

Blachstein (A. G.)

INTRAVENOUS INOCULATION OF RABBITS WITH THE
BACILLUS COLI COMMUNIS AND THE BACILLUS
TYPHI ABDOMINALIS.

BY ARTHUR G. BLACHSTEIN, M. D.,
Former Fellow in Pathology, Johns Hopkins University.



INTRAVENOUS INOCULATION OF RABBITS WITH THE BACILLUS COLI COMMUNIS AND THE BACILLUS TYPHI ABDOMINALIS.

BY ARTHUR G. BLACHSTEIN, M. D.,

Former Fellow in Pathology, Johns Hopkins University.

(FROM THE PATHOLOGICAL LABORATORY OF THE
JOHNS HOPKINS UNIVERSITY.)

Although a large number of experiments with the inoculation of animals with the bacillus coli communis (Escherich), and the bacillus typhi abdominalis have been made by different investigators, only those conditions which are produced after a few hours or days subsequent to the inoculation seem to have been observed.

INOCULATION OF BACILLUS COLI COMMUNIS.

Escherich,¹ to whom we owe the name and the first accurate characterization of the bacterium coli commune, and the demonstration that this is the prevailing micro-organism in healthy feces, observed that the subcutaneous inoculation of this bacillus in mice produced no alterations, and in guinea-pigs sometimes caused no symptoms or lesions, sometimes produced abscesses at the point of inoculation, and sometimes caused alterations such as more commonly follow intra-venous inoculation. The latter mode of inoculation caused death in less than twenty-four hours, and at the autopsy the most striking lesions were redness of the peritoneum and marked hyperaemia, combined often with ecchymoses, of the duodenum and small intestine, together with swelling and a reticulated appearance of Peyer's patches, the caecum and colon being normal or presenting some enlargement of the follicles. The peritoneum often contained an increased amount of serum, but no peritonitis was present. The small intestine contained bloody mucus. Similar results were

¹ Escherich, *Fortschritte der Medicin*, 1885, No. 15, u. 16, and *Arbeiten aus dem Pathologischen Institut zu München*, Stuttgart, 1886.



obtained by the intra-venous inoculation of rabbits, but these animals lived somewhat longer, and presented diarrhoeal stools during life. The bacilli were obtained in cultures from the blood and organs. The hyperaemia and catarrhal inflammation of the intestine, and the death of the animal, Escherich attributes to toxæmia. He says "Chronic forms of disease were never observed inasmuch as the animals which did not succumb in the first few days appeared to recover completely."

The inoculation experiments made by Emmerich, Coppola and Weisser¹ with the so-called Naples bacillus, and by Weisser with bacilli, obtained from human feces, probably belong to this same category, the bacilli employed being doubtless members of the group of colon bacteria. The results were similar to those of Escherich. According to Weisser's careful comparative experiments, only a certain number of the inoculated guinea pigs die after subcutaneous and intra-peritoneal inoculations with these organisms and these presented lesions of the intestine, similar to those described by Escherich, although often in slighter degree. Intestinal ulceration, such as was described by Emmerich in his experiments with the Naples bacillus, was not observed.

Tavel's² inoculation of guinea pigs and rabbits gave also results analogous to those of Escherich, only acute infection or intoxication being observed, and this not constantly.

Experiments have recently been published by French observers from a point of view, different from that in preceding experiments.

Laruelle,³ having found in two cases of perforative peritonitis caused by strangulated intestine the predominance of the bacterium coli commune in the peritoneal exudate, undertook to show that this bacillus is not indifferent in the production of the peritonitis. Although the intra-peritoneal injection of the colon bacillus suspended in salt solution caused no peritonitis, but sometimes death from intoxication, the injection into the peritoneal cavity of the same organism mixed with sterilized water, ox-bile or feces produced promptly sero-fibrinous or hemorrhagic peritonitis, the sterilized substances alone being incapable of producing peritonitis. In the exudation, the bacterium coli commune was found in pure culture.

¹ Weisser, *Zeitschrift f. Hygiene*, I. p. 315, containing references also to Emmerich's and Coppola's publications.

² Tavel, *Correspondenz-Blatt f. Schweizer Aerzte*, 1889.

³ Laruelle, quoted from *Centralbl. f. Bakteriologie*, 1890. Bd. viii, No. 3.

Gilbert and Girode¹ isolated in pure culture as the only organism present the bacillus coli communis from the gall bladder and the bile ducts of a case of suppurative cholecystitis and a case of suppurative cholecystitis, combined with suppurative angiocholitis, due to gall-stones. They likewise found the same bacillus in a gall bladder, containing gall-stones and normal bile removed by cholecystotomy. A similar observation led Charrin and Roger² to inoculate pure cultures of this bacillus into rabbits and guinea pigs. Subcutaneous injections in guinea pigs produced a local eschar with enormous oedema, cure resulting in a month; when cultures were injected into the abdominal cavity, the animal succumbed in fifteen days with hemorrhagic peritonitis. Three cubic centimeters injected into the veins were required to kill rabbits. Injected into the common bile duct of rabbits, the most virulent cultures killed in two or three days. Those cultures which had been growing for several generations were less virulent, and the animals were sacrificed at variable periods following the inoculation. After eight days the gall bladder was found filled with pus, and the liver presented disseminated miliary abscesses around the bile vessels; after a still longer period, multi-lobular cirrhosis developed. After inoculation of virulent cultures, peri-angiocholitis, small abscesses and necroses of liver cells were found. Although there is much to be desired in the description of this bacteriological work of Charrin and Roger, they consider that their experiments establish the role of the bacterium coli commune in the production of certain suppurative inflammations of the biliary passages. It can not be considered as beyond doubt that the organism with which their experiments were conducted was in reality the bacterium coli commune of Escherich, and nothing is said in their report as to the bacteriological examination of the purulent foci in the liver following the inoculations.

The experiments to be reported in this article have been made by inoculations of cultures of the bacterium coli commune into the ear-veins of rabbits. The cultures have been obtained by Dr. Booker from the stools of milk-fed infants, and their identity with Escherich's bacillus established by a study of all of their properties, and by comparison with a culture obtained from Escherich. They possessed the power of coagulating milk, and

¹ Gilbert et Girode, *Le Bulletin Médical*, 1890, No. 104.

