lesions found in experiment 4, except that the leucocytes in this instance are of the polymorphonuclear type.

Experiment 10.—Rat. June 19, 1912. 4 c.c. of a twenty-four hour broth culture from experiment 8 were given intraperitoneally. Death occurred within a few hours. The organism was recovered from the peritoneal cavity.

Experiment 11.—Rat. June 19, 1912. 3 c.c. of the same culture used in experiment 10 were injected. Death occurred the following night. The organism was recovered from the peritoneal cavity.

Experiment 12.—Rat. June 19, 1912. Intraperitoneal injection with 2 c.c. of the same culture used in experiment 10. Animal was sick the following day, but recovered. Increase in weight, 15 per cent. June 24. Killed. No lesions were found at autopsy.

Experiment 13.—Rat. June 19, 1912. 1 c.c. of the same culture used in experiment 10 was injected. No symptoms developed. Increase in weight, 5 per cent. June 24. Killed. No lesions found.

Experiment 14.—Rats. June 20, 1912. Three rats were each inoculated with 1.5 c.c. of a broth culture from experiment 8. All died the following night. The organism was recovered from the peritoneal cavity.

The preceding five experiments show that a *proteus* strain which had recently produced lesions in rats may lose this property entirely, although it may still be toxic when given in large doses.

Experiment 15.—Rats. June 21, 1912. Three rats were each inoculated intraperitoneally with 1 c.c. of a seven hour broth culture from experiment 14. All three were found dead the next morning. The organism was recovered from the peritoneal cavity.

Experiment 16.—Rats. June 22, 1912. Four rats were each injected intraperitoneally with 0.25 c.c. of a twenty-four hour broth culture from experiment 14. The first three died the following night. The organism was recovered from the peritoneal cavity. The fourth was sick the next day but recovered. No lesions were found when it was killed nine days later.

Experiments 15 and 16 show that the toxicity of strain A for rats had been markedly increased by a series of animal passages.

Experiment 17.—Rats. June 24, 1912. Two rats were injected intraperitoneally with 1 c.c. of a washed broth culture of *proteus* from experiment 16. No symptoms of infection appeared. Both were killed seven days later. No lesions were found at autopsy.

It appears from this experiment that the toxic properties of this *proteus* strain bear no relation to its ability to produce lesions. This is the same strain that produced lesions in experiment 8. By a series of animal passages the toxicity became markedly exalted, but, when the toxins were removed, the organism was harmless.

Experiment 18.—Rabbit. Aug. 2, 1912. Injected intravenously with one-fourth of an agar culture from experiment 9, which had stood in the laboratory since June 19. Aug. 8. Animal was in good condition. Aug. 22. Death from an intercurrent infection. No *proteus* lesions were found at autopsy.

Experiment 19.—Rabbit. Aug. 16, 1912. Inoculated intraperitoneally with one-half of a twenty-four hour culture of the same organism used in experiment 18. The animal gained in weight for several days. Death from pneumonia. Nov. 3. No *proteus* lesions were found at autopsy.

Experiments 18 and 19 demonstrate that strain A had lost its virulence entirely. No further experiments were made until April, 1913. The *proteus* cultures were kept in the laboratory during the intervening period.

Experiment 20.—Rabbits. One of the old cultures from strain A, which had long since lost its virulence, was injected into the anterior chamber of the eye of a rabbit (experiment 20 a). Twenty-four hours later it was recovered and this culture was injected into the peritoneal cavity of another rabbit (experiment 20 b), Apr. 26, 1913. The latter died the next day. *B. proteus* was recovered in pure culture from the peritoneal cavity and heart's blood.

Experiment 21.—Rabbit. Apr. 27, 1913. Injected intravenously with the same culture that was used intraperitoneally in experiment 20 b. No symptoms of infection developed.

Experiment 22.—Rabbit. Apr. 28, 1913. Intraperitoneal injection with one-

half of an agar culture from experiment 20 b. May 3. Animal was moribund. Killed. Loss of weight, 35 per cent. The omentum, both surfaces of the diaphragm, and the parietal pericardium were studded with soft whitish nodules. There were also a few nodules on the intestines. Emaciation was extreme.

Microscopic Examination.—The nodules are composed mainly of polymorphonuclear leucocytes. The central portions are necrotic. *B. proteus* was recovered in pure culture.

