

a marked disturbance in bone and tooth metabolism caused by chronic fluorine poisoning. No doubt it represents on the part of the animal body an effort to maintain and deposit normal skeletal structures. There seems to be a marked stimulation to elaborate this enzyme in the case of chronic fluorine poisoning similar to that found in such bone diseases as rickets and osteomalacia.

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# MICRO MOTION PICTURES OF *B. SHIGAE* GROWING UNDER CONDITIONS FAVOR- ING FILTERABILITY AND LIFE CYCLE FORMS

Two of the most important problems in present-day bacteriology deal with the reality of bacterial life cycles and the existence of a minute filter-passing form of bacterial life. With a simple and inexpensive micro motion picture apparatus developed primarily for following the action of X- and ultra-violet rays on bacteria, yeasts and molds we have accumulated records of the growth of the dysentery bacillus (*B. shigae*) which bear directly on both questions.

These records, on 6,000 feet of film and covering a period of 600 hours, picture the growth of *B. shigae* in microculture during and after cultivation in ordinary beef infusion media, in media consisting exclusively of either peptones or proteins (gelatine or K media) and in media containing varying quantities of lithium chloride. They show the formation and the subsequent fate of the various abnormally shaped cells which have previously been described as typical stages in a bacterial life cycle. Hadley<sup>1</sup> has filtered LiCl-treated Shiga cultures and Kendall<sup>2</sup> has stated that the use of his K medium gives filterable stages to the related *B. typhosus*. Many photographs have been made of "filterable" cultures obtained in these ways.

Shiga bacilli when rapidly multiplying in ordinary beef infusion media are rods about  $5\mu \times 1\mu$ , but by aging these cultures or by changing their food substances it is easy to obtain all the supposed life cycle forms. Real branching occurs early in many special media; in old cultures growth practically always takes place from long filamentous cells. Except for the micrococci to be mentioned later all the other life cycle forms appear merely as stages in the adaptation of the bacillus to new and somewhat unfavorable

media. When in an environment which permits of their growth and multiplication the progeny of these organisms always tend to become more and more normal in shape and size. None of them has shown any special function analogous to the more elaborate reproductive processes of the molds.

The micrococci split from certain bacilli constitute the only type of *B. shigae* growing under the conditions of these experiments which the pictures may not have completely described. These spheres, having a diameter about equal to that of the cells from which they arise, are present in nearly all cultures but are most numerous after several LiCl passages. Many appear in the photographs and some have been followed for long periods of time. No growth has ever been seen and it must be concluded that if they are alive and not mere bits of coagulated protoplasm eliminated by injured organisms, their lag period is long.

In discussing filterability through porcelain filters, a distinction may be drawn between quick and slow outgrowths, or reversions, in the filtrates. By quick reversions are meant those which show massive growth after 48-72 hours; in the slow reversions a period of weeks is required before many bacteria can be found. All efforts to get slow reversions in microculture have thus far failed. Many filtrates have been incubated and examined for periods of two and three weeks but in none of them have we been able to see or photograph anything which could properly be interpreted as alive.

More positive information has been gained concerning the quick reversions. Growth of Shiga in the K medium of Kendall<sup>3</sup> results in a large number of small, almost coccoid, cells. Similar dwarfed bacteria are present in LiCl cultures during those passages when they are supposedly filterable. Whenever a quick reversion was observed after filtration through a candle tested by the usual methods, it was always possible to find a few such stunted organisms in the fresh filtrate. After a more or less prolonged lag period they always grew into and multiplied as normal Shiga bacilli. This fact combined with the recent observation<sup>4</sup> that K medium itself aids the passage of normal bacilli through a Berkefeld filter seems to offer an adequate explanation of the quick reversions.

The details of these experiments will appear in the *Journal of Experimental Medicine*.

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<sup>1</sup> P. Hadley, E. Delves and J. Klimek, *Jour. Infect. Dis.*, 48: 1, 1931.

<sup>2</sup> A. I. Kendall, *SCIENCE*, 74: 129, 1931; 75: 295, 1932.

<sup>3</sup> A. I. Kendall, *Northwestern Univ. Bull.*, 32: 8, 1931.

<sup>4</sup> P. L. Varney and J. Bronfenbrenner, *Proc. Soc. Exp. Biol. Med.*, 29: 804, 1932.