CLOSTRIDIUM PUTRIFICUM (B. PUTRIFICUS BIENSTOCK), A DISTINCT SPECIES

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There has been much confusion regarding the identity of the organism which was discovered by Bienstock (1884) and which he named *Bacillus putrificus*. According to his own admission, Bienstock for a while worked with impure cultures of this anaerobe. However, he described its morphology and putrefactive properties, in his first publication (1884), and at a later date published a more complete description (1899).

Bienstock described his *B. putrificus* as a long, slender rod with a terminal spore. His photographs show both round and more or less oval spores. Some investigators have interpreted his phrase "baguette de tambour" and the word "Trommelschlägenform" as meaning literally *drumstick* and implying an oval form of the spore. This interpretation does not appear to the writers to be correct. In its sporulating stage *Clostridium tetani* is described quite generally in the literature as a drumstick form, and this term is generally applied to organisms possessing a round, terminal spore. Tissier and Martelly (1902) liken the spore of *C. putrificum* to that of *C. tetani*. Rettger (1906) also points out a strong resemblance, and further draws attention to the ease with which *C. putrificum* may, by morphology alone, be mistaken for the tetanus bacillus. However, certain writers have in recent years referred to *C. putrificum* as producing a terminal oval spore. Even so late a work as that of the British Medical Research Committee (1917) has included this organism among the putrefactive anaerobes which develop such spores. Some American writers have fallen into the same error. Sturges and Rettger (1919), on the other hand, state emphatically as the result of a continued study of several pure

strains which had been isolated by recently devised methods, that the spores of this organism are round.

The writers have employed the strains isolated by Sturges, in their morphological, biochemical and cultural studies of *C. putrificum*, and have corroborated the earlier observations of Rettger and those of Sturges and Rettger, particularly the points regarding morphology. In all of the stock strains ex-

FIG. 1. SHOWING RODS, CHAINS AND CHARACTERISTIC DRUM STICKS OF *C. putrificum*. INCUBATED 3 WEEKS AT 37°C. IN EGG-MEAT MEDIUM. FUCHSIN STAIN. × 1000

amined (four) the microscopic appearances of the organism were the same. The bacilli were long and slender, frequently slightly curved, and in the sporulated condition possessed a terminal, round spore which in the fully developed drumsticks appeared very large as compared with the thickness of the rods. The rods were as a rule longer and much more slender than those of *C. tetani* grown on the same medium (fig. 1). Colonies also are characteristic (fig. 2).

There has been considerable discussion as to whether *C. putrificum* attacks carbohydrates. Bienstock did not deal with this phase in his earlier work. Tissier and Martelly (1902) showed that it does not act upon glucose, or at most only slightly. In a later publication (1906) Bienstock confirms the observations of Tissier and Martelly, and of Rodella (1905), and holds that *C. putrificum* does not attack any of the carbohydrates, and that the minute quantities of acid formed in sugar media arise from the decomposition of protein. This organism occupies a unique position, therefore, among the anaerobes, in that it attacks proteins but not carbohydrates appreciably if at all. Bienstock's

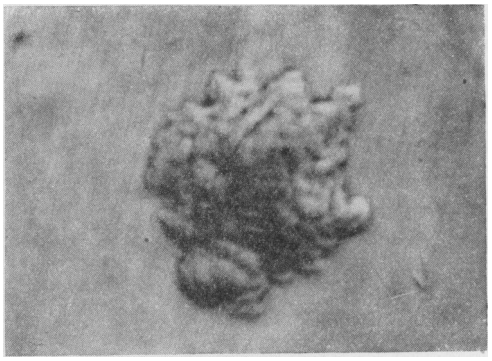

FIG. 2. COLONY ON 1.6 PER CENT GLUCOSE AGAR. INCUBATED 10 DAYS AT 37°C.

"*B. paraputrificus*," on the other hand, did attack carbohydrates with acid and gas formation.

In the recent reports of the British Medical Research Committee, *C. putrificum* is described as being saccharolytic to the extent of acting upon glucose, maltose, lactose, sucrose and starch. They even go further (1919) and consider it to be, not an entity or distinct species, but a mixture perhaps of *C. sporogenes* and *C. tertium* or of *C. sporogenes* and *C. cochleareus*.

In our own study of this organism its unique position appears to be so clearly defined and its characters are found to be so outstanding that we see no reason for mistaking it for any other anaerobe or mixture of anaerobes. Because of the fact that

the above mentioned work of the British Medical Research Committee has exerted and is exerting a commanding influence on present-day attempts at re-classification of the anaerobes, particularly those which were found in war wounds, we feel prompted to defend *C. putrificum* (*B. putrificus*) as a distinct species and to attempt to place it in its proper classification group.

