BACILLUS EGENS. A NEW PATHOGENIC ANAEROBE.

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The bacillus described in this paper occurred in a fatal case of gas gangrene. The cultures were derived from infected muscle at a considerable distance from the wound surface, and no other anaerobes were present in the muscle. The bacillus proved markedly pathogenic for guinea pigs, producing extensive muscle lesions from which pure cultures of the bacillus were always obtained. Thus there is good proof that the bacillus found was the responsible agent in the fatal human case.

Morphologically the similarity of the bacillus to *Bacillus welchii* is marked. Its cultural reactions are such that in a mixed culture with *Bacillus welchii* it would escape notice, unless the most careful plating were done. When it has once been isolated, however, it is culturally and pathologically distinct.

Case.—X, age 20 years.

Day 1. Wounded. No Casualty Clearing Station note.

Day 2. Arrival at Base with multiple wounds of legs. There is a penetrating wound of the right thigh, and the whole thigh is much distended and tense. Clinically it is considered an obvious gas bacillus infection. X-ray examination shows two small foreign bodies, one in the right knee and one in the right thigh.

Day 3. A severe gas bacillus infection, extending up the abdominal wall. The thigh is blown up tightly, with gas crackles everywhere. Incision into upper thigh muscles reveals complete gas bacillus infection of the outer part of the thigh. Amputation is considered useless. 10 cc. of anti-gas bacillus serum are given intravenously in 200 cc. of saline solution, followed by 90 cc. undiluted.

Day 4. Condition is about the same, with slight increase in the extent of infection.

Day 5. 110 cc. of serum given intravenously. The gaseous emphysema now extends to the level of the costal margin, but the patient's general condition is not very bad. The thigh is still very foully gaseous. The patient vomits practically everything taken.

Day 7. Died at 1.15 a.m.

Autopsy.—Performed 9 hours after death. Extensive gas gangrene of the right thigh. The muscles are greatly swollen and gaseous, gangrenous in the oldest part of the lesion, showing the usual variations in hue according to the age of the lesion. There is marked friability of the muscles. There is a small amount of subcutaneous edema, and edema between muscles, with reddish fluid. Iliopsoas muscles within the abdomen pale and soft but not gaseous. *Kidneys.*—Slightly swollen and pale on section, with markings obscure. *Spleen.*—Small. Appears normal on section. *Liver.*—Acute degenerative changes. *Lungs.*—Normal. *Heart.*—Normal. *Peritoneum.*—Normal.

Specimen Used for Cultures.—A large piece of muscle was excised aseptically from the right thigh at operation, before treatment was started. None of the original wound surface was included in the specimen. On arrival at the laboratory the surface was seared and pieces were cut with a sterile knife from within the mass, planted in chopped meat medium, smeared on egg slants, and incubated anaerobically in McIntosh and Fildes' jars.

Cultures.—In 24 hours numbers of isolated colonies were present on the egg slant. All appeared alike. Several were fished into milk, but gave no reaction within 4 to 5 days. Slides of the colonies showed a bacillus resembling *Bacillus welchii*, varying more in length, however, than is usual with *Bacillus welchii* under similar conditions. Further incubation and observation of the egg slants showed no new developments. The milk test was repeated on numerous colonies, but was always negative.

The chopped meat culture gave a bacillus resembling *Bacillus welchii*, but heavily planted subcultures in milk gave no reaction in spite of good growth. Streaked on egg slants, it gave only colonies like those from the original muscle planted on egg.

Colonies from the original egg slant and from the meat subcultures were then plated on egg slants and single colonies were picked and immediately replated. The resulting colonies were always alike in gross and microscopically. After the plating had been repeated a number of times, several colonies were picked and run through media in parallel for determination of characteristics.

The above account of the cultural methods shows that *Bacillus welchii*, if present, could not have been missed, for it would have given the characteristic milk reaction in the plantings from the original

cultures. The milk used was tested by running a *Bacillus welchii* in parallel. It also proves that the original muscle contained a pure culture of the bacillus in question.

Characteristics of Bacillus egens.

