

# THE IDENTITY OF BACILLUS PUTRIFICUS BIENSTOCK

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In the literature on the putrefactive, anaerobic bacilli there is, as Reddish and Rettger (1922) have pointed out, much confusion regarding the identity of *Bacillus putrificus* Bienstock. This is largely due to the fact that Bienstock's first publication (1884) refers to cultures which were impure, as he himself later admitted, and that the subsequent papers of this author (1899, 1901, 1906) contain imperfect descriptions of his organism which are to some extent mutually contradictory.

The majority of investigators are agreed that Bienstock's bacillus is an anaerobic, motile, sporulating rod which liquefies gelatin, peptonises milk and is non-pathogenic. In the published descriptions of this bacillus there is some diversity of opinion as to the rate at which the organism is capable of liquefying gelatin and peptonising milk. The main characteristics about which there is disagreement, however, are the shape and position of the spores, the size of the rods and the ability of the organism to ferment carbohydrates. Certain workers describe *Bac. putrificus* as a slender rod, which produces more or less spherical spores, always strictly terminal and which is incapable of attacking sugars to any appreciable extent. The chief advocates of this view are Reddish and Rettger (1922, 1923, 1924). On the other hand several investigators have referred to the fermentation of certain carbohydrates by strains of *Bac. putrificus* obtained from type culture collections (Achalme (1902); Reddish (1924)) or by cultures believed to be identical with Bienstock's bacillus (Kendall, Day and Walker (1922); Kahn (1922)).

In the course of an investigation of certain anaerobic bacilli

a number of strains of sporulating anaerobes which resemble more or less closely the forms referred to in the literature as *Bac. putrificus* have been examined. The organisms studied have already been described in detail in a previous paper (1931) under the numbers B<sub>4a</sub>, B<sub>4b</sub>, and B<sub>5</sub>. B<sub>5</sub> (fig. 3) is the form which Reddish and Rettger consider to be identical with Bienstock's bacillus; B<sub>4b</sub> (fig. 4), often referred to as *Bac. sporogenes* Metchnikoff, is the gas-producing type which certain workers regard as *Bac. putrificus*, (see Medical Research Committee, 1919, p. 94). In some of its characteristics B<sub>4a</sub> (figs. 1 and 2) is intermediate between B<sub>5</sub> and B<sub>4b</sub> and it corresponds more closely to Bienstock's description than either of these forms.

The studies on which the descriptions referred to (1931) are based were made on six strains of B<sub>4a</sub>, four strains of B<sub>4b</sub> and five strains of B<sub>5</sub>, isolated during the investigation, and on the following type cultures:

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|---|---|---|
| (1) <i>Bac. putrificus verrucosus</i> Stamm 22.   | } | Received from Prof. Zeissler, Altona-Elbe.                      |
| (2) <i>Bac. putrificus verrucosus</i> Stamm 58.   |   |   |
| (3) <i>Clostridium putrificum</i> (Bienstock) Bergey et al. No. 3559 (Hall collection No. 38) | } | Obtained from the American Type Culture Collection.             |
| (4) <i>Clostridium putrificum</i> (Bienstock) Bergey et al. No. 679                           |   |   |
| (5) <i>Clostridium putrificum</i> Sturges No. 2221  | } | Received from the National Collection of Type Cultures, London. |
| (6) <i>Bac. cochlearius</i> Douglas, Fleming and Colebrook. T. M. I. H. C. No. 535            |   |   |

Nos. 1 and 2 were found to be identical with B<sub>4b</sub>; Nos. 3 to 6 with B<sub>5</sub>.

Reddish and Rettger (1923) reject Bienstock's 1884, 1899 and 1901 descriptions as worthless on the ground that his cultures were impure. Nevertheless they state that these descriptions certainly "had reference to an anaerobe having round terminal spores." If the cultures on which the descriptions are based were impure, it is impossible to assert that the spores observed belonged to *Bac. putrificus* and not to a contaminant. Reddish and Rettger, however, accept the 1906 description, regarding which Reddish (1924) states that it furnishes "the final criteria

for our guidance in this work." Bienstock's admission that in 1884 he worked with impure cultures, has already been noted. If his 1906 characterisation is accepted as accurate the objection raised by Reddish and Rettger to the 1899 paper also appears to be justified, because the 1899 and 1906 descriptions are not in agreement. In Bienstock's 1899 paper, although growth in ordinary agar is referred to, no mention is made of gas formation in this medium, yet the organism is described as frequently producing gas in glucose agar. In the 1906 publication, however, the fact that *Bac. putrificus* is incapable of fermenting glucose is emphasised. These two statements appear to be irreconcilable. The 1901 paper contains little of diagnostic value.

