

growth whatever will ever occur on the gentian violet side of the plate. The organism will, indeed, refuse to grow up to the dividing line between plain and gentian violet agar, ceasing sharply at a point about $\frac{1}{2}$ to 1 cm. from this line.

If, however, the gentian violet half of the plate be repeatedly re-inoculated with thick smears, a moderate growth—in some instances a fairly vigorous growth—occurs. This is not due to acquisition by the bacteria of resistance to the dye, for if the organisms, which have thus grown, be transplanted to gentian violet agar they will not grow in the presence of the dye. Nor is it due to insufficiency of dye, for in the plates used in the experiments dye was used in strengths greatly in excess (1–100,000) of the strength necessary to prevent the growth of *B. subtilis* (1–1,000,000).

The explanation of the phenomenon is not at present perfectly clear, but there is some reason for believing that it may be due to a property of bacteria—not hitherto recognized—of communal action. A few studies of single stained bacterial cells, made by the method of Barbour, lend support to this explanation.

II (1593)

The selective action of gentian violet in relation to chemotherapy.

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It is clear from the facts stated in the four previous communications that selective bacteriostasis is a complex process. The method of divided plates presents a perfect machinery for studying the workings of this process; and in the selective property of gentian violet we have a means of observing the various elements of the process under perfect control on a single plate. On such a plate we will find that

- (a) Thick suspension of the Gram positive *B. subtilis* will not grow in the presence of the dye.
- (b) By repeated re-inoculations of this organism a moderate growth can be procured on the gentian violet agar.

- (c) Thick suspension of the Gram negative *B. coli* grow equally well on the two halves of the plate.
- (d) If very weak dilutions of suspension of the Gram negative *B. coli* be stroked across a divided plate a few colonies appear on the plain agar, none at all on the gentian violet agar.
- (e) From a suspension of the Gram negative *B. coli*, a gentian negative and a gentian positive strain can be cultivated.
- (f) A thick suspension of the gentian negative strain of *B. coli* will grow equally well on the plain agar and on the gentian violet agar; if a weak dilution of the suspension be used a few colonies will appear on the plain agar, none at all on the gentian violet agar.
- (g) If a thick suspension of the gentian positive strain of *B. coli* be stroked across the plate no growth whatever occurs on the gentian agar.

These observations indicate a number of the pitfalls which beset those who attempt to transfer laboratory observations into therapeutics. No conclusion as to the probable effect of a selective therapeutic agent is justified unless the experiments on which this conclusion is based have taken into consideration the quantities of bacteria used.

12 (1594)

The communal activity of bacteria.

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It has been shown above that, while *B. coli* like—most of the gram negative organisms—is apparently uninfluenced in growth by gentian violet, a careful study of thick suspension will demonstrate the presence of many individuals which *are* susceptible to the dye.

The isolation of a strain of *B. coli* entirely fast to gentian violet—that is to say, containing no individuals susceptible to the bacteriostatic effects of the dye—has made it possible to study quantitatively the reaction between this bacteriostatic agent