Experiment 23.—Rabbit. Apr. 28, 1913. Injected intraperitoneally with one-half of an agar culture from experiment 20 b. The animal lost weight for a few days, but began to regain it. May 6. Killed. Large, yellowish white nodules were found in the omentum, mesocolon, and parietal peritoneum.

Microscopic Examination.—The lesions consist of masses of polymorphonuclear leucocytes surrounded by a zone of newly formed connective tissue. They are healing abscesses. *B. proteus* was recovered in pure culture.

Experiment 24.—Rabbits. May 5, 1913. Two rabbits were each injected intraperitoneally with one loop of an agar culture from experiment 22. No symptoms of infection developed.

Experiment 25.—Rabbit. May 7, 1913. Inoculated subcutaneously with one-half of an agar culture from experiment 23. May 9. Death from an intercurrent infection. A small abscess was found at the site of the injection, from which *B. proteus* was recovered in pure culture. Heart's blood negative.

Experiment 26.—Rabbit. May 7, 1913. Inoculated subcutaneously with an old culture of strain A which had been made virulent by passage through the anterior chamber of the eye of a rabbit, as in experiment 20 a. May 15. Killed. Loss of weight, 41 per cent. A large subcutaneous abscess was found at the site of the inoculation, from which *B. proteus* was recovered in pure culture.

Experiments 20 to 26 show that an avirulent *proteus* strain can be made virulent by inoculation into the anterior chamber of the eye. It is also seen that strain A does not now produce proliferative lesions.

Strain B.—This strain was isolated from an eye infection, following a cataract operation which necessitated removal of the eyeball.

Experiment 27.—Rabbit. May 28, 1912. Injected intraperitoneally with 1 c.c. of a twenty-four hour broth culture of this organism. Death occurred on June 4. Loss of weight, 16 per cent. Autopsy revealed several rather large abscesses in the omentum, from which *B. proteus* was recovered in pure culture.

Microscopic Examination.—The nodules were found to be typical abscesses.

Strain C.—Strain C was obtained from an infection of a finger, which was contracted while assisting at an autopsy.

Experiment 28.—Rabbit. July 29, 1913. Injected intraperitoneally with this culture. No symptoms of infection developed. Aug. 11. Laparotomy revealed a large number of peritoneal lesions. There had been only a slight loss of weight. Aug. 15. Killed. At autopsy a number of small whitish nodules were found on the omentum and colon.

Microscopic Examination.—The nodules showed a proliferative lesion similar to that described in experiment 2.

Experiment 29.—Rabbit. Aug. 18, 1913. Injected intraperitoneally with one-half of a five hour agar culture from experiment 28. Death occurred the following night.

Experiment 30.—Rabbit. Aug. 19, 1913. Injected intraperitoneally with a culture from experiment 28. Animal became ill and lost nearly 200 gm. in weight, but gradually recovered.

Experiment 31.—Rabbit. Sept. 4, 1913. Injected intraperitoneally with a culture from experiment 28. No symptoms of infection developed.

Experiment 32.—Rabbit. Sept. 26, 1913. Injected subcutaneously and intraperitoneally with a culture from experiment 28. No symptoms of infection appeared.

Experiment 33.—Rabbit. Sept. 26, 1913. Injected intraperitoneally with a culture from experiment 28. Sept. 30. Animal dying of an intercurrent disease. Killed. No lesions were found in the peritoneal cavity.

Experiment 28 shows that strain C had the power to produce lesions in rabbits when it was first recovered from human tissue. Experiments 31 to 33 show that this property rapidly disappeared when the organisms were grown in the laboratory.

Strain D.—Strain D was obtained from the heart's blood in a human autopsy. The main autopsy findings were a gunshot wound through the abdomen, producing multiple perforations of the intestines, general peritonitis, and lobar pneumonia. *Bacillus proteus* was also isolated from the peritoneal cavity.

Experiment 34.—Rabbit. Sept. 23, 1913. Injected intraperitoneally with one-half of a twenty-four hour agar culture of strain D. Sept. 24. Died. The peritoneal cavity contained a considerable amount of thin blood-stained fluid. *B. proteus* was recovered from this fluid. The plates contained an occasional staphylococcus colony.

Experiment 35.—Rabbit. Sept. 24, 1913. Injected intraperitoneally with a small dose of the culture used in experiment 34. Sept. 29. Killed. No lesions were found at autopsy.

Experiment 36.—Rabbit. Sept. 24, 1913. Inoculated subcutaneously with one-half of a five hour agar culture from experiment 34. Death occurred during the night.

