9 (1591)

Relation of the gentian violet reaction to dilution of implanted suspension.

By JOHN W. CHURCHMAN.

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It has been stated above that if increasingly weak dilutions of suspension of a Gram negative organism (*B. coli*) be stroked on a divided gentian violet plate relatively few colonies appear on the gentian violet side of the plate and, when very weak dilutions are used, none at all. It has been shown that this is, in part, due to the fact that in a suspension of a Gram negative organism by no means all, indeed, only a small proportion of the organisms may be gentian negative.

This can hardly, however, be the whole explanation. For if the experiment be repeated, using an emulsion of organisms which have already grown in the presence of gentian violet and have thus proven their resistance to the dye, a similar quantitative phenomenon is observed; far fewer colonies appear on the gentian violet agar than on the plain agar when weak dilutions of the suspension are stroked across the plate. The explanation of this fact is not clear; it may be due to some sort of communal property which enables bacteria, instead of pursuing individual careers, to aid each other in their growth and thus to accomplish in large groups what they cannot accomplish singly.

10 (1592)

The effect of repeated re-inoculations of gentian violet agar with gentian positive organisms.

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If a divided gentian violet plate be stroked with a thick suspension of the Gram positive and gentian positive *B. subtilis* no

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 $^1/_2$ 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.