² Charrin et Roger, *Le Bulletin Médical*, 1891, No. 16.

presented the characteristic appearances of Escherich's bacillus in their growths on gelatine, potato and other media.

Cultures have been injected in various forms and quantities, suspensions in bouillon or in physiological salt solution of the organisms grown upon solid media, as well as cultures in bouillon of varying composition having been employed.

Acute Intoxication with B. Coli Com.—In conformity with the experience of preceding experimenters, we have repeatedly observed acute intoxication leading to the death of the rabbit in a few hours up to two days after intra-venous injection.

The conditions under which acute intoxication with speedy death follows intra-venous inoculation, relate to the quantity of the culture inoculated, to the age and character of the culture, to the virulence of the organism used, and to the special susceptibility of the animal.

As regards the quantity inoculated, we have observed acute fatal intoxication in rabbits to follow only occasionally the injection of one cubic centimeter of a bouillon culture 24 to 48 hours old; more frequently the injection of the same quantity of a cloudy suspension in salt solution of a fresh culture. The injection of 2 cc. of a 48-hour-old bouillon culture has caused fatal intoxication in a larger number of cases; but to insure this result injections of at least 3 cc. are generally necessary, and with some cultures even this amount is not fatal.

It has seemed to us that cultures 24 hours old (grown at 37° C.) in neutral or feebly acid bouillon containing 2 per cent. of grape sugar are less likely to cause death by rapid intoxication than cultures in ordinary bouillon without sugar. A large number of the chronic cases to be described presently were inoculated with 2 cc. of the fresh sugar-bouillon cultures, and although exceptionally acute intoxications followed such inoculation, they were less frequent than when the ordinary bouillon cultures of the same age were employed. It is probable that in the presence of sugar which is fermented by the colon bacterium toxic substances are not so quickly or so abundantly produced as in ordinary bouillon, devoid of glucose.

Cultures of the bacterium coli commune obtained from different sources have shown more or less marked differences in virulence. The most virulent cultures were obtained by Professor Welch from a case of multiple fat necrosis, in which this organism was found at autopsy in large numbers in the necrotic foci, as well as

in the bile, liver and spleen. Most of my experiments have been made with cultures derived from the stools of infants either healthy or with slight diarrhoeal affections. These cultures have in some instances shown no recognizable alteration in virulence when cultivated in successive generations for a year, but in other instances the later generations have exhibited a distinct decline in pathogenic power. A bacillus obtained from gutter water, and presenting all of the morphological and cultural characters of the colon bacillus, was found to be devoid of pathogenic effect in doses of 2 cc. injected into the veins of rabbits.

There appear to be also decided differences in susceptibility of different rabbits to intra-venous injections of the colon bacillus. If several rabbits of the same size as nearly as possible be inoculated in the ear-veins, with 1 to 2 cc. of the same culture, some may die of acute intoxication, some will develop a chronic affection, and some may recover completely and remain well, showing, if killed after several weeks or months, no evidences of preceding inoculation. Differences in susceptibility to acute intoxication have been noticed also by Escherich, and especially by Weisser.

It is apparent that there are many conditions which influence the result following the intra-venous inoculation of rabbits with cultures of the bacillus coli communis, and that, while some of these conditions can be controlled, other can not. These differences it is necessary to bear in mind in comparing the results of various experimenters.

As regards the symptoms and lesions resulting from acute intoxication with the colon bacillus, we have little to add to the observations of other experimenters.

In this group of cases, the death of the animal has occurred in 3 to 40 hours after the injection. The main symptoms observed have been increasing drowsiness, amounting to stupor, prostration and diarrhoea. If the abdominal cavity be opened during life, very active intestinal peristalsis is observed. At the autopsy, the small intestine, especially in the upper part, is hyperaemic and sometimes ecchymoses are present in the mucous membrane. The contents of the upper part of the small intestine may be stained with blood. The colon and rectum contain soft feces in many cases. Peyer's patches may be somewhat swollen, but we have not noticed this swelling as often as would appear from Escherich's experiments. It may here be noted that in healthy rabbits the Peyer's patches are often prominent, forming distinct elevations,

even when viewed through the unopened intestine. The spleen is often, although not invariably, moderately swollen, hyperaemic and soft. There may be some swelling and redness of the lymphatic glands. There may be slight fatty degeneration of the myocardium. Other organs appear normal. Cultures of the organism injected can be obtained from the blood and organs, and in some cases from the bile, and the bacilli can be demonstrated on cover slip preparations from the organs.

The following extracts from protocols of experiments will suffice to illustrate the cases of acute intoxication.

1. Large black rabbit. At 10 a. m. injected into ear vein 1 cc. of a suspension in 10 cc. sterilized salt solution (0.5 per cent.) of a small quantity of potato culture 3 days old. Rabbit dead at 5 p. m. Diarrhoeal feces in the cage. Duodenum and upper ileum reddened. Spleen rather large (weight 650 mgm.). Roll cultures showed the presence of a moderate number of bacilli in the blood, liver and kidney and of a much larger number in the spleen. No growth in culture tubes from the bile.

2. Large black rabbit. At 10.30 a. m. injected 1 cc. of a suspension similar to that used in preceding experiment. Rabbit dead at 6 p. m. Duodenum and jejunum reddened. Spleen large (1200 mgm.). Gall bladder nearly empty. Cultures from spleen, liver, kidney positive, organisms in moderate number, from bile negative.

3. Medium-sized white rabbit. 10.30 a. m. injected 1 cc. of a culture in ordinary alkaline bouillon, 24 hours growth at 37° C. Rabbit dead at 3 p. m. Duodenum and jejunum hyperaemic and ecchymosed. Spleen large and soft. Liver hyperaemic. Bile dark greenish-yellow, viscid. Cultures from liver and spleen positive, the larger number of colonies developing in spleen tube, from bile negative.

4. Large white rabbit. 6.30 p. m. injected 2 cc. of a cloudy suspension in sterilized bouillon of culture on slanting agar. The next morning rabbit appears weak and stupid with diarrhoeal discharges. Died during second night following inoculation, between 26 and 36 hours after inoculation. Inguinal and axillary lymphatic glands swollen and dark red. Spleen moderately swollen and dark red and dry on section. Liver contains coecidia nodules. Duodenal mucous membrane presents a few ecchymoses. Peyer's patches not noticeably swollen. A few small ecchymoses in mucosa of processus vermiformis. Soft feces in rectum and colon. Muscle of heart dotted with numerous fine oil globules. Tracheal mucosa dark red, lungs oedematous and mottled. Bacilli found on cover slips from spleen, liver, kidney and bile, most abundant in preparations from spleen, none found in cover-slips of blood. Roll cultures from blood, lymphatic glands (inguinal and mesenteric), spleen, kidney, liver, bile, lung positive, the spleen tube being crowded with closely set colonies as well as the bile culture. All are pure cultures of the bacterium coli commune.