Experiment 37.—Rabbit. Oct. 8, 1913. Injected subcutaneously with aggressin from a culture of strain D. Two hours later the rabbit was inoculated subcutaneously with a loopful of a culture of the same strain. Symptoms of severe infection appeared. A large subcutaneous abscess developed at the site of the inoculation. Oct. 14. The abdominal cavity was accidentally opened while exploring the abscess. Oct. 16. Death occurred from peritonitis. Loss of weight, 21 per cent. *B. proteus* was recovered in pure culture from the peritoneum as well as from the abscess.

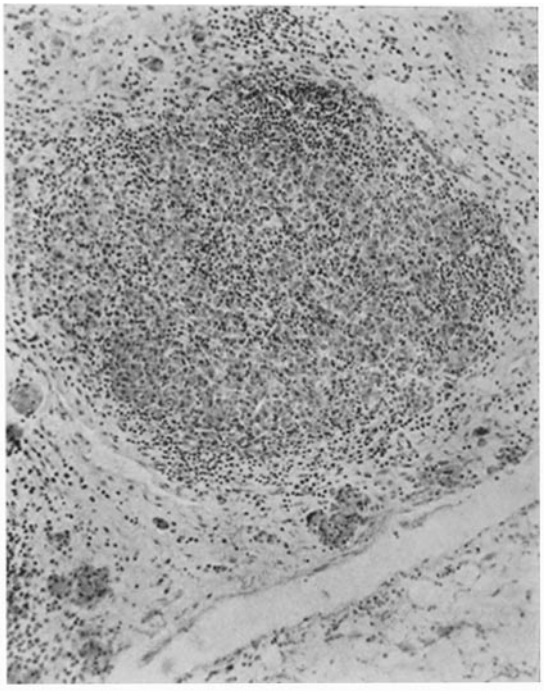

FIG. 1.

(Larson and Bell: Properties of *Bacillus proteus*.)

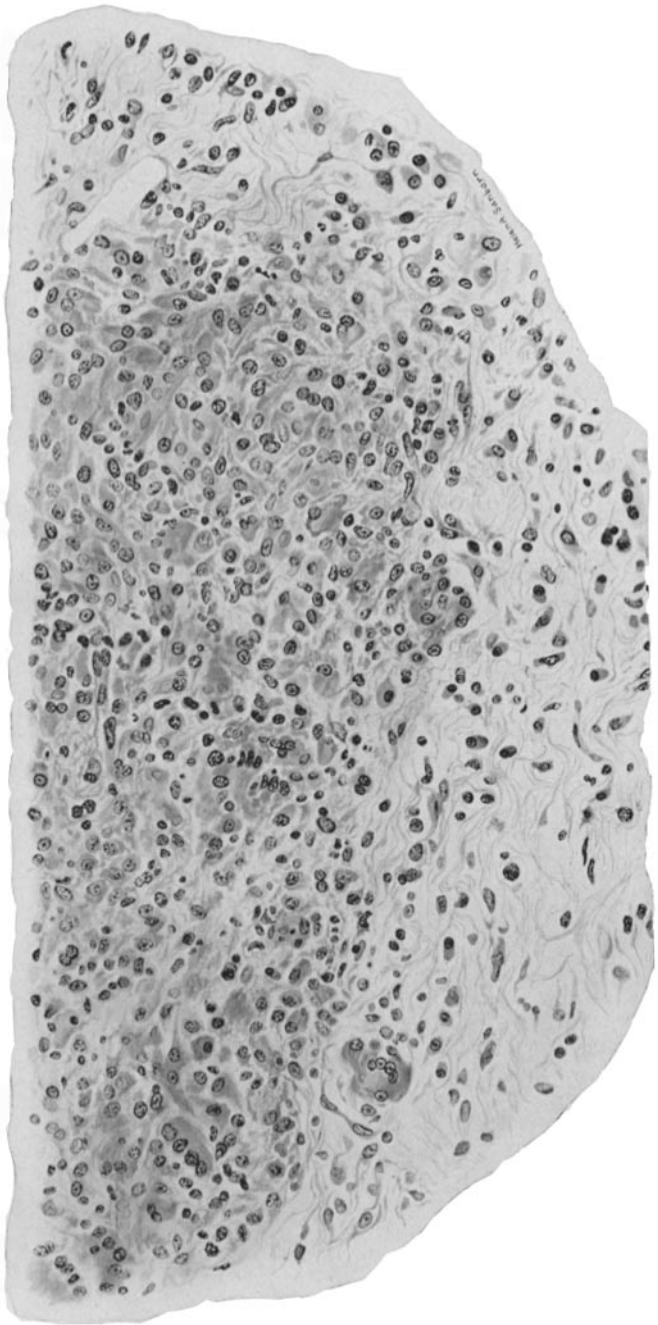

FIG. 2.