A careful study of the 1906 paper indicates that there are several respects in which Reddish and Rettger's organism does not correspond with Bienstock's description. The latter author states that in certain media ("l'albumine cuite") his bacillus frequently produces, in addition to drumstick shaped rods, the clostridium type of sporulation (that is, central spores). *B<sub>s</sub>* (Reddish and Rettger's bacillus) has not been observed to produce clostridia and no reliable data on this type of sporulation in cultures of the organism have been encountered in the literature. On the other hand *B<sub>a</sub>* may produce a small proportion of clostridia when grown on beef-infusion agar (fig. 2). The majority of the spores of this organism are, however, formed very close to the ends of the rods and many are definitely terminal (figs. 1 and 2). The proportion of terminal to subterminal spores varies in different strains and even in the same strain grown on a variety of media, and in certain cases terminal spores may predominate.

Reddish and Rettger (1922) claim that Bienstock's expression "baguette de tambour" should be interpreted as meaning that the spores of *Bac. putrificus* are terminal and spherical. They appear to base their opinion on the fact that in its sporulating stage *Clostridium tetani* is described quite generally in the literature as a drumstick form. It is clear, however, that this usage of the term does not necessarily restrict its application to rods with spherical spores. At an earlier stage the authors themselves admit that some investigators have interpreted the phrase as

implying that the spore is ovoid. Sporangia containing either spherical or ovoid spores in a terminal position may be equally correctly referred to as "baguettes de tambour." Reddish and Rettger describe the spores of their organism as round and wider than the cells. When they are first formed they may be spherical but ultimately they become distinctly ovoid (fig. 3). The spores of  $B_{4a}$  are ovoid and in certain cases almost cylindrical; they are generally wider than the sporangia (figs. 1 and 2).

The action of Reddish and Rettger's organism on milk is not in accordance with the 1906 description of *Bac. putrificus*. Bienstock states that his bacillus produces putrefactive changes in milk. These begin after about twenty-four hours and are rapidly completed. The medium is peptonised, amino- and other organic acids are formed and a putrefactive odour develops. Observations made in the course of this investigation, as well as by Hall (1922), indicate that Reddish and Rettger's bacillus is incapable of producing rapid peptonisation of milk when examined by the methods generally in use. Hall examined two Sturges strains, isolated in Rettger's laboratory, and found that they had no action on milk. The majority of the strains of  $B_6$  when first isolated also produced no change in milk. Certain strains, however, when retested showed a slow peptonisation without curdling after one to two months' incubation at 37°C. In all, twenty-four tubes of milk were inoculated from pure cultures of the organisms. Of these, twenty remained unchanged in appearance at 37°C. for three weeks or longer; in the remainder a slow peptonisation took place. Reddish and Rettger (1923) have observed that in tubes of milk, each inoculated with a large loopful of a six weeks old egg-meat culture of their bacillus, slight digestion occurred at the end of twenty-four hours and practically complete digestion within four days. The rapidity of the action is attributed to the presence of preformed enzymes carried over in the inoculum. Such mass inoculations are, however, unusual in bacteriological practice. In a later paper Reddish (1924) describes his milk cultures as showing digestion only after two weeks' incubation. Type  $B_{4a}$  is much more active than  $B_6$ . Milk inoculated in the usual way from young cultures on glucose or ordinary agar shows,

after two to three days' incubation at 37°C., a soft curd with separation of a small quantity of clear liquid at the surface. After approximately twenty-four hours' incubation curdling has been observed in milk inoculated from old bullock's heart cultures and occasionally in that inoculated from young cultures on beef-infusion agar. Peptonisation becomes obvious almost immediately after the curd has been formed and proceeds more or less rapidly. The liquid becomes transparent in parts but is never completely cleared. It appears whitish and more or less turbid and has a cheesy odour. This stage is reached in from ten days to about one month, after which no further change takes place.