Whether any multiplication of the injected bacilli occurs in these cases of acute intoxication we have not determined, but there is no positive evidence of such multiplication. The blood serum of the rabbit possesses powerful destructive influence upon the colon bacterium outside of the body and the injected organisms in a short time disappear from the blood and eventually from the organs (with the exceptions noted below). We think there can be no doubt that the fatal issue in these cases of speedy death in rabbits is due to intoxication and not to infection.

Sub-Acute and Chronic Infection with B. Coli Com.—Thus far the experiments reported are essentially confirmatory of already recorded observations. Our interest, however, has been less in the study of the well known cases of acute intoxication than in the study of rabbits which have died at longer intervals after inoculation, inasmuch as the latter class of cases seem to have been hitherto overlooked and present points worthy of attention.

Rabbits inoculated into the veins with 1-2 cc. of bouillon cultures or dilute suspensions of the colon bacillus may die from the effects of the inoculation at any time between a few hours up to six weeks after the inoculation.

The least common cases in our series of experiments have been those which, not dying of acute intoxication, die upon the fourth or fifth day after the inoculation. Out of over 50 rabbits inoculated into the ear-vein, only three died at this period. These acute or, perhaps better called in contrast to the more acute toxic cases, sub-acute cases present a moderate enlargement of the spleen and the bacilli can be obtained in cultures from the bile, liver, spleen and in one instance also from the kidney and blood. In one of these cases extensive intestinal ulceration was found, reminding one of the results obtained by Emmerich with the bacillus Neapolitanus. The protocol of this case is as follows:

5. Maltese rabbit, weighing 1435 grms. 15 XII. Injected into ear-vein 2 cc. of a culture of *b. coli com.* in bouillon, containing 2 per cent. glucose, 24 hours old. (This culture was derived from the spleen of a dog, which had received 5 cc. of a sugar bouillon culture in the saphenous vein, and had died after 36 hours with intestinal hyperaemia as the only noticeable lesion. Plate cultures showed a moderate number of scattered colonies of colon bacteria from the spleen, liver and bile, the culture used for the rabbit being obtained from a single typical colony on the spleen plate.) Rabbit died 19 XII, weighing 1300 grms.

The spleen was found slightly enlarged (weight 670 mgm.). The liver presents a small irregular yellowish-white patch on the surface. In the

coecum there are numerous scattered ulcerations, extending throughout the entire thickness of the mucosa, of irregular size and shape, some 15 mm. in extent, with hemorrhagic margins and grayish yellow floor. The gall bladder is distended with pale brownish bile. *B. coli* com. in moderate number in roll cultures from spleen, liver and kidney; in large number from bile, absent from blood.

Our attention was first directed to the occurrence of more chronic cases with peculiar changes in the bile and liver by Prof. Welch in his experiments with the intra-venous inoculation of rabbits with a bacillus, identical in all respects with the *b. coli* com., obtained from the case of multiple fat necrosis already mentioned. As these experiments have not been published, the protocols of two are here introduced:

6. Experiment of Prof. Welch.—Rather small white rabbit. 21 IV. At 4.30 p. m. injected into marginal vein of right ear 1 cc. slightly cloudy suspension in salt solution from agar culture 2 weeks old of *b. coli* com. isolated from focus of parapancreatic fat necrosis. Rabbit dead 29 IV, having become much emaciated, and appearing weak and stupid for 24 hours before death. Autopsy. Red thrombus 6 mm. long in vein at site of inoculation. Spleen very small, pale reddish-brown color, apparently atrophied. Kidneys, cortex reddish-brown, pyramids pale. Liver appears normal to naked eye, but fresh frozen sections show a number of scattered small opaque foci of necrotic liver cells. Bile, of light yellowish tint, much paler and thinner than normal, with absence of greenish hue and containing many small brownish-yellow particles. The quantity of bile is about normal. Stomach, intestines and lungs normal. The heart muscle not fatty. Very large number of oval bacilli, resembling the *b. coli* com. found in cover slips from bile; none detected in cover slips from spleen, liver or blood.

Gelatine roll cultures from thrombus in inoculated vein shows the first tube full of minute grayish colonies; in the second tube typical spread out bluish-white thin colonies with irregular margins.

First gelatine roll tube, made with one oese of bile, is crowded with minute gray colonies; No. 2, from preceding, presents well distributed colonies with the characters of *b. coli* com.

Cultures made from blood, spleen and liver show no growth.

7. Experiment of Prof. Welch.—Rabbit. 2 V. Injected into ear vein 0.3 cc. suspension in sterilized bouillon of agar culture of *b. coli* commune, obtained from same source as in preceding case. The culture is 3 days old. In the attempt to enter the vein, a small quantity of the culture at first escaped into the tissues by the side of the vein; then the vein was penetrated successfully at another point with the needle of the syringe and the injection went smoothly. Rabbit dead 12 V. The rabbit appeared dull and weak, eating but little for three days before death. It has become much emaciated.

Autopsy. Fibrino-purulent exudation at site where culture entered subcutaneous tissue of ear and extending, with inflammatory oedema to root of ear. There is therefore marked inflammatory local reaction.

Spleen small. Liver presents, in addition to a few psorosperm nodules, several large and small opaque whitish or yellowish-white mottled areas, the largest being 13 mm. in diameter. These occur independently of the psorosperm nodules and on fresh frozen sections are seen to contain large numbers of fatty leucocytes and necrotic liver cells with a reticulum of fibrin-like bands evident in places. Bile—gall-bladder distended with thin, light brownish bile, containing small yellowish particles. Stomach contains no food, only mucus. Small intestine filled with gas and yellowish fluid. Rectum and colon contain soft fecal material in place of the usual hard fecal balls. Heart muscle not fatty. Lungs and trachea normal. A large number of bacilli, apparently identical in all respects with *b. coli* com. in the subcutaneous exudate in the ear, in the necrotic foci in the liver and in the bile, found both on cover slips and in cultures. None detected in blood or spleen.

