CLOSTRIDIUM PUTRIFICUM

III. A COMPARISON OF STRAINS OBTAINED FROM COLLECTIONS IN THIS COUNTRY AND ABROAD

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There is still a certain amount of uncertainty in the minds of many bacteriologists concerning the identity of *C. putrificum*. In the early work of Bienstock, impure cultures were used, but by 1906 he was working with pure strains and his descriptions at this time are those of his type strain. These descriptions appearing in the Annales de l'Institut Pasteur are the final criteria for our guidance in this work. In one specific character Bienstock is emphatic; namely, the non-fermentative property of *C. putrificum* on any sugar. Since practically all other anaerobes ferment glucose, the inability of *C. putrificum* to form gas from this sugar is one of its most outstanding features. However, in the face of these definite records of the specific characteristics of this peculiar anaerobe, many workers in this country and abroad have described cultures which differ in very many respects from Bienstock's type strain.

In two recent papers on this anaerobe (Reddish and Rettger, 1922, 1923) a detailed study of its more important distinguishing features was made, emphasizing the morphology, the cultural characteristics and three phases of its biochemical properties. An attempt was then made to establish the organism as a distinct species, and to compare our own stock strains, isolated by Sturges, with those of Bienstock as described in 1906. The results were sufficiently constant and conclusive. Hall (1922), also, in his recent classification of the sporulating anaerobes, gives to *C. putrificum* its proper place in the round, terminal spore group

which digest albumin and brain slowly, and which do not ferment glucose. This places *C. putrificum* in the class intended by Bienstock when he suggested a separate group in which to include this species. In his study, the only difference he makes between *C. putrificum* and *C. tetani* is in the pathogenicity of the latter. This is in marked contrast to the descriptions given by the British Medical Research Committee (1917), which places this organism in the group characterized as having subterminal spores, as being actively proteolytic and also fermenting several of the common sugars with gas production. Later (1919), this Committee refused to recognize the existence of such a species, and claimed it to be a mixture of *C. sporogenes* and *C. cochlearum* or *C. tertium*. It is consistent with good practice to uphold the type strain of any organism as given by the discoverer, provided it is shown to be in pure culture when described. Bienstock's 1906 paper gives him this priority.

In the present investigation, the object has been to determine in just what respects the various strains of *C. putrificum* from representative collections differ and in what respects they resemble the strain described by its discoverer. At the same time, it was hoped to bring out some definite criteria on which to base identification. If we are to accept Bienstock's organism, those strains not complying with his descriptions must be discarded, or at least given another name.

The following strains were studied:

1. *C. putrificum* Hygienic Laboratory from Hall No. 22.
2. *C. putrificum* Hall No. 38.
3. *C. putrificum* (Sturges).
4. *C. putrificum* Pasteur Institute. Strain "B". No history.
5. *C. putrificum* Pasteur Institute Strain "M". No history.
6. *C. putrificum* Pasteur Institute Strain "G". No history.
7. *C. putrificum* Kral Collection. Strain E. Primban (Plant).
8. *C. putrificum* Kral Collection. Strain Car. verrucosus (navel of child).
9. *C. putrificum* Kral Collection. Strain No. 3—Bienstock Zeisler, Berlin.

Kendall's cultures had been disposed of, and were not available for the present study. I was unable, also, to obtain cultures from the Lister Institute, because they do not consider the organism to be a distinct species.

After preliminary study, I was convinced that the cultures received from Hall, the Hygienic Laboratory, and our own stock strains were all similar, and they did, in fact, come from the same original cultures of Sturges. Therefore, I included but two strains, one of our own and one from the Hygienic Laboratory. A stock strain of *C. sporogenes* (obtained originally from the Army Medical School) was included in the series for control. For convenience, the characteristics of these eight strains will be considered collectively.

In many outstanding respects, the strains fall into two distinct groups: (1) the round, terminal spore group, which digests protein slowly and does not form gas from any sugar; and (2) the oval, subterminal group, which breaks down the various kinds of protein readily and ferments various sugars with gas and acid formation. We shall consider these groups as nos. I and II, respectively.

MORPHOLOGY

Group I is characterized by having round, strictly terminal spores on the end of long, slender rods, which in old cultures form filaments. The Pasteur Strain "B" and our own stock strains belong to this group; the other two Pasteur strains and all three of the Kral strains fall into the second group, having oval subterminal spores. The vegetative rods of those in group II are about the same size as the rods of *C. sporogenes*, and do not form filaments in old cultures. It is very apparent here that we are dealing with two distinct groups of organisms, one with round terminal spores and the other with oval subterminal spores.