(Larson and Bell: Properties of *Bacillus proteus.*)

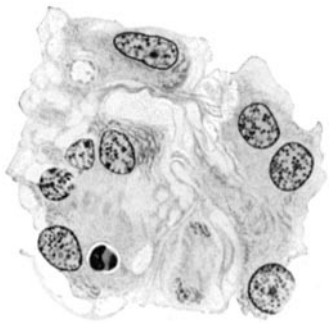

FIG. 3.

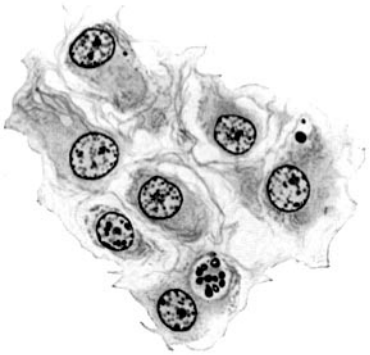

FIG. 4.

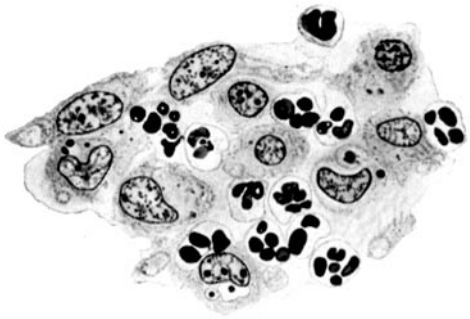

FIG. 5.

(Larson and Bell: Properties of *Bacillus proteus*.)

Experiment 38.—Rabbit. Oct. 8, 1913. Injected subcutaneously with aggressin from a culture of strain D. One hour later inoculated intraperitoneally with a loopful of a culture of the same strain. A large subcutaneous abscess developed at the site of the inoculation. Loss of weight in eight days, 22 per cent. After about four weeks the abscess began to disappear. Dec. 17. Death from an intercurrent disease. The abscess had disappeared entirely by this time. There were no *proteus* lesions present.

Experiment 39.—Rabbit. Oct. 15, 1913. Inoculated intraperitoneally with one-half of a twenty-four hour agar culture taken from experiment 37 at operation. Oct. 24. Died. Loss of weight, 30 per cent. Autopsy revealed a considerable number of whitish nodules, 1 mm. to 3 mm. in diameter, on the mesentery, omentum, diaphragm, and posterior abdominal wall.

Microscopic Examination.—Most of the nodules are ordinary abscesses; but a few show the proliferative type of lesion.

Strain E.—Strain E was obtained from a gangrenous human lung.

Experiment 40.—Rabbit. Dec. 3, 1913. Inoculated intraperitoneally with a twenty-four hour culture of this organism. Dec. 8. Died. At autopsy a large number of characteristic nodules were found on the peritoneal surfaces. Microscopically, some of the nodules were found to be ordinary abscesses. Other nodules show proliferative inflammation. *B. proteus* was recovered from the lesions.

Experiment 41.—Rabbit. Dec. 10, 1913. Inoculated intraperitoneally with one-half of an agar culture from experiment 40. Dec. 18. Killed. Loss of weight, 13 per cent. Numerous soft, whitish nodules were found on the peritoneal surfaces.

Microscopic Examination.—Some of the nodules are ordinary abscesses surrounded by a well developed wall of fibroblasts. Other nodules show mainly the proliferative lesion. There are masses of large connective tissue cells of the epithelioid type among which a considerable number of polymorphonuclear leucocytes are interspersed (figure 5).

Experiment 42.—Rabbit. Dec. 19, 1913. Inoculated intravenously with one-half of an agar culture from experiment 41. Died the next day. *Proteus* recovered from the heart's blood.

Experiment 43.—Rabbit. Dec. 23, 1913. Inoculated intraperitoneally with one-half of an agar culture from experiment 42. Dec. 30. Laparotomy showed no lesions in the peritoneal cavity.