In these two experiments the most interesting results are the survival of the animal until 8 and 10 days after the inoculation, and the occurrence of necrotic and inflammatory foci in the liver together with a peculiar alteration in the bile which became lighter in color and thinner than normal, with loss of green tint and presence in it of small yellowish particles like a precipitate, also the atrophy of the spleen and general emaciation. The presence of the bacilli in the necrotic foci in the liver and in especially large number in the bile, together with their absence from other organs and the blood is also noteworthy.

Inasmuch as the cultures with which these experiments were made appeared altogether identical with those of the ordinary *b. coli* com. of Escherich, and when injected in larger quantity produced the same form of acute intoxication, it became necessary to determine whether cultures of the ordinary colon bacillus as obtained from the stools, are possessed of similar pathogenic properties, which may have escaped the observation of previous experimenters. As the following experiments prove, cultures of *b. coli* com. obtained from stools, are capable of producing the same sort of relatively chronic infection as was observed by Prof. Welch in his experiments, although this result is obtained more uniformly and with smaller doses with the cultures from the case of fat necrosis and as already mentioned cultures from some cases and especially fresh cultures have appeared to us more pathogenic than from other cases and than older cultures, but we do not consider that a sufficient number of comparative

experiments have been made to warrant positive statements on this point.

Regarding the method employed in the experiments the only point which may need any especial statement is that the bacteriological examination at the autopsies of the rabbits consisted in making Esmarch or roll gelatine cultures with an oese from the substance of each organ and from the bile and blood. The autopsies in nearly all instances were soon after death. The platinum loop was inserted through a spot on the surface of each organ which had been just before thoroughly burnt and sterilized with a hot piece of metal, usually the heated broad surface of the blade of a scalpel. The surface of the gall bladder was thus thoroughly burnt at the spot through which the platinum oese was inserted. The usual order in which the cultures were made was spleen, kidney, liver, blood, bile, the organs remaining in situ until after the cultures had been made. Unless otherwise stated the organisms obtained in culture were only colon bacteria. Cover-slip preparations were made after the culture tubes had been inoculated. All of the inoculations are by injection into one of the ear-veins by means of a sterilized syringe. The following experiments illustrating the more chronic forms of infection were all made with pure cultures of unquestionable bacterium coli commune obtained from infant's stools by Dr. Booker. When cultures in sugar-bouillon are mentioned it is understood that the culture medium is ordinary neutral or feebly acid meat-extract bouillon containing 2 per cent. glucose and that they have grown at 35 to 37° C.

8. Rabbit. Weight 1575 grm. 12 XII. 2 cc. 24 hour culture in sugar-bouillon, derived from a potato culture.

+ 20 XII. Weight 1245 grm. Death in 8 days.

Spleen slightly swollen (weight 610 mgm.). Intestine normal. Liver appears normal. Gall bladder large, bile looks normal. Cover-slip from bile shows a few bacilli. Culture from bile shows a considerable number of colonies. Cultures from blood, liver, spleen, kidney negative.

9. Rabbit. 23 V. 1 cc. dilute suspension in 10 cc. 0.5 per cent. sterilized NaCl solution from fresh potato culture.

+ 2 VI. 10 days after inoculation.

Spleen about normal in size. Liver, full of psorosperm nodules. Gall bladder very large, and distended with pale, nearly colorless, bile containing yellowish white particles. Coccidia present in bile. Cover-slips show large number of bacilli in bile. Cultures show very large number of crowded colonies in Esmarch tube from bile, a much smaller number in tube from liver, and none in tubes from blood, spleen and kidney.

10. Rabbit. Weight 1980 grms. 15 XI. 2 cc. slightly acid sugar bouillon culture 24 hours old.

+ 25 XI. 10 days after inoculation. Weight 1425 grms.

Autopsy immediately after death. Extreme emaciation. Spleen apparently normal. Numerous mottled white necrotic areas in liver. No psorosperms. Gall bladder distended with thin pale bile in which many bacilli are found by cover-slip preparations. Cultures show very large number of colonies in bile tube, smaller number in liver tube and a few in the spleen tube. Cultures from blood and kidney negative.

11. Rabbit. Weight 925 grms. 28 XI. 2 cc. slightly acid sugar bouillon culture 24 hours old.

+ 8 XI. 10 days after inoculation. Weight 735 grms.

Spleen small and pale. Intestine normal. Liver presents several irregular mottled whitish areas 2 to 8 mm. in diameter. No psorosperms. Gall bladder distended with pale, greenish-yellow bile without precipitate.

Cover-slip preparation shows bacilli in bile and liver. Cultures present very large number of colonies in bile tube, a smaller number in liver tube and none in tubes inoculated from blood, kidney and spleen.

12. Rabbit. Weight 1155 grms. 15 V. 1 cc. of bouillon culture, 10 days old, containing in 10 cc. 5 drops of 1:10,000 solution of hydroxylamin.¹

+ 30 V. 15 days after inoculation. Weight day preceding death 995 grms.

Spleen, small and pale. Intestine normal. Liver presents numerous small necrotic areas. Gall bladder distended with pale bile. Very large number of colonies in culture from bile, smaller number in culture from liver. Cultures from spleen, kidney, blood negative.

13. Rabbit. Weight 1755 grms. 19 XI. 2 cc. 24 hours old, sugar bouillon culture.

+ 8 XII. 19 days after inoculation. Weight 1030 grms.

Spleen small, pale reddish. Duodenum hyperaemic, rest of intestine normal. Liver presents small mottled whitish areas. Gall bladder very large, bile pale and thin.

Cultures show very large number of colonies in bile tube, smaller number in liver tube and none in blood, spleen or kidney.

14. Rabbit. 5 V. 1 cc. 3 days culture in bouillon containing 2 drops strong solution of naphthaline² in glycerine. Culture is markedly cloudy.

+ 30 V. 25 days after inoculation.

Great emaciation. Spleen small. Intestine normal. Liver presents a few spots of grayish mottling. Gall bladder very much distended with pale, thin bile.

Bacilli numerous on cover-slips from bile. Culture tube from bile crowded with colonies, from liver a smaller number, from other organs and blood negative.

15. Rabbit. Weight 1520 grms. 23 XI. 2 cc. 24 hours old sugar bouillon culture.

+ 24 XII. 31 days after inoculation.

