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The Isolation of "Bacteroides" Organisms from a Specimen of Pleural Fluid (Human)

by

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History

OBLIGATORY anaerobic, non-sporing bacilli capable of causing disease in man were first isolated in the last few years of the nineteenth century (see Dible, 1951). In the year 1919, Castellani and Chalmers suggested that all such bacteria should be grouped in a new genus for which the name "Bacteroides" was coined.

Dible mentions the investigations of Eggerth, Weiss and Rettger, and Lewis and Rettger in this connection. Their investigations showed that it would be necessary to separate the gram positive and gram negative species at least into separate genera and possibly to make more drastic sub-divisions.

Laboratory Examination

Pus aspirated from the pleural cavity of a man aged 40, who is said to have developed acute left Empyema Thoracis following on a left sided subphrenic abscess was received at the Medical Research Institute on 11.9.59. It was reported that the patient had a high (E.S.R.) erythrocytic sedimentation rate and a high W.B.C. count.

W. B. C.	:	20,300 per cu. ml.
D. C.	:	N=88%
		L=11%
		E=1%
E. S. R.	:	1st hour .. 66 mm.
		2nd hour .. 88 mm.

Smears were made direct from the specimen, and stained by Gram's method and Ziehl Neelsen's method of staining. There was no evidence of organisms in the smears examined microscopically.

The specimen was inoculated into :—

(1) Blood Agar
(2) Lactose Agar
(3) Robertson's cooked meat medium
(4) Thiol-Broth (Difco laboratories).

and incubated aerobically. After 48 to 72 hours incubation of the inoculated media, there was no growth on the Blood and Lactose Agar. The cooked meat medium and Thiol-Broth however appeared turbid. The broths were then plated on to two sets of Blood and Lactose Agar plates. One set was incubated aerobically, and the other anaerobically.

There was no growth on the plates incubated aerobically.

The plates incubated anaerobically, however, had evidence of growth. The inoculations of the cooked meat and Thiol-Broth on to the Blood and Lactose Agar plates incubated anaerobically led to the isolation of two organisms, one being a gram positive bacillus from the cooked meat and the other a gram negative bacillus from Thiol-Broth.

ORGANISM No. I

The colonies on Lactose Agar inoculated from the cooked meat appeared pink and moist. The colonies on Blood Agar inoculated from the cooked meat were haemolytic, submerged colonies, about 1 mm. in diameter.

Smears made from these colonies showed gram positive thick bacilli.

The organism was non-motile and non-sporing.

Repeated smears made from the colonies on Blood Agar did not show any spores. Sub-cultures of the haemolytic colonies were made into cooked meat for evidence of digestion of meat and production of gas.

There was no gas produced and no blackening of the meat. The meat turned pink (indicating that the organism was saccharolytic in nature) and, like in the case of most anaerobes, there was a foul odour.

Clostridium welchii, the non-motile clostridium organism of the gas gangrene group was excluded by inoculating broth cultures of the organism into guinea-pigs. The animals were alive and well even after forty-eight hours with no apparent ill effect. The organism also failed to produce a " stormy " clot reaction in litmus milk, nor did it liquefy gelatin.

Repeated attempts to make the organisms sporulate failed to produce results. The organism was inoculated into salt agar, Lactose Agar with 6.5 % NaCl, and finally a salt agar plate with 2.5 % NaCl. There was no evidence of growth on these plates, either aerobically or anaerobically. Storage at room temperature failed to cause sporulation.

Biochemical Tests

The organism from the cooked meat was inoculated into parallel sets of peptone sugars with and without iron nails. Its sugar reactions were as follows :—

	Lactose	Sucrose	Maltose	Glucose	Dulcite	Mannite	Salicyn
With nail:	+	+	+	+	0	0	0
Without nail:	–	–	–	–	–	–	–

+ = Acid and gas produced
± = Acid only
0 = Growth
– = No growth.

The organism did not produce Indole from Casein ; it also failed to liquefy gelatin ; and the reaction in litmus milk was neutral. It did not produce H_2S.

The organism did not grow on Tomato Juice Agar at a p^H5, but it did grow on this medium at a p^H6. Colonies were large and moist, and smears showed gram-positive bacilli.

