LACTIC ACID BACILLI—THE RELATIVE ADVANTAGES OF COMMERCIAL LIQUID AND SOLID PREPARATIONS, THEIR MODE OF ADMINISTRATION, AND THEIR EFFECT ON CERTAIN PATHOGENIC AND PUTREFACTIVE ORGANISMS.

PRELIMINARY PAPER.

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COMMERCIAL LIQUID PREPARATIONS.

We examined preparations sent to us by Messrs. Oppenheimer and Son, London, and Messrs. Millet et Veillard, Paris. The former is retailed as a semi-fluid "clot of milk," in a sterile glass vial; the latter in the form of a malt extract. Both of these preparations contain a bacillus in pure culture, whose morphological and cultural characters are identical with those of the Bulgarian bacillus of Grigoroff, described by Massol and Cohendy: It is an immobile rod, varying in length from 2 μ to 20μ, somewhat similar in appearance to the anthrax bacillus, but differing from it in being non-sporing. In cultures it grows in pairs or in chains. The bacillus retains the stain by Gram's method in preparations prepared from living cultures, but it fails to do so in cultures which have been desiccated or heated to 65°C. It does not liquify gelatine, and grows but feebly on ordinary laboratory media at 37°C, or at room temperature on any media. It grows abundantly both aerobically and anaerobically in slightly acid whey, sterilised milk, and glucose media at 37°C, but ceases to grow when the acidity of the media exceeds 2 per cent. It ferments lactose, glucose, and maltose readily at 37°C. Its action on sucrose, mannite, and dulcite is less marked, and only appears after several days' incubation. The acid-producing qualities are much greater than those of other lactose fermenters found in solid lactic acid bacilli preparations. The difference is marked after twenty-four hours' incubation at 37°C. No contaminations are found in these preparations.

COMMERCIAL SOLID PREPARATIONS.

We received from Messrs. Allen and Hanbury a sample of their tablets of sauerin; from Messrs. Parke, Davis and Co., lactowe
F. G. Bushnell and G. Dansey-Browning

tables; from W. Martindale, trilactine tablets; from the Anglo-American Pharmaceutical Company, fermenlactyl tablets; from the Société le Ferment of Paris, lactobacilline tablets; and from the Laboratoire de Biologie, Paris, a preparation of lacteol.

At first sight, these tablets appeared to be most advantageous, on account of their portability and their moderate price. Subsequent examination, however, showed that in every case contaminating organisms were present. In most of the preparations examined we noted the presence of an impure culture of Bulgarian lactic acid bacilli, some of which failed to stain by Gram, or to reproduce themselves in subcultures, and were regarded by us as having been killed by desiccation. The subcultures contained at first living Bulgarian bacilli, but in most cases these were quickly overgrown by other Gram-staining and non-Gram-staining organisms. The type of organism which predominated in subcultures on sterilised milk, and on Coheney's whey medium, was a sporing Gram-staining rod. It appeared to be identical with a form of butyric acid bacillus.

The presence of these contaminations was not accidental, as they were found in every subculture made on milk sterilised by heat for one hour on five successive days. We noticed, in addition, varying numbers of micro-organisms, some of which fermented lactose and clotted milk, and others which failed to do so. Their presence appeared to inhibit the growth of the Bulgarian bacillus. Amongst these we noticed most constantly a streptococcus, a diplococcus, and a bacillus similar to Bacillus acidi lactici of Hueppe.

The Importance of the Contaminating Organisms.

There are no experimental data to prove that any of the contaminating organisms referred to above are of pathogenic importance, and it is probable that in some cases some of the lactose fermenters found may have been purposely introduced. Apart from any remote possibility of pathogenicity, the presence of these contaminating organisms appears to us to be undesirable. Whether they ferment or fail to ferment lactose, they have undoubtedly an inhibitory effect on the growth of the partially desiccated Bulgarian bacillus. In our opinion, the portability of the tablets does not compensate for their cultural impurities. The fact that they all clotted milk in twenty-four hours is obviously no special recommendation, as virulent cultivations of B. coli communis would do so equally.
Methods of Administering Lactic Acid Bacilli.