¹ In a number of experiments small quantities of various substances were added to the culture medium with the idea that they might restrain the production of toxic substances so that the animals would be less likely to die from acute intoxication.

² See preceding footnote.

Emaciation. Subcutaneous tissue dry. All organs anaemic. Spleen small. Liver presents a few psorosperm nodules but no necrotic mottling can be detected. Gall bladder distended with pale, watery bile.

Colonies very numerous in cultures from bile, fewer from liver and spleen, and none in blood and kidney.

16. Rabbit. 4 V. 1 cc. bouillon culture, 24 hours old.

+ 8 VI. 35 days after inoculation.

Extreme emaciation. Spleen very small, atrophic, pale. Liver presents several small mottled necrotic areas. No psorosperms. Gall bladder distended with colorless bile containing small grayish yellow particles. Walls of gall bladder thickened.

Very numerous colonies in culture from bile, smaller number from liver and none in cultures from blood, spleen, kidney.

17.¹ Rabbit. Weight 740 grms. 21 III. 1 cc. 24 hours old, bouillon culture (not containing sugar).

+ 28 IV. 38 days after inoculation. Weight on day preceding death was 545 grms.

Spleen very small and thin and of pale grayish red color. Pericardial sac contained an increased amount of serum. Myocardium not fatty. Lungs normal. Kidneys pale, anaemic. Liver contains a large number of psorosperm nodules. Bile transparent, thin, colorless, containing a number of small yellow clumps. Intestine normal.

Very large number of bacilli in bile. None found in cover-slip preparations from other parts. Cultures from bile contain very large number of colonies, from liver, spleen, blood, kidney negative.

A large number of rabbits have been inoculated in the same manner as in the preceding series, with entirely negative results. With cultures from the case of fat necrosis, nearly all of the inoculated animals died; with cultures from other sources, the ratio of positive to negative results has varied from about two-thirds to one-sixth of the animals inoculated. A sufficient number of experiments with cultures from different sources, and with different ages, for the purpose of determining their relative virulence, has not been made to permit any positive statements on this point.

The two following protocols will suffice to illustrate the negative cases:

18. Rabbit. Weight, 1220 grms. 19 II. 1 cc. 48-hour-old bouillon culture (without sugar). The rabbit presented no symptoms of illness. Its weight gradually fell during the first ten days, reaching 1010 grms., after which it rose, and exceeded in three weeks the original weight. In apparently healthy condition, it was killed on 26 VI, over four months after inoculation.

¹ This experiment was made by Dr. Abbott, Assistant in Bacteriology and Hygiene, Johns Hopkins University, and is published with his kind consent.

Save a few psorosperm nodules in liver and cysticerci in peritoneal cavity, all of the organs are normal. The bile is yellowish-green, viscid, normal in appearance, containing a few coccidia. No bacteria found in cover-slips from bile or other organs, and cultures from bile, liver, blood, spleen and kidney are all negative.

19. Rabbit. Weight, 850 grms. 21 III. 1 cc. 24-hour-old bouillon culture with sugar. Rabbit lost weight for five days, falling to 750 grms., after which the weight rose, reaching 890 grms. ten days after inoculation, and continued at about 950 to 975 grms. No symptoms. Killed 27 VI, over three months after inoculation.

Bile clear, light green, viscid. With the exception of a few small psorosperm nodules in liver, organs are normal. Cover-slips preparations and cultures from bile, liver, blood, spleen, kidney negative.

We have also observed a few cases in which, after intra-venous inoculation of 1-2 cc. bouillon culture of the colon bacterium, the rabbit has progressively or irregularly lost in weight, wasted away, and died after several weeks, without having presented marked symptoms during life and without showing after death evident lesions of a nature to account for the fatal issue. In these cases bacteria could not be found in the bile, liver or other organs, and the relation between the inoculation and the death of the animals remains obscure. The following are cases of this kind:

20. Rabbit. Weight, 1482 grms. 21 II. 1 cc. sugar bouillon culture 13 days old.

+ 6 IV. 44 days after inoculation.

For eleven days after the inoculation, the rabbit steadily lost in weight, on 4 III the weight being 1215 grms. During the next 4 days the weight rose each day, reaching on 8 III 1390 grms. It fluctuated between 1320 and 1450 grms. during the following two weeks, after which it was not again taken.

Save considerable emaciation and a few small psorosperm nodules in the liver, no alterations were found at the autopsy. The bile was pale green and viscid. The spleen was of normal dimensions. Cover-slip preparations and cultures from the bile, liver, blood, kidney and spleen were all negative.

21. Rabbit. Weight, 870 grms. 13 III. 0.5 cc. 48-hour-old sugar bouillon culture.

+ 21 III, 8 days after inoculation.

The daily weights ran as follows: 870, 810, 790, 790, 775, 740, 690, 660, 650.

All of the organs appeared normal. Bile green and viscid. Cultures from bile and organs negative.

With the aid of the cases which have now been briefly reported we will summarize the principal features of the fatal ones which run a more or less chronic course. In the majority of cases fresh

bouillon cultures either with or without glucose have been used, but the same results have been obtained by dilute suspensions in salt solution or bouillon of cultures on solid media. The quantity injected has been usually either one or two cubic centimeters, but the same result has been obtained by the injection of 0.3 cc. (case 7). The duration of life has varied from 4 days to 6 weeks, the largest number of deaths occurring between the 8th and the 20th day after inoculation. There have been as a rule no marked symptoms during the first few hours following the inoculation; exceptionally somnolence and diarrhoea have been observed at this period, indicating acute intoxication from which the animal has recovered. The affection is unattended by fever. Occasionally temperatures of 40° C. to 40.6° C. have been observed, but the same degrees may be met with in healthy rabbits. The most marked symptom is the loss of weight. This is usually progressive from the first or second day after inoculation, with slight fluctuations until death. Sometimes the animal may after a few days gain in weight and then lose, but usually when there is any considerable and steady gain in weight for a number of successive days the animal recovers. A diminution of 15 to 25 per cent. in weight is not uncommon, and the loss may exceed even 40 per cent. in less than three weeks (case 13). The animal generally presents no further objective symptoms until two or three days before death, when it is found to eat little or nothing, to appear dull and weak and finally unable to rise. It dies apparently from exhaustion. Sometimes death occurs unexpectedly without manifest symptoms, other than loss of weight, having been observed.