Bienstock states that the putrefactive activity of his bacillus is retarded or inhibited by the presence of *Bac. coli* or *Bac. lactis aerogenes*. In order to study the effect of the latter organisms on the putrefactive changes produced by B<sub>4a</sub> a number of tubes of milk were inoculated with this bacillus. Half of the tubes then received a further inoculation of *Bac. coli* or *Bac. aerogenes* and all were incubated anaerobically at 37°C. In all the cultures prompt precipitation of the casein took place. After a total incubation period of about three weeks the appearance of the curd in those tubes which had received inoculations of *Bac. coli* or *Bac. aerogenes* remained practically as it was when curdling first took place, whereas in the tubes containing B<sub>4a</sub> alone the characteristic peptonisation change was complete. It appears, therefore, that *Bac. coli* and *Bac. aerogenes* are capable of inhibiting the peptonisation of milk by B<sub>4a</sub>.

Owing to the variation in the rate of liquefaction of gelatin by different strains of the same type as well as by the same strain at different times, it is difficult to give definite comparative figures for B<sub>4a</sub> and B<sub>5</sub>. In general, however, it may be stated that the former liquefies gelatin more rapidly than the latter. In the case of B<sub>4a</sub> liquefaction of gelatin frequently takes place after three to four days' incubation at 22°C. whereas in that of B<sub>5</sub> it has not been observed to occur in less than about ten days. Gelatin, inoculated from one of the B<sub>5</sub> strains and incubated at 37°C., solidified on cooling even after an incubation period of more than

three months. It may be recalled that *Bac. cochlearius*, since identified with Reddish and Rettger's organism (Hall (1922); Fildes (1929); Cunningham (1931)) was originally described as incapable of liquefying gelatin. The latter observation was attributed by Hall to the difficulty in obtaining growth of the organism in the medium. Strain No. 535 from the National Collection of Type Cultures, London, examined during this investigation also showed some reluctance to grow in gelatin at 37°C. Absence of liquefaction of gelatin by the strain of B<sub>5</sub> referred to above was not, however, due to its failure to grow in the medium.

In motility, Gram-staining reaction and the characteristics of their growth in bouillon, bullock's heart medium, brain and liver-liver bouillon types B<sub>4a</sub> and B<sub>5</sub> show marked similarity. B<sub>5</sub>, however, grows much more slowly than B<sub>4a</sub> and, therefore, it is improbable that organisms of the former type could have produced the changes in certain media described by Bienstock in the comparatively short time during which it was usual to keep cultures under observation at that period.

Strains of B<sub>5</sub> are difficult to isolate in pure culture. Thus, Sturges and Rettger (1919) and Würcker (1909), whose *Bac. postumus* was probably also of this type (Zeissler (1928)), experienced difficulty in obtaining colonies of the organism on solid media and had to devise special methods for its isolation. Würcker states that he failed to obtain growth of his bacillus on agar (from the context it appears that glucose agar was used, p. 230) in plate and shake cultures. When, however, he employed as the basis of his agar medium horse liver, previously digested for fourteen days by a putrefactive anaerobic bacillus, he secured colonies and succeeded in isolating the organism. Sturges and Rettger observed that *Clostridium putrificum* only developed on ordinary agar plates incubated in a Novy jar when it was associated with other organisms. They obtained the bacillus in pure culture by growing it with *Bac. coli* or *Staphylococcus aureus* until spores were produced in the mixed colonies so formed and heating the cultures to destroy the non-sporing organisms. Hall (1922) emphasises the tedious nature of the work involved

in obtaining colonies of this bacillus on solid media in order to verify the purity of cultures.  $B_{4a}$ , however, can be readily isolated by anaerobic plating on ordinary agar.