Experiment 44.—Rabbit. Dec. 25, 1913. Injected intravenously with one-half of an agar culture from experiment 42. No symptoms of infection developed.

The proliferative lesion appeared with strain E in experiment 40, and again in experiment 41. It was not followed further, since the strain suddenly lost its virulence.

DISCUSSION.

From the above experiments it will be seen that some strains of *Bacillus proteus* obtained from human lesions are definitely patho-

genic for rabbits, rats, and guinea pigs, producing either typical abscesses or a granulomatous type of lesion. Not all strains of the *proteus* are pathogenic, however. During the past three years we have experimented with twenty other strains which were obtained from various sources, such as decaying organic material and routine autopsy blood cultures where they were probably merely postmortem invaders. In one case a non-pathogenic strain was isolated from an amebic abscess of the liver. None of these proved to be pathogenic for laboratory animals. Rabbits injected either intravenously or intraperitoneally with one to two cubic centimeters of broth cultures of such strains suffered little or no inconvenience. Where excessive doses were given the animals died with symptoms of acute toxemia.

The five strains of *proteus* which were pathogenic for laboratory animals were obtained from human lesions from which, with one exception (strain E), no other organisms were recovered. It is, therefore, probable that these organisms were responsible for the lesions in which they were found. Only in two cases (strains C and E) was it possible to examine microscopically the tissues from which the organisms were obtained. Sections from the infected finger (strain C) showed atypical tubercles, but repeated examinations failed to reveal acid-fast bacilli. However, the possibility of a tuberculous infection in this case cannot be excluded. Strain E was obtained from a gangrenous lung in association with pneumococci. No cellular structure was visible in the area from which the culture was taken. Outside the gangrenous area the lung showed typical lobar pneumonia.

From our work covering a study of twenty-five different *proteus* strains we have come to regard a positive rabbit inoculation as strong evidence that the strain is pathogenic for man. It must be borne in mind that a positive inoculation does not necessarily cause the death of the animal, since some animals may recover from rather severe infections. The method of inoculation is very important. The intraperitoneal inoculation is the method of choice, since intraperitoneal infections are more severe than subcutaneous infections. Inoculations made intravenously rarely result in the production of the characteristic lesions.

The toxic properties of the *proteus* bacilli must not be confused with their power of producing the characteristic inflammatory reactions described in our experiments; in fact, these two characters bear no relation to each other. As indicated in experiments 10 to 16, the toxicity of the organism may become most markedly exalted, while its power to produce the lesions is entirely lost.

Proteus cultures lose their virulence rapidly when kept on laboratory media. The original culture of strain A, which was at first very virulent, became non-pathogenic in about two months (experiments 5, 6, and 7). Another culture of this strain lost its virulence in six weeks (experiments 18 and 19). Strain C became avirulent in three weeks (experiment 31); and strain E in less than one week (experiments 43 and 44). This rapid loss of virulence may explain the fact that *Bacillus proteus* is seldom regarded by bacteriologists as an important pathogenic organism.

A non-pathogenic *proteus* may be made pathogenic by inoculation into the anterior chamber of the eye. In this situation the organisms grow rapidly, there being neither antibodies nor complement present. If recovered from the anterior chamber after twenty-four hours, the culture will usually be found to be pathogenic (experiments 20, 22, and 23). Non-pathogenic strains of streptococci may also become very virulent for rabbits when grown in the anterior chamber, as just described.

A non-pathogenic *proteus* may be enabled to produce lesions by the use of aggressins. The aggressin is injected one or two hours before the animal is inoculated with the bacteria (experiments 37 and 38).

Strains of *proteus,* made pathogenic by either of the above described procedures, produce lesions similar to those produced by strains that were pathogenic when obtained from human lesions.

SYMPTOMS OF PROTEUS INFECTION IN LABORATORY ANIMALS.

The clinical symptoms of experimental *proteus* infection in animals present marked variations, depending mainly upon the virulence of the bacteria. In the mildest cases the animals may not show any signs of infection. In one case (experiment 28), which presented no clinical symptoms, laparotomy revealed rather extensive peritoneal lesions. Mild cases show a temporary loss of weight, weakness, and

decreased appetite, after which they gradually recover. Occasionally an animal will recover from a very severe infection (experiment 38).

Several of the rabbits that were killed might otherwise have recovered from the *proteus* infection.