At the autopsy the animal is found to be emaciated. The subcutaneous tissue and the muscles appear pale and dry. The serous cavities, particularly the pericardial, may contain some excess of serum. The viscera are anaemic. The spleen is enlarged only when the animal dies within a few days and then only to a moderate degree. More frequently and in more chronic cases it is generally small, thin, and pale. The kidneys present no especial changes. The stomach may be nearly or quite empty of the partly digested food so constantly found in rabbits' stomachs and may contain only mucus. Exceptionally ulcers and ecchymoses have been observed in the caecum, but generally there are no lesions of the intestinal tract and the usual hard fecal balls are found in the rectum, although in their place soft feces may be present. The lungs are pale, rarely oedematous. The right cav-

ities of the heart contain dark red, soft coagula; the left ventricle is generally contracted. The heart muscle is usually free from fatty degeneration.

The most striking and constant lesions, those most characteristic of the affection, are in the bile and in the liver. The quantity of bile may not exceed the normal, but often the gall bladder is abnormally distended. The bile is nearly colorless or has a pale yellowish or brownish tint, with little or none of a greenish color. Its consistence is much less viscid than normal, and is often thin and watery. It usually contains small opaque yellowish particles or clumps which can be seen floating in it even through the thin walls of the gall bladder. These clumps microscopically consist of bile-stained, apparently necrotic, epithelial cells, leucocytes in small number, amorphous masses of bile pigment and bacteria often in zoogloea-like clumps. The walls of the gall bladder are sometimes thicker and more opaque than normal. Similar material is found in the larger bile ducts.

In the liver are found frequently, although by no means constantly, opaque whitish or yellowish-white spots and streaks, of irregular size and shape, which give a peculiar mottling to the organ when present in large number. Only one or two such areas may be noticed or they may occur in large numbers. They vary from foci scarcely visible to the naked eye to areas 2 to 3 cm. in extent, the latter appearing to result from coalescence of smaller areas. These areas occur both on the surface and in the depth of the liver, and often appear slightly elevated above the surrounding liver substance. On microscopical section these areas are found to be places where the liver cells have undergone necrosis accompanied with emigration of leucocytes, and are usually surrounded with fatty degenerated hepatic cells. The first step in the process appears to be coagulation necrosis of the liver cells accompanied with an appearance of a fibrinous network between and in the place of the cells. This is accompanied and followed by an emigration of leucocytes. In later stages most of the necrotic cells and leucocytes may have disappeared and a network, possibly representing the walls of capillaries, be left behind. These necrotic and inflammatory changes appear to be of essentially the same nature as have been observed after ligation of the common bile duct (Foa and Salvioli, Pick and others), and occur in other infectious processes, such as after inoculation with typhoid bacilli and with hog cholera and Schweine-Pest bacilli. They

may be so small as to be recognized only upon microscopical examination. Changes in the bile similar to those described may also occur in other affections.

As regards the distribution of the bacteria the rule is that they are found only in the liver and the bile, but they may be present in the spleen. They disappear in a short time from the blood. In the bile the bacilli are present in large number occurring both singly, in pairs and in clumps and are readily demonstrable on cover-slips. They stain with the usual aniline dyes, being decolorized by Gram's method. They sometimes have a tendency to stain somewhat irregularly, especially to stain more deeply around the margin than in the centre. Roll cultures made with nutrient gelatine or agar with one small oese of bile will generally show the original tube packed full of minute colonies and the second and third dilutions presenting well distributed bluish-gray translucent spread-out colonies with notched irregular margins. Tested in milk, on potato and other media the organisms have all the characters of the colon bacillus. The bacilli are often, but not invariably, found in cover-slips and cultures from the liver. Inasmuch as sections show an irregular distribution of the bacilli in the liver, the failure to find them in cover-slip preparations or cultures from this organ can not be considered conclusive evidence of their absence. On sections of the liver stained with Löffler's methylene blue we have repeatedly discovered masses of the bacilli in or near the necrotic inflammatory foci already described. They occur both within and outside of the vessels. If present in the spleen, they are generally in such small number as not to be detected on cover-slip preparations, although they may be demonstrable in cultures. No other species of bacteria than the *b. coli com.* was found in any of the cases.

The possibility that the presence of psorosperms in the liver may have had something to do with the development of the peculiar lesions described occurred to us at one time but this can be positively excluded. Psorospermiasis is common in rabbits of this region, but, although many of our cases were of rabbits presenting this affection, in many others there was no trace of psorosperms in the liver or elsewhere. Likewise in cases which recovered after inoculation psorosperms were as frequently found as in those which died. Only on very superficial examination could the necrotic inflammatory foci in the liver occurring after chronic infection with the *b. coli com.* be confounded with the white

psorosperm nodules, and care was taken to guard against possible mistake by microscopic examination, for which purpose extensive use was made of fresh frozen sections.

There can be no doubt that in the group of sub-acute and chronic cases described the bacillus coli communis is proven to be in a peculiar and hitherto undescribed manner pathogenic for rabbits by intravenous inoculation. Similar results we have not been able to obtain by subcutaneous inoculations, which may or may not be followed by abscesses at the point of inoculation. It would appear to be necessary that the bacilli should be introduced in considerable number directly into the blood current. We have not attempted direct inoculation of the liver, or bile passages which it is possible may yield positive results. Why some animals should succumb and others recover is not clear. The blood of the rabbit possesses powerful germicide powers over the colon bacterium and is capable of destroying a large number of the bacilli and the conditions enabling some of the injected bacilli to escape this influence in the liver and in the bile may be in a sense accidental or out of the control of the experimenter. Although care was taken not to inject large clumps, it is manifest that in using unfiltered bouillon cultures or dilute suspensions small masses sufficient to form emboli may be injected, but the view that the lesions result primarily from emboli of bacteria in the liver is only hypothetical.

The use of the term infection in describing the more or less chronic affection produced by injection of the colon bacterium may appear open to question. The justification of this term depends upon whether or not there is evidence that the bacteria introduced by inoculation actually multiply within the body. Upon this point we think that the occurrence of clumps of the bacilli in the liver, both within and without the vessels and the large number of bacilli, also often in clumps, present in the bile speak for actual multiplication. It would be difficult to account for the number found in the bile even upon the assumption that all introduced were eliminated by this channel, and the evidence is that by far the greater number of the bacilli injected are not thus eliminated but are quickly destroyed by the blood. That the bacilli or at least most of them present in the bile are actually living is shown by the multitude of colonies which develop in the cultures. Without, however, wishing to place too much emphasis

upon this question as to infection, it is certainly of great interest that the colon bacteria may preserve their vitality for many weeks in the bile and liver and exert a pathogenic influence upon the animal.