STAINING

All strains are Gram-positive and stain well with ordinary dyes.

MOTILITY

All strains are motile.

COLONY FORM

The strains in group II were found to have sporogenes-like colonies, both on the surface and in deep shake agar tubes. The Pasteur strain "B" grows readily in the depths of shake agar cultures and the colonies grow up to within half an inch of the surface of the medium. They are woolly and have no outstanding distinguishing features. Surface colonies of Pasteur strain "B" are delicate and somewhat similar to those of *C. putrificum* (Sturges) (see Reddish and Rettger, 1922, 1923). There is little difference between the strains as to the depth colonies.

CULTURAL CHARACTERS

Egg-meat. The organisms in group I grow slowly in egg-meat medium, showing very little change within ten days. After this time, there is digestion of the meat and putrefactive odors develop. The Pasteur strain "B" resembles very closely our stock strains in this respect. The organisms in group II attack meat medium very readily, showing gas formation within twenty-four hours, a turbid supernatant liquid and a slight odor; after thirty-six hours, definite digestion of the meat takes place with the evolution of distinct putrefactive odors. In this respect, they resemble *C. sporogenes*, and were equally as active as the control *C. sporogenes* culture in simultaneous growths.

Milk. The group II strains digest milk readily under solid paraffin, showing definite signs of growth within 36 hours. The action takes place quite as rapidly as does that of the *C. sporogenes* control. The organisms in group I attack milk very slowly and show definite digestion only after two weeks. This difference in types is again very marked in ordinary culture media.

Broth. There is abundant growth of all strains in twenty-four hours, excepting the Sturges strains, in both plain and glucose broth.

Gelatin. All strains liquified gelatin, the Pasteur "B" and our own doing so more slowing than the oval sporing group.

BIOCHEMICAL REACTIONS

Fermentation reactions. Briefly, the fermentation reactions of these strains are as follows: the organisms in group I do not form gas from any of the 18 test substances used, when tested by the shake agar method. The strains in group II ferment with gas production all the sugars that *C. sporogenes* ferments. This is one of the most significant features of the present study. It shows in another way, that we have as stock strains in some of the most reliable collections in the world two very different groups of organisms all of which are supposed to be true to the type strain of Bienstock. The Sturges strains and the "B" strain from the Pasteur Institute coincide with the description given by Bienstock in this respect also.

Glucose-consuming power. Here again a marked difference between the two groups is apparent. The Sturges strain reduces glucose 0.07 per cent in four days, the Pasteur "B" culture 0.06 per cent in the same period, while the oval sporing group consume from 0.37 to 0.42 per cent in the same time under like conditions. *C. sporogenes* in this test consumed 0.49 per cent; the similarity to the above cultures in group II is striking.

Peptolytic property. The simple Sörensen test was used for determining the relative amounts of peptolytic action by these strains on plain broth. Readings were made after growth for four days at 37°C. The figures are not of much assistance in separating the groups. In fact the "B" strain from the Pasteur Institute gives a Sörensen figure only slightly lower than the members of the other group, while the Sturges strains showed a distinct delayed action on peptone and gave a much lower figure than the other cultures at the end of four days' incubation.

Pathogenicity. All strains were found to be non-pathogenic to guinea-pigs, rabbits, and white mice.

DISCUSSION

From these very simple comparisons, it is evident that we have two groups of anaerobes, all of which are labeled *C. putrificum.* It is inconsistent with good practice that this condi-

tion should continue, especially since there are on record in explicit terms the distinguishing features of the organism as given by its discoverer, and as clearly substantiated by Reddish and Rettger, with other detailed descriptions now on record. We must of necessity accept the organisms included here in Group I as *C. putrificum*, and discard those in group II. The members of the first group are the only ones resembling the original strain.

REFERENCES

HALL, I. C. 1922 Differentiation and identification of the sporulating anaerobes. Jour. Inf. Dis., **30**, 445–504.

Medical Research Committee 1917 National Health Insurance. Special Report Series 12. The classification and study of the anaerobic bacteria of war wounds. London, pp. 74.

Medical Research Committee 1919 National Health Insurance. Special Report Series 39. Reports of the committee upon anaerobic bacteria and infection. London, pp. 182.

REDDISH, G. F., AND RETTGER, L. F. 1922 *Clostridium putrificum* (*B. putrificus* Bienstock), a distinct species. Jour. Bact., **7**, 505–510.

REDDISH, G. F., AND RETTGER, L. F. 1922 *Clostridium putrificum*. II. A morphological, cultural and biochemical study. Jour. Bact., **8**, 375–386.