

REPORT OF A LABORATORY EPIZOOTIC AMONG
GUINEA-PIGS, ASSOCIATED WITH GASEOUS EM-
PHYSEMA OF THE LIVER, SPLEEN AND KIDNEYS,
DUE TO BACILLUS MUCOSUS CAPSULATUS.

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During the summer of 1899, an epizootic broke out among the stock guinea-pigs belonging to the laboratory, in the course of which twenty-five animals were affected. Careful watch was kept over the stock in order that such animals as showed symptoms of illness might be at once isolated; in this manner the course and duration of the infectious process in the various cases could be carefully watched and noted.

The symptoms were similar in all cases; the animals ceased to eat, their hair became much ruffled, and their condition grew rapidly worse, culminating in coma, which existed for some time before death. During the coma there were intervals of muscular twitching, which in one or two of the animals were sufficiently marked to be called convulsions. In the animals autopsied as soon as respiration and cardiac impulse had ceased, so far as external examination could determine, the heart was found to be still beating very slowly. The heart as a whole, the separate divisions, and even strips of the ventricular walls, responded readily to stimuli for fifteen minutes or more after removal from the body, though no precautions were taken to prevent drying.

The duration of illness in the fatal cases, from the first symptoms noted to the time of death, varied between 12 and 48 hours, but by far the greater proportion died in from 18 to 24 hours after the onset of the disease. Two of the infected animals recovered after an illness of seven or eight days. In these cases the condition of coma was not reached, but it was over a week before the animals began to eat, and between two and three weeks before they recovered their normal weight and health.

Careful autopsies were made in all the fatal cases from 15 minutes to 24 hours after death. The bodies were kept in the cold chamber until autopsied, at a temperature well below the freezing point.

Summary of autopsy protocols.—None of the bodies showed any swelling after death, nor could emphysema be detected by external examination. Further examination of the skin and subcutaneous tissues failed to demonstrate any emphysematous areas. The glands of the groin and axilla were slightly swollen.

Thorax.—The pleural cavities showed no adhesions and no excess of pleural fluid. The lungs were normal except in one instance, where there was a lax consolidation of the posterior part of the right lower lobe. The pericardium and heart were uniformly negative.

Abdomen.—In 15 cases, or 65% of the animals autopsied, there was a well-marked peritonitis, chiefly of the sero-purulent type, though a few flakes of fibrin were usually to be found in the dependent parts.

The liver in every case showed marked congestion and cloudy swelling. In 14 animals, or 60%, there was a general gaseous emphysema of this organ which in 4 instances was so extensive as to resemble closely the "Schaumleber" described in cases of invasion by *B. aërogenes capsulatus*. No areas of degeneration were noticed macroscopically.

In 6 of the animals there was associated with this hepatic emphysema a like condition of the spleen. This organ was markedly congested in all cases. In 8, or 39% of all, there was gaseous emphysema like that described in the liver. In 2 of these cases, the emphysema was so extensive that the splenic capsule was distended until the organ was cylindrical in contour, resembling a small sausage.

The kidneys and adrenals showed no macroscopic changes other than well-marked congestion and cloudy swelling.

The gastro-intestinal tract contained much gas, but no lesions could be demonstrated.

Bacteriological examination.—Coverslip preparations were made at each autopsy from the heart's blood, the liver and the spleen, and from the peritoneum in cases with inflammatory exudate. The coverslips were stained in various ways, both with the usual aniline dyes and with Welch's capsule stain. In all instances of peritonitis, large numbers of organisms were found in the exudate, and in most cases the blood and organs showed a small number of bacteria of the same type. The organism uniformly seen in the stained smears was a short bacillus, with rounded ends, 0.5μ to 3μ in length, and about 0.5μ in diameter. These occurred most frequently in pairs. Examination by the hanging-drop method showed absence of motility. Capsules were readily demonstrated by Welch's method. The organism stained easily with the aniline dyes, and decolorized rapidly when treated according to Gram.

There was no pleomorphism observed in stained specimens from the organs and exudates.

Cultures were made at each autopsy from the heart's blood, the liver, the spleen, and from the peritoneum when an exudate was present. The usual precautions were taken to prevent contamination, the surface of the organs being seared with a hot knife before the insertion of the platinum loop. Glycerine-agar slants were used, and from these, when the organism had grown out, transplantations were made into glucose-agar, glucose-bouillon, gelatin, milk, and on potato. With two exceptions, each of these being from a peritoneal exudate, the original tubes showed pure cultures of the organism to be described. In the two mixed cultures there were a few colonies of *Staphylococcus aureus* in addition to the characteristic bacillus. The tubes inoculated from the peritoneum showed a profuse growth, while in those inoculated from the blood and organs the colonies were fewer and often separate. The superficial colonies were round, soft, grayish-white, smooth in outline, and somewhat raised above the surface of the medium. Under the low power they were seen to be darker in the centre than at the margin, and finely granular. The type of growth in various media, using a number of cultures from different animals for comparison, was carefully studied, and proved to be similar in all cases.