Bienstock does not describe his isolation methods in the 1906 paper. In a previous publication (1899, p. 351) he gives an account of the isolation of his bacillus, from which it appears that he experienced no particular difficulty in obtaining colonies in deep tubes of glucose agar or gelatin. The negative results obtained by Würeker when he used glucose agar shake cultures, the difficulty experienced by Sturges and Rettger in obtaining colonies of their organism and the tedious nature of the technique involved in the isolation of  $B_6$  even when modern methods are employed, all raise doubt as to whether Bienstock's methods were capable of producing growths of an organism of this type on the solid media employed by him.

Bienstock's 1906 description of *Bac. putrificus* is also at variance with the opinion of those workers who believe that this organism is capable of fermenting certain sugars. Bienstock is most emphatic in declaring that his bacillus was incapable of attacking carbohydrates. When first isolated, the strains of  $B_{4a}$  showed no action upon the common sugars and other substances generally employed in fermentation tests. The same strains, when retested after they had been in pure culture for some time, produced slight acidity and minute amounts of gas in peptone water containing certain sugars including glucose. When, however, they were grown in glucose agar stabs, they consistently failed to produce gas. It may, therefore, be concluded that they show no appreciable fermentative activity towards the usual test substances.

The facts already recorded indicate clearly that the characteristics of  $B_{4a}$  correspond more closely to those of Bienstock's organism, as described in his 1906 paper, than do those of *Clostridium putrificum* of Reddish and Rettger or the gas-producing bacillus considered by certain other workers to be identical with *Bac. putrificus*. If Bienstock's incomplete description can be accepted at all as an adequate characterisation of his organism,  $B_{4a}$  is, of all the anaerobic bacilli which have hitherto been ac-

curately and fully described, the one which agrees most closely with this characterisation. It is suggested, therefore, that the name *Bac. putrificus* Bienstock should be reserved for this organism.

The literature on the anaerobic bacilli contains very few descriptions of organisms of this type. The bacillus referred to by Hempl (1918) as organism I, which was isolated from septic wounds and completely described, appears to be identical with *B<sub>4a</sub>*. *Bac. sporogenes-regularis* of Distaso (1911) and *Bac. sporogenes-parvus* of Choukevitch (1913) are probably also closely allied to, or identical with, this organism but the meagre descriptions of the authors referred to do not permit identification to be made with certainty.

The adoption of the name *Bac. putrificus* for organisms of the *B<sub>4a</sub>* type will necessitate the renaming of *Clostridium putrificum*. In 1919 the Medical Research Committee described an anaerobic bacillus which they named *Bac. cochlearius* Douglas, Fleming and Colebrook. As already indicated, original strains of this organism have been identified with *Clostridium putrificum* by a number of workers (Hall (1922); Fildes (1929); Cunningham (1931)). There appears, therefore, to be justification for using the name *Bac. cochlearius* for organisms of this type. The Medical Research Committee's description, however, requires to be modified in at least two particulars. It contains the statements that the organism does not liquefy gelatin and that it has no action on milk. The majority of strains (including original strains of *Bac. cochlearius*) however, liquefy gelatin slowly, and some are also capable of producing slow peptonisation of milk. The Medical Research Committee's description should, therefore, be modified to include strains which produce slow liquefaction and peptonisation. In general, organisms which produce slow liquefaction of gelatin and peptonisation of milk have more in common with those which are without action on these media than with cultures which bring about rapid liquefaction and peptonisation. They should, therefore, be classed with the former.



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## SUMMARY

An anaerobic sporing bacillus is described, the characteristics of which correspond more closely with Bienstock's final description of *Bac. putrificus* than those of any other organism hitherto described. It is proposed that the name *Bac. putrificus* Bienstock should be reserved for this organism.

The adoption of this name for the organism in question will involve the renaming of *Clostridium putrificum* Reddish and Rettger. It is suggested that the name *Bac. cochlearius* Douglas, Fleming and Colebrook should be used when reference is made to this bacillus.