Morphology.—The bacillus is morphologically practically identical with *Bacillus welchii* when grown in most liquid media. It is of the same length, thickness, and shape. In animal tissues and in the first subcultures in bouillon the bacillus shows a well defined capsule, easily stained by gentian violet according to the Welch method. The bacillus is strongly Gram-positive in young cultures.

The differences in form between *Bacillus egens* and *Bacillus welchii* are evident in surface colonies of 2 days or more on a medium which is not alkaline. Occasionally *Bacillus welchii* produces long thread-like forms in old cultures on alkaline sugar-free media, but there is little variation in length on 0.5 per cent acid glucose agar or on the neutral egg slants. *Bacillus egens*, however, in cultures over 1 day old produces long thread-like forms on these media, often in great abundance. They are especially marked in the condensation water of egg slants and on glucose agar. These long forms are tortuous, unjointed, and Gram-positive. From forms five to ten times the length of *Bacillus welchii* they soon grow to a length extending from one to three times the diameter of an oil immersion field.

The second principal difference in appearance consists in the early appearance of Gram-negative forms of *Bacillus egens*. Often 50 per cent of the bacilli in surface colonies appear Gram-negative in 2 days. In cultures 4 to 5 days old nearly all the bacilli except some of the long threads are Gram-negative, and a great many of them are greatly degenerated, forming small, non-refractive, swollen, nearly spherical masses. *Bacillus welchii* shows few if any Gram-negative forms under corresponding conditions, and no similar degenerated forms. This tendency to Gram-negative forms is less evident in liquid media.

Another less definite difference consists in an unevenness of outline in 1 day milk cultures which *Bacillus welchii* does not show.

Motility.—The bacillus is absolutely non-motile in cultures, and in the infected fluids of animals. *Bacillus fallax* and *vibrion septique*

were used for a test of method, and both were found definitely motile in the medium used (condensation fluid of egg slants).

Spores.—No evidence was found of spore formation.

Cultural Characteristics.

Anaerobiosis.—*Bacillus egens* is a fairly strict anaerobe. No growth could be obtained aerobically on slants, and an almost imperceptible cloud formed in the depths of a glucose bouillon tube which had been shaken with air.

Proteolytic Action.—No proteolytic action occurs on the casein of milk, coagulated egg albumin, cooked muscle, or coagulated horse serum, even after long exposure. This is in contrast to its softening effect on living muscle in infected animals. *Bacillus welchii* shows the same contrast in less degree.

Saccharolytic Action.—The only sugar fermented quickly and vigorously is glucose, in which marked acid and gas formation occurs in 24 hours. Some of the other sugars are feebly attacked. Often they show no change or a very slight one unless a heavy planting is made. Serum water media were used, and a culture of *Bacillus welchii* run as a control.

Maltose and saccharose under favorable conditions show slight acidity and gas in 24 to 48 hours. Lactose is fermented with great slowness and feebleness, never showing more than a trace of acidity to litmus. In milk not enough acid is produced to cause an acid clot in less than 12 to 14 days, even with a heavy growth.

Galactose and raffinose are fermented to the same degree as saccharose.

Mannite, dulcite, inulin, glycerol, and salicin are not changed.

Indol Production.—No indol is produced.

Hemolytic Action.—Surface and deep colonies on blood agar show a definite hemolytic zone in 24 hours.

Odor.—No noticeable odor was produced in any cultures.

Appearance of Cultures.

Milk.—No gross change occurs in milk in less than 12 days, and usually not for 15 to 20 days, when a slow precipitation of casein

occurs followed by acid clot formation with little or no gas. The fluid above the clot is cloudy and gray. No digestion of the casein or further change occurs.

This absence of the stormy fermentation seen with *Bacillus welchii* is a constant and definite character of *Bacillus egens*, whether the cultures are freshly isolated from animals or have been under cultivation for 6 to 7 weeks. Tests were made with numerous cultures with heavy plantings, and good growth occurred within 24 hours. *Bacillus welchii*, run in parallel on the same medium, always produced the characteristic stormy clot.

Chopped Meat.—A few gas bubbles, but no other change. No digestion, blackening, or other color production.