Glycerine agar.—24 hours at 37° C. Profuse, grayish-white, moist porcelain-like growth, with wavy edges, distinctly raised above the surface. The water of condensation contained a rather heavy, flocculent sediment, and was markedly viscid. Some tubes showed gas-bubbles in the growth itself, and in all there was gas-formation along the lines of the stab, sometimes in sufficient quantity to raise the whole slant half an inch or more from the bottom of the tube. There was no liquefaction or discoloration of the medium.

Glucose agar.—24 hours at 37° C. The growth was almost exactly like that on glycerine agar, though the gas-formation was more marked.

Gelatin.—5 days at 22° C. The surface growth closely resembled that on agar, but was less profuse. The growth along the stab was slight, and at the point of inoculation there was a raised, nail-head growth, not extending far from the centre. There was no liquefaction nor gas formation.

Litmus milk.—24 hours at 37° C. There was complete coagulation in every case, with acid reaction. The beginning of the acid reaction was noted in about 15 hours from the time of inoculation. During 20 days there was no peptonization.

Bouillon.—24 hours at 37° C. There was diffuse cloudiness, with a heavy grayish-white, flocculent sediment. Later, the bouillon became very viscid, hanging in strings from the loop.

Glucose bouillon.—24 hours at 37° C. Fermentation tubes, containing 1% glucose bouillon, invariably showed gas-formation. Unfortunately the reaction to lactose and saccharose was not tested, as the cultures were inadvertently destroyed during my vacation.

Potato.—24 hours at 37° C. There was a profuse, moist, viscid growth, grayish in color, containing numerous gas-bubbles.

Indol reaction.—Peptone bouillon kept for ten days at 37° C. and tested with 10% sulphuric acid and 1% sodium nitrite for the formation of indol gave a slight positive reaction in all cases.

Morphology and staining reactions in coverslips from artificial media.—Stained specimens showed a pleomorphic organism, most frequently in the form of a short bacillus, with rounded ends. Special stains for capsules gave negative results, except sometimes with coverslips from litmus milk. The organisms stained readily with the aniline dyes, and decolorized rapidly with Gram's stain. Examination by the hanging-drop method failed to show motility.

Exclusion of other organisms.—In the cases which showed marked gaseous emphysema of the organs, control experiments were made by cultures and by animal inoculations, to ascertain the presence or absence of *B. aërogenes capsulatus*.

Anaërobic cultures in glucose agar were made from the liver and spleen, and kept in an atmosphere of hydrogen in Novy's jars for 48 hours. Examination of the tubes showed growths similar in all respects to those noted above, except that they were somewhat less luxuriant. No organisms answering in any way to the morphology or staining reactions of *B. aërogenes capsulatus* were seen.

Bouillon suspensions from the emphysematous livers were made and inoculated into the ear veins of rabbits. The animals were killed by a blow on the back of the neck after a short interval, and kept at body temperature for 48 hours. No emphysema or swelling was noticed, and no gas bacilli were found in coverslips. Cultures from the blood and organs of the animals showed the organism described in detail above.

Animal experiments.—Inoculations of 1 cc. of 24-hour cultures in bouillon into the peritoneal cavities of guinea-pigs which had never been exposed to the infection caused death in from 12 to 186 hours, with symptoms similar to those described in animals dying from the original epizootic. At autopsy there was in every case a sero-purulent

peritonitis, and the organs showed marked congestion and cloudy swelling, but none of the experimental cases showed any gaseous emphysema in either subcutaneous tissue or organs.

Further experiments were made on the two animals which had survived the original infection, to find out if any degree of immunity had been conferred by the previous attack. When they had completely recovered from the first infection, 1 cc. of a 24-hour culture of the organism described was injected into the peritoneal cavity of each. The animals showed no signs of illness on the succeeding day, and the inoculation was repeated, in the same manner and quantity. This was done on the third day also, but the animals showed no sign of any discomfort. In order to make sure that the organism had not diminished in virulence, control inoculations were made at the same time, from the same tubes, into guinea-pigs not previously exposed to the infection. These animals died within 24 hours with the same clinical symptoms, post-mortem findings and cultural results as described.

This observation agrees with that of Howard,¹ who found that by starting with small, non-fatal doses both of living and of sterilized cultures and filtrates of bouillon cultures of a bacillus of the *B. mucosus capsulatus* group, obtained from a case of hæmorrhagic septicæmia in man, both guinea-pigs and rabbits could be accustomed to withstand doses fatal to untreated animals.

Microscopic examination of hardened sections.—Portions of the heart, lungs, liver, spleen and kidneys were removed at each autopsy, and hardened in Zenker's fluid and in alcohol. Sections were stained by the following methods: (1) Hæmatoxylin and eosin; (2) methylene blue and eosin; (3) Van Gieson's picric acid and fuchsin; (4) Weigert's fibrin stain; (5) Lugol's solution.

Sections of the heart showed marked congestion in all, and cloudy swelling in most cases. The lungs showed marked congestion.

