

substance in the culture broth appeared, in some cases, as early as 4 hours after incubation was begun. The titer of the lytic substance, as indicated by the amount of culture broth required to produce an appreciable amount of hemolysis of 1 cc. of 1% washed sheep cells, was usually greatest after from 16 to 24 hours' incubation.

Culture broth of a group-IV pneumococcus strain, comparatively avirulent in its present form but capable of having its virulence raised by animal passage, and of a completely avirulent, atypical pneumococcus strain, No. 189, isolated from a horse which died during immunization with the standard type-I strain, No. 5, also contained the hemolytic substance.

The hemolytic substance in the culture broth, like that of Cole, is thermolabile and is destroyed by heating for 30 minutes at 55° C. Its activity is diminished by standing at cold room temperature and also, in a much shorter time, at 37° C.

Red cells, stroma, and leucocytes absorb the substance from the culture broth. The substance is also absorbed from the culture broth by shaking with animal charcoal and alumina. Shaking alone, however, greatly reduced the lytic power of the culture broth.

Normal horse serum has an inhibitory effect on the hemolytic power of the substance, while pneumococcus immune serum has a much more marked effect, which is apparently species rather than type specific.

### 3993

#### **Comparative Study of Certain Aciduric Organisms of Intestinal and Dental Origin.**

T. MORISHITA. (Introduced by L. F. Rettger.)

*From the Laboratory of General Bacteriology, Yale University.*

Therapeutic studies of certain intestinal disorders have increasingly directed attention toward a bacterial rôle played in the intestinal tract by naturally inhabiting types of organisms. This field has been advanced particularly through the work of Rettger and Cheplin<sup>1</sup> and the administration of *B. acidophilus* milk. These observations have in the main been supported.

Unfortunately this intestinal organism, *B. acidophilus*, has recently been confused with somewhat similar organisms which are associated with and may bear some relation to dental caries. The

---

<sup>1</sup> Rettger, L. F., and Cheplin, H. A., *Arch. Int. Med.*, 1922, xxix, 357.

confusion has been increased not a little by the recent publications of Bunting and his co-workers<sup>2</sup> on a high acid-producing and aciduric organism in dental caries. They called this bacterium "*B. acidophilus*" without having apparently made any comparative studies of it and established strains of *B. acidophilus*. McIntosh and his co-workers,<sup>3</sup> and Rodriguez,<sup>4</sup> had previously reported the presence of an acid producing organism in dental caries which is seemingly identical with that of Bunting; they applied the name "*B. acidophilus odontolyticus*". More recently investigators have reported organisms associated with dental caries under the name of *B. acidophilus*, as Bunting had done.

The determination of the relationship of the intestinal and the dental organisms appeared to the writer to be of extreme importance. This paper\* presents a certain phase of the experimental work which has been conducted on more than 100 strains of acid-forming organisms isolated from dental caries, together with some known strains of *B. acidophilus* of intestinal origin. The results may be summarized briefly as follows:

(1) The occurrence of *B. acidophilus* of the intestinal type in dental caries is relatively rare compared with the other aciduric organisms found, and may be regarded as merely adventitious.

(2) A medium containing Klim-digest-casein and 1% glucose proved most satisfactory in this study.

(3) Morphologically the common aciduric tooth organisms appear as short, small rods occurring in chains, as compared with the larger and longer rods of *B. acidophilus* which under the given conditions tended to grow in long chains or threads.

(4) Colony formation of the 2 types is markedly different. The teeth organisms form solid, smooth, rather large colonies, while *B. acidophilus* has a tendency to produce the fuzzy or X type colonies, though at times the so-called Y type is quite apparent. The stroke plate method and the plating of the organisms in low dilutions will as a rule also show pronounced differences. Furthermore, the tooth organisms produce a general turbidity in broth culture, while with *B. acidophilus* the supernatant broth usually appears clear, along with distinct sedimentation in the bottom of the tube.

<sup>2</sup> Bunting, R. W., and Palmell, *J. Am. Dent. Assn.*, 1925, xii, 381.

<sup>3</sup> McIntosh, J., James, W. W., and Lazarus, Barlow, *Brit. J. Exp. Path.*, 1922, iii, 138.

\* Only 1 of the 5 groups, the one most frequently confused with *B. acidophilus*, is considered.

<sup>4</sup> Rodriguez, F. E., *Military Dent. J.*, 1922, v, 199.

(5) In sugar broth there is much greater acid production by the tooth organisms than by *B. acidophilus*, the former attaining a final pH of 3.8-3.0, and the other 4.4-3.8.

(6) In the determination of limiting pH for the growth of the 2 types, the tooth organisms grew in a medium having a far greater range of pH (both acid and alkaline), than did *B. acidophilus*.

(7) In the study of the reduction of methylene blue in milk, except in a few instances, the tooth organisms reduced the indicator before coagulation of milk took place. On the other hand *B. acidophilus* first produced a milk coagulation and then brought about reduction gradually in the depth of the tubes.

(8) The tooth organisms were able to grow at temperatures as low as 12-14° C., while *B. acidophilus* failed to do so even at 18° C. This suggests, perhaps, one of the most important means of separating these 2 types.

(9) The ability or inability of the 2 types of organisms to ferment certain sugars, to coagulate milk, and to grow at low temperatures was studied. Twenty-two different sugars were used.

Both of the representative tooth strains belonging to this group coagulated milk, and produced acid from mannose, trehalose, melzitose, mannit, sorbitol and salicin. They did not attack raffinose. Sucrose was fermented by some strains, but by relatively few of the non-raffinose fermenters, compared with other groups of tooth organisms. On the other hand, *B. acidophilus* fermented raffinose and sucrose, but did not attack mannite and sorbitol.

(10) Agglutination experiments also showed differences. There was no cross agglutination between the 2 groups in dilutions of immune serum higher than 1:100, while both types were agglutinated by their homologous sera.

*Conclusion:* The present comparative study seems to the writer to warrant the conclusion that the tooth organisms discussed here constitute a type entirely separate from *B. acidophilus* of the intestinal tract.