In very severe *proteus* infections the animals become extremely emaciated. In one case a loss of weight of 41 per cent. was recorded. Losses of 20 to 30 per cent. are common. Emaciation is a striking feature of severe *proteus* infections. The animals refuse to eat and become progressively weaker. Death usually results in about one week. Deaths from toxemia, without the production of lesions, usually occur within the first twenty-four hours.

GROSS AND MICROSCOPIC PATHOLOGY.

The characteristic lesions resulting from intraperitoneal inoculation are fairly firm, whitish nodules, one to five millimeters in diameter, sometimes larger. They may occur on any part of the peritoneal surface. They were most frequently found in the omentum in our experiments, apparently because the injecting needle came into contact with this structure. The number and size of the lesions usually corresponded to the clinical severity of the infection. In one case the infection passed through the diaphragm and involved the lungs and pleuræ. In no other instance did the lesions resulting from an intraperitoneal inoculation extend beyond the peritoneal cavity.

The large nodules with softened centers always show the exudative type of lesion histologically; but, as a rule, the exudative and proliferative lesions cannot be distinguished by macroscopic examination. When subcutaneous inoculation is successful it usually results in the formation of an abscess of varying size. Death may result from a subcutaneous lesion; but an intraperitoneal infection advances more rapidly and is more apt to terminate fatally.

By intravenous inoculation we succeeded in producing the characteristic lesions in only one case (experiment 3). In this instance there were abscesses in the liver, both kidneys, and the right lung. *Bacillus proteus* disappears rapidly from the blood stream. After intravenous inoculation it was recovered only in those cases that died within the first twenty-four hours. It was never recovered from

the heart's blood after subcutaneous inoculations, and only once after intraperitoneal injection.

As regards histological structure the *proteus* lesions show an exudative or a proliferative type of inflammation, or a mixture of these two forms of tissue reaction. The exudative lesions occur more frequently. They are simple abscesses composed mainly of polymorphonuclear leucocytes. If the infection is not too severe, the abscesses become walled off by a zone of newly formed connective tissue. In the one case in which intravenous inoculation was successful, typical abscesses were formed. The subcutaneous lesions in every case were of the exudative type. The exudative lesions have no special histological interest since they are ordinary abscesses.

The proliferative lesions, however, demand particular attention. Grossly, the nodules are usually smaller and firmer than the abscesses, but it is often not possible to distinguish them from the exudative lesions by gross examination. Figure 1 represents a small nodule of the proliferative type under low magnification. It consists mainly of large cells resembling the so called epithelioid cells of a tubercle. This lesion may indeed be grouped with the granulomata. Masses of small lymphocytes are seen around the periphery of the nodule and in certain places within it. Below the large nodule are seen several smaller ones which consist of a few epithelioid cells. In a section through one of the macroscopic omental nodules of this case, one sees several masses resembling in structure the one shown in figure 1, but some of them are many times larger.

A higher magnification of a part of a proliferative lesion is shown in figure 2. There is a gradual transition from the dense part of the nodule to the adjacent fibrous connective tissue. The cytoplasm of some of the epithelioid cells fuses with that of adjacent cells. Blood vessels are not present in the denser parts of the lesion.

Figures 3 and 4 represent small areas from figure 2 under very high magnification. Multinucleated cells are shown in figure 3. The epithelioid cells are frequently found fused, in this way forming giant-cells, but no giant-cells of the Langhans type have ever been observed in any case. Figure 4 represents an area in which the cells are all distinct. In both figures delicate connective tissue fibrillæ are shown running between the cells and apparently con-

nected to their cytoplasm. By means of Mallory's phosphotungstic hematoxylin stain, it is easy to see that these fibrillæ are present everywhere among the epithelioid cells.

The smallest recognizable nodules, such as are shown in the lower part of figure 1, consist of a few epithelioid cells. The delicate fibrillæ are always present except where the cytoplasm of the cells is fused. Careful examination of the peripheral parts of the nodules (figure 2) will show that there is a gradual transition between the epithelioid cells and the adjacent fixed connective tissue cells. There is no evidence that the epithelioid cells are derived from the endothelium of blood or lymph vessels. All the evidence available indicates that they develop from the fixed cells of the connective tissue. The black masses shown in the cytoplasm of the cells in figures 3 and 4 are fragments of the nuclei of leucocytes. In some proliferative lesions all the epithelioid cells are crowded with coarse, deeply stained nuclear fragments. These cells readily ingest the leucocytes that enter the lesion.