C. putrificum bears a more or less close resemblance to *C. tetani*, and *C. tetanoides* morphologically, but differs markedly

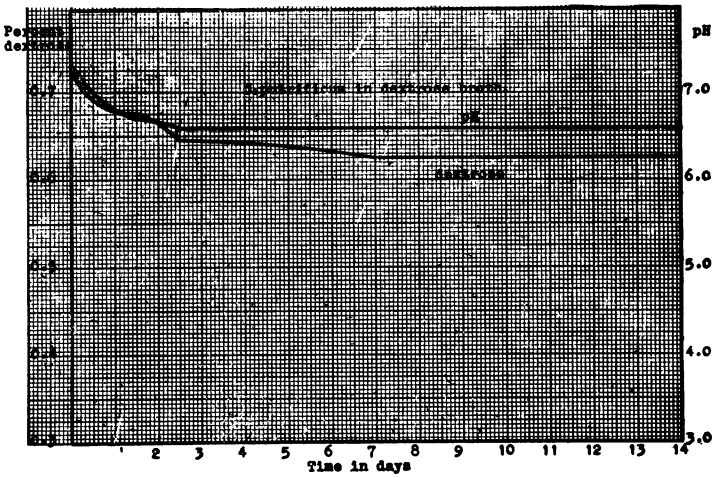

CHART 1. PLOTTINGS OF pH AND GLUCOSE FIGURES FOR *C. putrificum* IN GLUCOSE BROTH

from them in certain important respects. Pure strains of *C. tetani* and *C. tetanoides* are non-putrefactive, and are unable to digest meat or egg-meat medium even during months of incubation. *C. tetani* is pathogenic, whereas the other two organisms are entirely void of this property. Furthermore, *C. putrificum* does not attack carbohydrates or at the most exerts but a feeble action. No gas was produced from any of the 25 carbohydrates, alcohols and glucosides employed by us, and only slight amounts of acid from three or four, one of these being glucose. Quantitative estimations of the amounts of glucose consumed were made, and the results expressed in plotted curves (chart 1).

This organism constitutes a unique division, therefore, among the known anaerobes, and differs from all others in that it is powerfully putrefactive, but non-saccharolytic, or at the most but feebly saccharolytic.

While *C. putrificum* is an active proteolytic and putrefactive organism, it differs from *C. sporogenes* in that in pure culture in meat or egg-meat medium it develops slowly and produces little or no apparent change in less than a week or ten days, even at the most favorable temperature. After the long incubation period, however, the protein is vigorously attacked with the formation of the usual putrefaction products. When mixed with other organisms, as for example *Staphylococcus aureus* or *Proteus vulgaris*, the preliminary period is relatively short and putrefactive decomposition takes place much sooner. These observations are in accord with those of Sturges and Rettger (1919).

C. putrificum has a very marked peptolytic action in ordinary peptone broth, as may be shown readily with the aid of the ammonia, Sörenson, Van Slyke and quantitative biuret tests. The complex nitrogenous substances in the commercial peptone are destroyed rapidly, with the formation of large amounts of ammonia, but with little permanent increase in amino nitrogen. The biuret figures drop sharply (chart 2).

Important points of difference between *C. putrificum* and *C. sporogenes*, *C. tertium* and *C. cochleareus*, organisms with which the Bienstock anaerobe has been confused, are as follows: *C. sporogenes* is actively proteolytic and saccharolytic; *C. tertium* is saccharolytic and peptolytic, but not proteolytic; and *C. cochleareus* (according to the statements of the British Medical Research Committee) is saccharolytic and non-proteolytic. *C. putrificum* occupies an entirely different position in that it is proteolytic and but very slightly or not at all saccharolytic. There are sufficient morphological and cultural differences to set the Bienstock anaerobe apart from the others, and not only should it be easy to distinguish *C. putrificum* from these three organisms, but from all known anaerobes by a combination of the morphological, cultural and biochemical characters, which have been mentioned above.

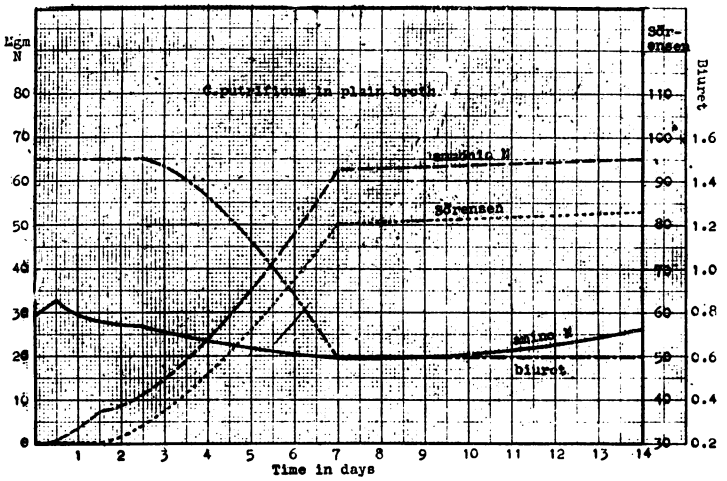

CHART 2. PLOTTINGS OF AMMONIA, SÖRENSEN, AMINO NITROGEN AND BIURET FIGURES FOR *C. putrificum* IN PLAIN BROTH

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