Egg Slants.—No blackening or softening.

Glucose Bouillon.—Diffuse cloudiness with fine sediment. Diffuse cloud persists indefinitely.

Plain Bouillon.—Faint diffuse cloud.

Colonies.

Glucose Agar.—In 24 hours the colonies are 0.5 to 2 mm., rather flat, circular, with a sharp even edge. By transmitted light against a black background they appear a bluish gray with scarcely greater opacity in the center. With a hand lens the colony is seen to have a very fine grayish grating in a clear translucent stratum. With the light at the proper angle reddish, purplish, and green iridescent tints are visible with a hand lens. They are probably due to refraction of the light by the fine internal structure of the colony. *Bacillus welchii* colonies under similar conditions have a large, dense, opaque, yellowish white central portion, with a translucent border filled with coarse white flecks, and show no iridescence. *Fallax* colonies are like *Bacillus welchii*, but a little less opaque and coarse.

Further incubation produces a slight increase in size and the refractive qualities decrease, but otherwise there is no special change.

Deep colonies on glucose agar are very small ($\frac{1}{3}$ mm.), opaque, white, lenticular specks without processes. They change little in size. *Bacillus welchii* produces much larger colonies and forms a larger amount of gas. On egg slants the colonies are circular with a

sharp regular outline. Within 24 hours they are pale gray but soon become a light yellow color. The surface is extremely smooth, even, and shiny. The size varies from 1 to 2 mm. at first, to 4 to 5 mm. in 2 to 3 days. *Bacillus welchii* in comparison shows after 2 to 3 days a grayish white, elevated center, with a flat, dirty yellow, slightly crenated border. Both have a surrounding zone of light discoloration of the egg medium.

Deep colonies in blood agar become brown in 2 to 3 days with a small, central, darker brown dot.

Pathogenic Action.

Bacillus egens proved highly pathogenic for guinea pigs and somewhat less so for rabbits. After 6 weeks of subculture 0.1 cc. of a 24 hour bouillon culture was fatal to guinea pigs. After passage through guinea pigs 0.02 cc. of a 24 hour glucose bouillon culture was fatal within 30 hours. Experiments in rabbits were few, but 2 cc. of a 24 hour glucose bouillon culture was fatal to a rabbit in 2 days.

The disease in guinea pigs has the following characters.

Clinical Characteristics.—1. Constitutional symptoms of dullness, apathy, and sluggishness of reflexes. Fever is followed by subnormal temperature.

2. Local signs consisting in a marked soft fluctuant swelling at the site of inoculation with brownish or slightly greenish discoloration of the skin. There is local tenderness and warmth.

3. Soft sodden edema over abdomen, chest, and neck.

4. Slight gas crepitus over site of inoculation, abdomen, and chest.

Pathological Characteristics.—1. Extensive and complete muscle destruction in the region of inoculation. Many of the muscles are reduced to soft, pale, pulpy fragments lying in a thin red fluid. On picking up one of the pieces it falls apart by its own weight. Muscles less affected are easily torn. All the lesions are characterized by extreme pallor of the muscles. *Bacillus welchii* produces some local muscle destruction but not to such a degree, and the muscles have a different color.

2. Extensive muscle lesions distant from the point of inoculation, especially over the abdominal wall and chest. Sometimes the muscles

of the opposite leg are affected. The muscles are pale with a faint, light, purplish pink tinge, are very soft, and tear with great ease.

3. A pseudogelatinous dull red edema, usually marked in the flanks and axillæ, and often thick over the sides of the abdominal wall. It appears gelatinous to sight and touch, but on cutting it a thin, slightly brownish red fluid escapes and the immediate area entirely collapses. It therefore simply consists in a thin fluid held in very small compartments made up of thin translucent recent adhesions.

4. Gas bubbles thinly scattered in the subcutaneous tissue of abdomen and chest.

5. Absence of any odor different from that of a normal dead guinea pig.

6. No destruction of skin or loosening of hair.

7. Invasion of peritoneal cavity and blood stream.

A positive blood culture at death, or soon after in the cases where autopsy was not done immediately, was always obtained and usually bacilli could be found in blood films.