The liver showed extensive changes. There was enormous congestion, both of the hepatic and portal systems. The intralobular capillaries were distended so that the liver cells were often separated into bands and small clumps. In a large number of the vessels no abnormal elements were present, but there was much granular detritus, which stained deeply with eosin. Some of the capillaries were irregularly dilated, and their lumina were empty, or at most contained a small amount of granular material. Many of the central veins of the lobules as well as many of the hepatic veins were much wider than normal. Some of them

¹ *Journal of Experimental Medicine*, 1899, iv, p. 164.

contained granular detritus, but most were empty. The liver cells about these distended veins and capillaries were elongated and flattened. This change often affected several rows of liver cells about these spaces. Some of these cavities were very large, and occupied nearly the whole field of a Zeiss DD lens. In some of these abnormally distended vessels, fibrinous thrombi were found, the thrombus often occupying a portion of the lumen, so as to leave an empty space, round or oval in outline. The vascular changes above described were clearly due to the gaseous emphysema found in the gross sections of the organ. Scattered through the sections capillaries were found, containing fibrinous thrombi. The liver cells were cloudy, and often contained large fat drops; the protoplasm was granular, but the nuclei as a rule stained normally.

Scattered through the sections from two cases were areas of varying size, in which there was more or less complete necrosis of the liver cells, which in many places was typical coagulative necrosis. In some places the cell-protoplasm was hyaline in appearance, while the nuclei stained well and showed but little change. In other places both cells and nuclei took a deep eosin stain, though the outlines of the nuclei could be still readily made out. The necrotic portions bore no special relation to the lobules, though perhaps more of them lay at the periphery than at the centres of the lobules. They varied in size from those including only one or two cells to those as large as or larger than a single lobule; these larger ones were often markedly irregular in shape. In some of these areas both veins and capillaries were filled completely with either hyaline or fibrinous thrombi, in some of the latter of which leucocytes were seen. The liver cells in the immediate vicinity of these degenerated areas, especially of the smaller ones, frequently showed dropsical degeneration. In and about these areas there were numerous spaces similar to those noted in the blood-vessels. Sections treated with Lugol's solution failed to show any amyloid reaction.

Sections of the spleen showed marked congestion. There was a diffuse emphysema, and the vessels were distended as in the liver. The cells of the splenic pulp showed no special changes, and no areas of necrosis were seen.

Sections of the kidney showed marked congestion, extensive cloudy swelling and granular degeneration of the epithelial cells of the convoluted tubules. In many places the cell-outlines could not be made out, but the nuclei throughout the sections stained uniformly sharply. In some sections there was diffuse emphysema, and the vessels were dis-

tended as in the liver and spleen. No areas of necrosis or of cell-infiltration could be seen in any section.

Sections from the liver, spleen and kidney, stained by Weigert's method for fibrin, showed small amounts of fibrin in some of the vessels, but nowhere else, and no organisms of any kind.

Sections were stained with eosin and methylene blue to determine the presence of organisms decolorizing with Gram. In the blood-vessels, especially the veins, and in and about the emphysematous areas, numerous bacilli of from $1\ \mu$ to $3\ \mu$ in length, and about $0.5\ \mu$ in width, were seen. They frequently occurred in pairs, and capsules could often be seen.

The fact that they stained with methylene blue but not by Gram's method, as well as their morphology, confirm the results in the cultures, excluding the possibility of the presence of *B. aërogenes capsulatus* or other bacteria in the emphysematous areas.

The observations recorded in this paper are of especial interest in view of Howard's² report in a recent number of this journal of an instance in a human being of general gaseous emphysema with gas-cysts in the brain, formed after death, and due to a member of the *B. mucosus capsulatus* group (variety "aërogenes" of Strong). He further found that these two bacilli and three other bacilli of the same group and variety as the latter, obtained at autopsy from various lesions, caused general gaseous emphysema in the cadavers of rabbits most abundantly with, but also without, intravenous injections of lactose or glucose solutions before killing the animals.

There are three facts of special importance connected with the observations herein reported:

1. The spontaneous occurrence of a very fatal epizootic among laboratory guinea-pigs due to *B. mucosus capsulatus*.
2. The development of immunity of a high grade from otherwise fatal doses of the organism causing the epizootic.
3. The development of gaseous emphysema of various organs in 56 per cent of the animals either just after or 24 hours after death, due to the same bacillus, the usual cause of such emphysema, namely *B. aërogenes capsulatus*, being carefully excluded.

² *Journal of Experimental Medicine*, 1900, v, p. 139.

It is to be regretted that by an accident during my absence the cultures of the bacillus were destroyed before I had an opportunity of determining its exact position in the "mucosus capsulatus" group, according to the amount of gas formed in glucose, lactose and saccharose bouillon. There seems to be no doubt, however, that it belongs to Strong's³ "aërogenes" variety of the *B. mucosus capsulatus* group, on account of its great production of gas in the animal body, and the rapid coagulation of milk. This view is further supported by Howard's already cited observation in this laboratory of gas-formation in both human and rabbit cadavers, caused by bacilli of this variety.

³ *Journal of the Boston Society of the Medical Sciences*, 1889, iii, p. 185.