Concerning the bearing of our experiments upon the doctrine of Wyssokowitsch, that bacteria are eliminated by secretions only when the organ producing the secretion presents some lesion, it is to be said that in the majority of our cases the requisite lesion existed in the liver in the form of the necrotic and inflammatory foci described, and when such lesion was not observed with the naked eye careful microscopic examination occasionally revealed similar minute foci. Where no lesion was found we cannot of course be certain that it may not have existed in some part of the liver.

We can only speculate as to the actual cause of the wasting affection and death of the animals. Three theories suggest themselves. It is possible that death is due to toxic substances introduced with the inoculated fluid or resulting from the destruction of the bacilli by the blood. This explanation, which is probable for the cases of acute intoxication, seems highly improbable for the chronic cases, although the slow poisoning following the injection of toxic albumens produced by the bacillus diphtheriae has given more plausibility to the suggestion than it could otherwise claim. We are more inclined to adopt one of the two following theories, either the bacilli produce deleterious substances by their presence and probable multiplication in the liver and bile and the symptoms and death are due to the absorption of these substances or else the properties of the bile are so altered as to cause emaciation and death. Circumstances having compelled for the present interruption of the work, we are obliged to leave these and many other points which suggest themselves open for future investigation.

Inasmuch as the bacillus coli communis is not a very uncommon invader of certain organs of the human body in a certain class of diseases, as will appear from investigations on this subject to be published by Prof. Welch, the experiments here presented, aside from their scientific interest, may not be without their bearing on human pathology, although it would be entirely unjustifiable without corresponding observations in human beings to transfer to them the results of experiments on animals.

INOCULATION OF *BACILLUS TYPHI ABDOMINALIS*.

As the acute intoxication following the intravenous inoculation of large quantities of cultures of the typhoid bacillus strikingly resembles that produced by cultures of the colon bacillus the question suggested itself whether a chronic affection similar to that described may not be caused by the intravenous injection also of typhoid cultures. The similarity in many respects, notwithstanding important differences, between the typhoid bacillus and the colon bacillus rendered the suggestion not improbable.

As the result of the experiments of numerous investigators the prevailing opinion is that intravenous injections of cultures of the typhoid bacillus cause, if in sufficient amount, rapidly fatal intoxication, if in smaller amount, temporary intoxication from which the animal recovers and remains well. The evidence is that the bacilli do not multiply but are destroyed within the body. While this view seems to us correct for the majority of cases, we deem it probable in the light of our experiments that some of the rabbits dismissed as fully recovered after this mode of inoculation may have subsequently died from chronic infection of the character to be described. Deaths several days, or even weeks, after such inoculation have indeed been mentioned in the reports of some writers, but so far as we have been able to find the lesions and the results of the bacteriological examination after death which we have found in some cases have not been observed by others.

A larger number of late deaths are reported after introduction of typhoid cultures into the alimentary canal either by feeding or by direct injection into the intestine and in some of these cases interesting intestinal lesions and the presence of bacilli in the spleen or mesenteric glands have been noted (A. Fraenkel, Cygnaeus, Kilcher).

E. Fraenkel and Simmonds, Pavone and Cygnaeus, have observed changes in the livers of the inoculated animals described variously as small-celled infiltration or lymphomata, foci of coagulation necrosis and fatty degeneration. Pavone also mentions the presence of typhoid bacilli in the bile in some cases.

As the following experiments show, we have found that the intravenous injections of cultures of the typhoid bacillus are capable of producing the same chronic effects as those which have been described for the colon bacillus.

The cultures used were derived from single colonies growing in Esmarch roll cultures of nutrient gelatine inoculated from the spleens of three cases of typhoid fever. The cultures grew in the characteristic invisible or hardly visible manner on potato, grew in milk without changing its appearance, and in a word presented all of the characters of the typhoid bacillus. The bouillon used for the injections was usually inoculated directly from an invisible growth on sterilized potato. The inoculations were all into the ear-vein of rabbits with sterilized syringes. The culture medium in the majority of cases was ordinary bouillon containing 2 per cent. glucose. Cases of acute intoxication are not reported as we have nothing to add to the extensive literature on this subject.

(a). Rabbit. Weight 1285 grms. 28 XI. 2 cc. slightly acid sugar bouillon, 24 hours old, growing in thermostat at 35° C. Planted from typical invisible potato growth.

+ 10 XII. 12 days after inoculation. Weight 980 grms.

2 necrotic ulcers in caecum, one about the middle of the caecum opposite the mesenteric attachment, measuring 2 cm. long and 3-6 mm. broad, running obliquely across one of the projecting folds; edges which are slightly elevated and the floor have a yellowish necrotic appearance. A smaller similar ulcer is situated near the ileo-caecal valve, its long diameter running parallel to the folds. Surrounding mucous membrane ecchymosed. Spleen not enlarged. Liver presents no distinct mottling. Gall bladder distended with pale brown bile. Kidneys hyperaemic and presenting several small hemorrhages.

Cultures from bile, liver and spleen positive, a very large number of colonies appearing in the bile tube and a considerable number in the liver and spleen tubes. Further study of the cultures obtained from these colonies by planting on potato, etc., proves them to be typhoid.

(b). Rabbit. 13 XII. Weight 1160 grms. 2 cc. faintly cloudy slightly alkaline sugar bouillon, 2 days in thermostat, planted from invisible potato growth.

+ 5 I. 23 days after inoculation. Weight two days before death 750 grms.

Intestine normal. Spleen perhaps slightly swollen. Liver contains a few small psorosperm nodules and a number of small opaque yellowish-white spots of necrosis. Gall bladder enormously distended with pale yellow bile containing yellowish particles.

Bacilli found in large number on cover-slips from bile.

Cultures show original bile tube cloudy from closely packed minute grayish colonies, and a much smaller number of colonies in the liver and spleen. None in heart's blood or kidney. Cultures derived from the original Esmarch tubes grow characteristically upon potato.