In the following protocols of experiments all these details will not be given as they are qualitatively the same in each case.

Technique.

Each animal was shaved at the site of inoculation and the skin cleaned with methylated spirits followed by ether and iodine. The cultures were injected into the muscles of the thigh with a very fine, sharp needle, so that trauma of muscle was reduced to a minimum as a factor in the pathogenicity. Unless otherwise stated the cultures were 24 hour growths in 1 per cent glucose bouillon.

Guinea Pig 1.—2 cc. of a 2 day culture in chopped meat medium (rather thin growth). In 24 hours marked local signs of the usual character. The guinea pig appeared dull and sick. Died 36 hours after inoculation.

Autopsy.—Performed ½ hour post mortem. A large pocket is found at the site of inoculation filled with thin red fluid in which lie pulpy muscle fragments. Nearly all the muscles of the thigh are gone. Subcutaneous edema over abdomen and chest with a few gas bubbles. *Peritoneal Cavity.*—No fluid.

Cultures.—Pure cultures from the edema fluid, affected muscle, and heart's blood.

Films.—Films from the edema fluid show large numbers of the bacilli, which are short and resemble *B. welchii.* There are practically no cells. Blood films show a few bacilli.

Guinea Pig 2.—The same inoculation as Guinea Pig 1. Similar clinical course and pathological condition.

Guinea Pig 3.—0.3 cc. of a 24 hour glucose bouillon culture from a colony from the heart's blood of Guinea Pig 1. Died in 18 hours.

Autopsy.—Performed within ¾ hour after death. Lesions similar to those of Guinea Pigs 1 and 2 with a smaller local pocket of fluid. Cultures and films give similar results, with the addition of a positive culture from the peritoneal cavity.

Guinea Pig 4.—0.1 cc. from the original culture. (No passage through animals; under cultivation for 6 weeks.) Died in 28 to 36 hours.

Autopsy.—Performed 1 to 8 hours post mortem. Lesions of the usual type. Pure cultures from edema, muscle, heart, and peritoneum.

Guinea Pig 5.—The same inoculation as Guinea Pig 4. In 12 hours marked local swelling, tenderness, and discoloration of skin. Guinea pig sluggish and sick. In 36 hours local swelling is slightly less, the animal appearing better. Recovery later, with persistence of a slight local swelling.

Guinea Pig 6.—0.01 cc. of the same culture as Guinea Pig 4. No evident effects.

Guinea Pig 7.—0.1 cc. of a culture from a colony from Guinea Pig 4. Died in 29 hours.

Autopsy.—Performed ½ hour post mortem. Usual lesions. Pure cultures from edema, peritoneum, and heart.

Guinea Pig 8.—0.02 cc. of the same culture as Guinea Pig 7. Died in 29 hours.

Autopsy.—Performed within 1 hour post mortem. The usual lesions. Pure cultures from edema, peritoneum, and heart.

Rabbit 1.—2⅛ cc. of a 2 day chopped meat culture (from culture not passed through animals; rather thin growth). Local swelling in 24 hours. The animal appears somewhat sluggish and ill. Killed with chloroform in 48 hours.

Autopsy.—A local abscess in the muscles of the thigh, caused by destruction of muscle tissue, well walled off. The muscles nearby, however, were pale, nearly translucent, crisp, and friable. Further off the muscles were pale and easily torn. Edema was present in the flanks as in the guinea pigs.

Cultures.—Pure growth of *B. egens* from the muscle.

Rabbit 2.—2 cc. of a culture recently isolated from a guinea pig. In 24 hours slight local swelling. The rabbit appeared sluggish. In 48 hours the local swelling had increased, the leg was apparently paralyzed, and the rabbit appeared extremely sluggish and dull and felt cold to the touch. There was slight crepitation over the local swelling and over the abdomen. Death occurred that night.

Autopsy.—At the site of inoculation the muscle was pale externally and on section, friable. The muscles were slightly swollen, and between them were

a dull red pseudogelatinous edema and gas bubbles. A similar edema and gas extended over the abdominal wall and chest. There was marked edema in the flanks and axillæ. The muscles of the chest and abdomen were pale, soft, and friable. *Peritoneal Cavity.*—A small amount of slightly cloudy, pale fluid, containing numerous bacilli.