Discussion

The reason why this organism may still belong to the Bacteroides group and not be a Lactobacillus is that while the Lactobacilli are strictly anaerobic on primary isolation, they rapidly become aerobic in artificial culture. This gram positive bacillus isolated from pleural fluid remains strictly anaerobic.

At first it was thought that all gram positive Bacteroides would prove to be Lactobacilli, but later work by Eggerth and Orla-Jensen et al. (quoted by Dible, 1951) revealed the existence of organisms variously labelled " B. bifidus Group II " and " Bact. bifidum " which differed in several respects from the classical " Lact. bifidus " in such features as their morphology in subculture, in remaining strictly anaerobic, in colonial characters and fermentation reactions.

Barker and Hass (1944) suggested that a new genus be created—Butyribacterium, of which " B. bifidus Group II " is the type species.

Bergey (1948) states that Lactose and Sucrose are not fermented by Butyribacterium. This organism ferments Lactose and Sucrose with gas production. As distinguished from Ramibacterium ramosum which is a small slender rod, also non-motile and non-sporing in culture but produces branching forms, this gram positive organism is strongly saccharolytic, producing both acid and gas in Glucose, Maltose, Sucrose and Lactose.

The colonies of this organism on Blood Agar are haemolytic, moist, "milky-water" colonies unlike R. ramosum. R. ramosum is also stated to produce a true exotoxin which is not haemolytic (Weinberg et al. quoted by Dible, 1951).

ORGANISM No. II

The plates inoculated from the Thiol medium and incubated anaerobically showed gram negative short bacilli on Blood Agar. There was no growth on the Lactose Agar. The colonies on Blood Agar were tiny and non-haemolytic.

In connection with this gram negative organism which resembled similar gram negative cocco-bacilli, such as those of the Haemophilus group both in colonial and morphological appearances, tests were done to eliminate such a possibility. The Haemophilus organisms are not anaerobic and will grow aerobically as long as X and V factors of the blood are present, but will not grow on plain agar. This gram negative organism did not grow aerobically even when supplied with X and V factors. Though colonial and morphological appearances resemble the Haemophilus Group, the ability of the organism to grow without X and V factors and its dependence on strict anaerobic conditions excludes it from this group.

Biochemical Tests

Single non-haemolytic colonies were inoculated into cooked meat for evidence of digestion of native proteins and production of gas. There was no blackening of the meat. The meat turned slightly pink, and like in the case of most anaerobes gave a foul odour.

The organism was subsequently inoculated into parallel sets of peptone sugar with and without iron nails. Its sugar reactions were as follows :—

	Lactose	Sucrose	Maltose	Glucose	Dulcite	Mannite	Salicyn
With nail:	±	±	±	±	0	0	0
Without nail:	–	–	–	–	–	–	–

The organism did not produce indole from casein and it also failed to liquefy gelatin. The reaction in litmus milk was neutral. The organism when inoculated into Kliegler's medium showed a slight trace of H_2S production.

Discussion

Accounts of human infections of this type by similar organisms have been described by Shaw and Bigger (1934) reporting a lung abscess, and by Alston, Baker, and Bratton (1937) describing "a fatal infection by Bacillus (Actinomyces) Necrophorus." More recently Henderson (1953) has reported an organism similar to this gram negative anaerobic bacillus.

Organism II had cultural and biochemical characteristics which so far as they went agreed with the description of the Fusiformis organism in Henderson's paper.

Pathogenicity

Broth Cultures (12 to 24 hours of incubation) of the gram positive and gram negative organisms were injected subcutaneously into guinea-pigs, rabbits and white mice, with no apparent ill effect.

Conclusion

A number of non-sporing, non motile, anaerobic bacilli have been isolated by different workers from necrotic lesions in man and animals. They represent an ill-defined group of organisms classified under the genus Bacteroides—Bergey 1948. The term necrobacillosis is given to the condition they cause in man.

It is probable that both the organisms (gram positive and gram negative bacillus) can be included in the genus *Bacteroides*. The fundamental characteristics of the group being that they are non-sporing, non-motile, gram-variable, anaerobic organisms.

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