(c). Rabbit. 13 XII. Weight 1005 grms. 2 cc. sugar bouillon culture, 2 days old in thermostat, from invisible potato growth.

+ 5 I. 23 days after inoculation. Weight 690 grms.

Peritoneum and intestine normal. Spleen very small and pale, weighing only 100 mgms. Liver presents a few psorosperms. No necrotic foci recognized. Bile pale greenish, thinner than normal. A smaller number of bacilli found on cover-slips from bile than in preceding cases.

Original roll culture from bile contains a considerable number of colonies, but is not crowded, a still smaller number found in the liver. None in heart's blood, liver or kidney. Cultures grow characteristically on potato.

(d). Rabbit. 13 XII. Weight 1300 grms. 2 cc. 2 days old sugar bouillon culture from invisible growth on potato.

+ 23 XII. 10 days after inoculation. Weight 800 grms.

Intestine normal. Spleen extremely small. Liver presents a few psorosperm nodules. Gall bladder distended with colorless bile. Bacilli found on cover-slips of bile.

Very large number of colonies in original Esmarch tube from bile, a smaller number in liver tube, and none in spleen, blood or kidney cultures.

(e¹). Rabbit. 21 III. Weight 930 grms. 1 cc. bouillon culture without sugar, 24 hours old in thermostat.

+ 13 VI. 84 days after inoculation. Weight 732 grms.

The rabbit's weight fell to 820 grms, during the first three days after inoculation, it remained at about this point for the following four days, after which it rose to 950 grms. and remained in this neighborhood for a month after which the weight was not again taken until death.

Spleen small, kidneys appear rather small but normal, urinary bladder distended with clear urine. Stomach and intestine normal. Liver contains a number of small psorosperm nodules, but no necrotic foci can be recognized. The gall bladder is distended with pale, faintly yellow, thin watery bile containing small whitish specks.

Two cervical glands near the angle of the left jaw contain several small whitish foci, 2 to 3 mm. in diameter, of dry caseous-looking pus, which microscopically consists of disintegrated but still recognizable pus cells.

Cover-slips from bile show a large number of oval bacilli resembling the typhoid bacilli. Cover-slips from pus in cervical glands show no bacteria.

Roll cultures from bile show the original tube crowded with closely-set minute colonies. Cultures from the purulent foci in cervical lymph glands, the blood, spleen, liver and kidneys show no growth. Cultures planted from the bile tube on potato grow invisibly. They present all of the characters of cultures of typhoid bacilli.

(f). Rabbit. Weight 1175 grm. 19 II. 0.3 cc. 48 hours old bouillon culture without sugar.

+ 8 VI. 109 days after inoculation. Weight 1050 grms.

From 19 II to 2 III the animal rapidly lost weight, sinking to 960 grms. After this the weight steadily rose to 1210 grms. on 25 IV, after which the weight was not again taken until death. Intestine normal. Spleen small and dark in color. Liver presents an irregular firm white fibrous patch

¹ This and the following experiment were made by Dr. Abbott and are taken from his notes with his kind consent.

6 mm. in diameter near border. No psorosperms. Gall bladder large and filled with thin amber-colored fluid in which float yellow particles. Other organs appear normal.

Large number of bacilli found on cover-slips from bile, none in other parts.

Culture tube from bile filled with minute colonies. Cultures from liver, spleen and blood negative.

The cultures from the bile present the characters of typhoid cultures on potato and other media.

In one instance the rabbit died over three weeks after the inoculation without presenting lesions to account for death and with negative bacteriological examination.

(g). Rabbit. Weight 1240 grms. 15 XII. 2 cc. 48 hours old sugar bouillon culture.

+ 8 I. 24 days after inoculation. Weight 860 grms. Spleen small. All organs appear normal except a small white infarction with hemorrhagic border in the left kidney. Gall bladder and bile appear normal. Cultures from bile, spleen, liver, kidney, blood, negative.

The number of cases with entirely negative result, in which the animals have survived for months and when killed have shown no lesions and have been free from bacteria in the bile and organs, is large.¹ It is sufficient to report two such cases.

(h). Rabbit. Weight 1310 grms. 13 XII. 2 cc. 48 hours old sugar bouillon culture from invisible potato growth.

Killed 21 I. 38 days after inoculation. Rabbit at first lost weight and then regained it.

All of the organs and the bile appear normal.

Cultures from the bile and organs negative.

(i). Rabbit. Weight 2005 grms. 21 III. 1 cc. 24 hours old (at 37° C.) sugar bouillon culture.

Killed 27 VI. 98 days after inoculation.

Rabbit lost weight but little during the first week, after which the weight ran along for a month with some fluctuations at about 1900 to 1950. It appeared perfectly well when killed.

There are several psorosperm cysts in the liver and a few cysticerci in the peritoneal cavity, otherwise the organs appear normal. The bile is green, viscid and clear. Cultures from bile and organs negative.

The most striking feature of the experiments with positive result which are contained in our series, is the almost incredible

¹ Note by Prof. Welch. It is to be regretted that Dr. Blachstein does not give the ratio of positive to negative cases. His notes concerning the number of unsuccessful inoculations have not been left with me, and as he is now absent in Europe this omission cannot be supplied for the present publication. My impression is that more than one half of the inoculations yielded no result.—W. H. W.

length of time in which the typhoid bacilli in two instances remained in a living state in the bile of the rabbit, in one case (f) this period being $15\frac{1}{2}$ weeks. It need hardly be said that in this as well as in the other cases every means was taken to fully identify the organisms obtained in culture from the bile with the typhoid bacillus. It may also be added that we have never observed in uninoculated rabbits the changes in the bile which have been described and that in conformity with the results of many other investigators we have found the bile of healthy rabbits to be free from bacteria. A number of rabbits kept for weeks and months in the same kind of cages, and under the same conditions as those inoculated have been sacrificed with entirely negative result so far as the lesions and bacteriological results indicated are concerned.

The chronic affection which we have produced by intravenous inoculation of rabbits with the typhoid bacillus does not differ, so far as we have been able to observe, from that caused by the colon bacillus, and therefore no additional comment upon this group of experiments seems necessary.

The occasional production of intestinal ulcerations with each bacillus as well as the general resemblance and even apparent identity in their effects, robs the experimental results obtained with the typhoid bacillus of any specific character. It is by no means improbable that still other species of bacteria may be found which will produce the same effect.