Frequently the nodules consist of epithelioid cells among which are numerous polymorphonuclear leucocytes. Such a lesion is shown in figure 5. This may be regarded as a mixture of the exudative and proliferative types of inflammation. As in figure 5, the leucocytes in these lesions usually show pronounced signs of degeneration. They evidently degenerate readily and are ingested by the epithelioid cells.

Sections appropriately stained[2] show enormous numbers of bacilli everywhere in the proliferative lesion. The bacilli all seem to be extracellular in position.

There seems to be no relation between the clinical severity of the case and the histological type of the lesion. Neither does the histology of the lesion depend upon the particular strain of *proteus* used, since all types of lesions were produced at different times with strain A. The virulence of the particular culture employed, though it may be of importance, is not the only factor, since both exudative and proliferative lesions were sometimes found close together in

[2] To stain the bacilli the tissues should be fixed in Zenker's fluid, sectioned in paraffin, and stained by Mallory's eosin-methylene blue method (overstaining with the methylene blue). This method was used by Mallory to show the Bordet-Gengou bacillus in tissue sections.

the same experiment (experiments 39, 40, and 41). We did not discover the factors that control the histology of the lesion.

SUMMARY.

Some strains of *Bacillus proteus* obtained from human lesions are pathogenic for rabbits, rats, and guinea pigs. There is good evidence that these strains are also pathogenic for man.

All cultures obtained from sources other than human infections were non-pathogenic for the laboratory animals.

A non-pathogenic culture may be made pathogenic by the use of aggressins or by inoculation into the anterior chamber of the eye.

Proteus cultures lose their virulence rapidly when grown on artificial media.

The lesions produced in animals are either simple abscesses, proliferative lesions, or a mixed exudative and proliferative lesion.

The proliferative lesions consist mainly of epithelioid cells apparently of connective tissue origin. No giant-cells of the Langhans type are present.

The histological type of the lesion does not depend upon the strain employed. Neither does it bear any relation to the clinical severity of the case.

The ability to produce the characteristic lesions has no necessary connection with the toxicity of the bacteria.

The *proteus* bacteria probably play a more important part in human pathology than is generally believed.

BIBLIOGRAPHY.

1. Hauser, G., Ueber Fäulnissbacterien und deren Beziehungen zur Septicämie. Ein Beitrag zur Morphologie der Spaltpilze, Leipzig, 1885.
2. Jordan, E. O., *Jour. Hyg.*, 1903, iii, 1.
3. Foà, P., and Bonome, A., *Arch. ital. de biol.*, 1887, viii, 219.
4. Krogius, A., *Compt. rend. Soc. de biol.*, 1890, xlii, 65.
5. Schnitzler, J., *Centralbl. f. Bakteriol.*, 1890, viii, 789.
6. Jaeger, H., *Ztschr. f. Hyg. u. Infectionskrankh.*, 1892, xii, 525; *Deutsch. med. Wchnschr.*, 1895, xxi, 667.
7. Flexner, S., *Bull. Johns Hopkins Hosp.*, 1893, iv, 34.
8. Jensen, C. O., in Kolle, W., and von Wassermann, A., Handbuch der pathogenen Mikroorganismen, 2d edition, Jena, 1913, vi, 121.
9. Wyss, O., *Ztschr. f. Hyg. u. Infectionskrankh.*, 1898, xxvii, 143.
10. Larson, W. P., and Bell, E. T., *Jour. Infect. Dis.*, 1913, xiii, 510.

EXPLANATION OF PLATES.

PLATE 65.

FIG. 1. Experiment 2. Proliferative lesion from the omentum. Several very small nests of epithelioid cells are shown in the lower part of the figure. Microphotograph. × 120.

PLATE 66.

FIG. 2. Experiment 2. Proliferative lesion. One-half of a nodule similar to the one shown in figure 1. × 350.

PLATE 67.

FIG. 3. Experiment 2. Small area from figure 2. Note the fusion of the epithelioid cells. Delicate connective tissue fibrillæ are shown. The black mass in the cytoplasm is a pycnotic nucleus. × 900.

FIG. 4. Experiment 2. Small area from figure 2. Connective tissue fibrillæ are prominent. A disintegrating leucocyte is shown in the cytoplasm of one of the cells. × 900.

FIG. 5. Experiment 41. Mixed exudative and proliferative lesion. The polymorphonuclear leucocytes show various stages of degeneration. × 900.