In this country at the present time lactic acid bacilli are generally administered either in the form of curdled milk, or as tablets directly by the mouth.

The British Medical Journal of November 28th contains a well-timed warning from Dr. R. W. Allen as to the dangers of incubating possibly contaminated milk, even in the presence of living Bulgarian lactic acid bacilli. It would appear to us that this danger is much greater when the milk is incubated in the presence of non-vigorous growths of this bacillus.

We have shown that in solid preparations the bacillus is in a low state of vitality, and is frequently overgrown by contaminating organisms. The direct method of administering tablets is, therefore, useless.

The objections to incubated milk apply equally to Cohendy's sweetened whey. Herschell's malt extract solution seems unobjectionable, and is easily prepared. As milk diet is often indicated in cases in which the administration of lactic acid bacilli is advisable, we suggest the following solution of this difficulty: — Incubate the liquid preparation of living bacilli and then add it to boiled, but non-incubated milk or other sterile suitable vehicle which has been cooled to a temperature of 37° C. If an incubator be not available the liquid culture may be incubated unopened in a Thermos flask filled with water heated to 37° C. Malt extract solution or sweetened whey may be used instead of the milk if so desired. It is quite unnecessary to allow the latter to become clotted before administration.

Summary.

(1) The majority of both liquid and solid preparations contain true lactic acid bacilli, but these are only found in a state of pure culture in the liquid preparations.

(2) The liquid preparations, if retailed in smaller bulk and in hermetically-sealed glass capsules, would constitute the safest and most convenient method of administering pure lactic acid bacilli.

An ideal preparation has yet to be placed on the market.

(3) It is unnecessary to incubate the vehicle used for the administration of the lactic acid bacillus. It is sufficient to incubate the preparation itself, provided it be in the form of a fresh liquid culture, and to add this incubated preparation to the vehicle selected. By so doing, the danger of increasing the contamination of impure milk by incubation is practically avoided. In all cases,
however, milk, if selected as a vehicle, should previously be boiled.

We are indebted to Dr. Cohendy, Dr. R. Weiss, and others, for suggestions which have helped us in working out some of these problems, and also to the manufacturers of different proprietary preparations referred to in this paper, for their courtesy in placing at our disposal samples of their products.

In a later paper we hope to deal with some of the effects of the products of Bulgarian bacilli on the growth of pathogenic and putrefactive organisms.

**APPENDICES.**

I.—Method of Preparing Cohendy's Special Media.¹

(a) **Gelatine Litmus Whey** (modified from Cohendy's formula).—Prepare whey from 1 litre of milk by boiling it for five minutes in the presence of 1·5 cc. of hydrochloric acid, or glacial acetic acid. Strain off the casein, and neutralise to litmus. Add gelatine, 3 grammes; grape-sugar, 15 grammes; peptone, 1·5 grammes. Make it up to 1,000 cc. with distilled water. Sterilise for one hour in steamer, filter, transfer it to tubes, and re-sterilise by the intermittent method for five days. When required for use, add 1·5 cc. of sterile litmus solution to each 10 cc. of the medium. This medium will remain semi-fluid at the room temperature, and may be used for cultures in the cool or hot incubator. When flavoured with vanilla, it is used as a vehicle for administering the lactic acid bacilli.

(b) **Agar Litmus Whey** is prepared in the same manner, but 15 grammes of agar are substituted for the gelatine.

II.—**Herschell's Malt Extract Solution.²**

Take a tablespoonful of malt extract and add it to a pint of water, boil for a few minutes, allow it to stand, and pour off from it any sediment which may form. The medium is then cooled to 40° C., and is ready for the reception of the bacillary preparation.

¹ M. Cohendy, *Comptes rendus de la Société de Biologie*, 1906, vol. i.