Cultures.—Pure cultures from muscle, edema, peritoneum, and heart.

Pathology of the Lesions.

Sections of muscle from the human case showed muscle degeneration much as in *Bacillus welchii* infection, but without any hemorrhages. There was practically no cellular infiltration. Bacilli were not numerous except in a few areas. No other organisms could be found.

Kidney.—Sections showed marked acute tubular degeneration. The nuclei were pyknotic or had disappeared; the protoplasm was swollen and granular. No bacilli were found.

Liver.—Fairly well marked degeneration of the central type. No foci of infection.

Guinea Pig Muscle.—The infected muscle shows marked edema between muscle fibers, practically no cellular infiltration, and no hemorrhage. The muscle fibers degenerate either in mass, losing structural markings and becoming deep staining, or, and this very frequently, becoming edematous, with separation of the fibrillæ, which still appear sharp and distinct. Bacilli are very numerous between muscle fibers and invade them early.

Identity of the Bacillus.

From other anaerobes than *Bacillus welchii* the bacillus differs morphologically as *Bacillus welchii* does. It is needless to spend much time on this point. It also differs from others in the characters of its colonies. Further differences are given below.

Bacillus fallax is motile in culture under favorable conditions and highly motile in animal fluids. *Bacillus egens* is always absolutely immotile. *Bacillus fallax* has scarcely any pathogenic powers and these are quickly lost in subculture. *Bacillus egens* was markedly pathogenic after 6 weeks of subculture. *Fallax* is non-pathogenic for rabbits. When it does produce lesions in guinea pigs they are of a different character. The muscles are red and hyperemic and there

is no muscle lysis. Morphologically *fallax* is thinner, becomes Gram-negative more quickly, has more rounded ends, and does not produce long filaments as described for *Bacillus egens*.

Bacillus welchii differs fundamentally by its characteristic stormy fermentation of milk, its vigorous saccharolytic action on maltose, lactose, galactose, saccharose, and glycerol or inulin. Pathologically it does not produce such marked and extensive muscle lysis, and there is often hemorrhage in the lesions, giving a pink or red color to the affected muscles. It is not usually so pathogenic after long subculture, especially for rabbits. Morphologically it is characterized by the absence of filament formation under the conditions described for *Bacillus egens*. Its colonies are quite different.

Bacillus œdemaciens differs in morphology (chain formation in cultures and animal tissues; spore formation). In colony characteristics it is absolutely unlike *Bacillus egens*, and the pathological lesions are totally different. There is little or no muscle lysis, but an extensive white jelly-like edema.

Vibrion septique differs in motility, morphology, formation of long filaments in peritoneal fluid, spore formation, colony characters, and pathogenic action.

Bacillus aerofœtidus is practically non-pathogenic. It is a much smaller, thinner bacillus with much rounded ends, quickly becoming Gram-negative. Its colonies are different and it liquefies blood serum and ferments lactose vigorously.

Bacillus egens differs from all the other pathogenic anaerobes in the lack of spore formation and of proteolytic power.

The result of this case shows the importance of careful bacteriological control in testing the use of an anti-gas gangrene serum. It would have been easy to mistake the case for a *Bacillus welchii* infection, especially as *Bacillus welchii* was present on the wound surface, together with other aerobes and anaerobes. Of course the serum could not be expected to have therapeutic value in this particular case.

Whether such an infection as this will prove to be common is uncertain. Its presence would be extremely easy to overlook in the event of a coincident infection with *Bacillus welchii*, for *Bacillus egens* would add scarcely a single positive morphological or cultural character to mixed cultures.

Nomenclature.—Since the bacillus appears to be a new species, a name has been given it. Its most evident difference when compared to any other anaerobe consists in the lack of some characteristic; it differs from *Bacillus welchii* by lack of fermentative powers; from *fallax* by lack of motility; from *œdemaciens* and other anaerobes by lack of spore formation and of proteolytic power. Therefore the name *egens* has been chosen.

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