## REFERENCES

- ACHALME, P. 1902 *Ann. de l'Inst. Past.*, **16**, 641-662.  
BIENSTOCK 1884 *Zeitschr. f. klin. Med.*, **8**, 1, quoted by REDDISH AND RETTGER, 1922.  
BIENSTOCK 1899 *Arch. f. Hyg.*, **36**, 335-389.  
BIENSTOCK 1901 *Arch. f. Hyg.*, **39**, 390-427.  
BIENSTOCK 1906 *Ann. de l'Inst. Past.*, **20**, 407-415.  
CHOUKEVITCH, J. 1913 *Ann. de l'Inst. Past.*, **27**, 307-321.  
CUNNINGHAM, A. 1931 *Zentralbl. f. Bakt.*, II Abt., **83**, 1-21.  
DISTASO, A. 1911 *Zentralbl. f. Bakt.*, I Abt., Orig., **59**, 97-103.  
FILDES, P. 1929 *MEDICAL RESEARCH COUNCIL'S, A System of Bacteriology*, **3**, 365-367.  
HALL, I. C. 1922 *Jour. Infect. Dis.*, **30**, 445-504.  
HEMPL, HILDA 1918 *Jour. Hyg.*, **17**, 13-19.  
KAHN, M. C. 1922 *Jour. Med. Res.*, **43**, 155-206.  
KENDALL, A. I., DAY, A. A., AND WALKER, A. W. 1922. *Jour. Infect. Dis.*, **30**, 204-210.  
MEDICAL RESEARCH COMMITTEE 1919 *Special Report Ser. No. 39*, pp. 182. London (H. M. Stat. Office).  
REDDISH, G. F., AND RETTGER, L. F. 1922 *Jour. Bact.*, **7**, 505-510.  
REDDISH, G. F., AND RETTGER, L. F. 1923 *Jour. Bact.*, **8**, 375-386.  
REDDISH, G. F. 1924 *Jour. Bact.*, **9**, 321-326.  
STURGES, W. S., AND RETTGER, L. F. 1919 *Jour. Bact.*, **4**, 171-175.  
WÜRCKER, K. 1909 *Sitzungsber d. physik.-med. Soz. in Erlangen*, **41**, 209-257.  
ZEISSLER, J. 1928 *KOLTE, KRAUS U. UHLENHUTH'S Handbuch der pathogenen Mikroorganismen*, Jena, **4**, 1097-1212.

## PLATE 1

FIGS. 1 AND 2. *Bacillus putrificus*. Beef-infusion agar. Two days at 37°C. Rods and endospores. × 1000.

FIG. 3. *Clostridium putrificum*. Beef-infusion agar. Nine days at 37°C. Rods and endospores. × 1000.

FIG. 4. *Bacillus sporogenes*. Ordinary agar. Two days at 37°C. Rods and endospores. × 1100.



FIG. 1.

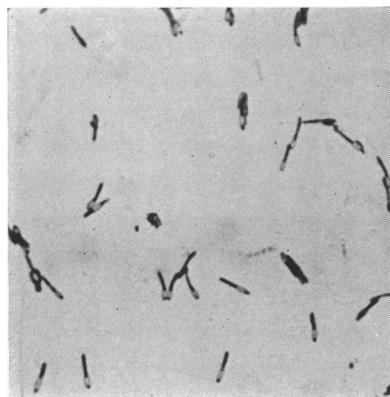


FIG. 2.

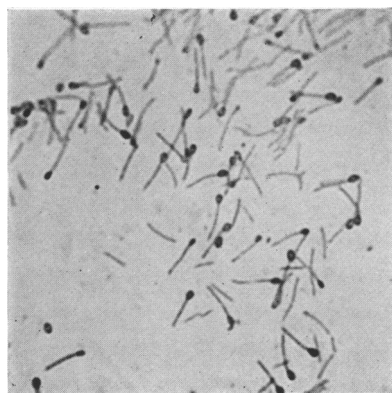


FIG. 3.

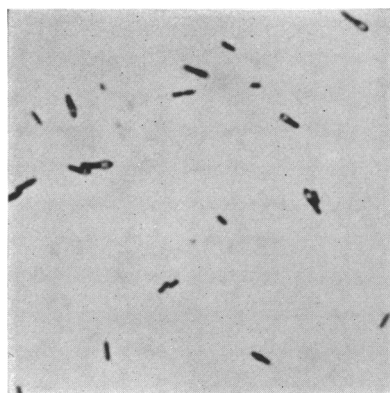


FIG. 4.

(Andrew Cunningham: Identity of *Bacillus putrificus* Bienstock)