COMMUNAL ACTIVITY OF BACTERIA.*

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PLATES 78 TO 80.

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The isolation of a strain of *Bacillus coli* fast to gentian violet, that is one containing no individuals susceptible to the bacteriostatic properties of the dye,[1] has made it possible to study quantitatively the reaction between this bacteriostatic agent and bacteria, without encountering the disturbing factor usually met in such studies which results from the variability in susceptibility of individual organisms to the chemical substance under examination. This strain —Strain X—had been isolated from a single colony growing on gentian violet agar, and had been kept growing, by frequent transplants, on media containing the dye, over a period of several weeks.[2] The ability of every individual to grow in the presence of the dye had therefore been proved.

With this strain a large number of single cell and small group transplants have been made in order to see whether any difference could be observed between the behavior of isolated individual organisms and that of very small aggregations of the same organisms. The experiments showed that a marked difference exists.

The technique used was that of Barber.[3] Only motile organisms were transplanted. The transplants were always made with young broth cultures 2 to 4 hours old, but it was found equally important to use for the stock cultures, from which the broth cultures for study were made, fresh agar transplants not more than 18 hours old; when

* A preliminary report of these observations was published in the *Proceedings of the Society of Experimental Biology and Medicine* (Churchman, J. W., *Proc. Soc. Exp. Biol. and Med.*, 1920, xviii, 22).

[1] Churchman, J. W., *J. Exp. Med.*, 1921, xxxiii, 569.

[2] See Figs. 5 and 6, of the preceding paper.[1]

[3] Barber, M. A., *Philippine J. Sc.*, 1914, ix, 307.

older agar transplants were used there were delay and inconstant results in the controls. We observed this type of lag, due to the age of the culture from which the subculture for the transplantation was made, independently, before knowing of Chesney's work.[4] To bear it in mind in single cell work is essential.

To determine the behavior of single cells in the presence of gentian violet, transplantations were made onto gentian violet agar and into gentian violet broth, and by way of comparison, similar transplants of small groups of cells were made into the same media.

Transplantation onto Gentian Violet Agar.

Although, as has been said, the organism with which the experiments were done was definitely gentian-negative and grew with apparently no inhibition when strokes of a heavy suspension were made onto agar containing the dye,[5] transplants of single cells almost never grew, although as high as 85 per cent positives were obtained in the controls. In the only two instances in the whole series of single cell transplants in which growth occurred, marked delay took place, a delay which was never observed in the controls. Moreover, transplants of small groups of organisms (five to fifteen) did not grow, though transplants of thirty individuals grew regularly (Figs. 1 to 5).

Transplantation into Gentian Violet Broth.

The experiments just cited made it seem likely that there was some fundamental difference between the behavior of a single cell and that of a small group of cells in the presence of gentian violet and this probability became a certainty as a result of the transplantations made into gentian violet broth in a dilution of 1:100,000, pH 7.2. The gentian negativeness of the organism used for this series of experiments was even more authentic than that of the one used in the agar experiments. It came from the colony marked *B* in Fig. 5. This was one of the only two colonies which ever appeared in our experiments after transplantation of a single cell onto gentian violet agar. Colony A on this plate was just visible at the end of 18 hours, though

[4] Chesney, A. M., *J. Exp. Med.*, 1916, xxiv, 387.

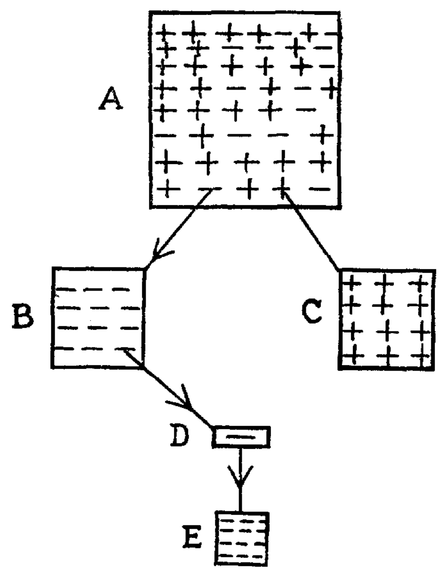
[5] See Fig. 5 of the preceding paper.[1]

the controls were well developed at this time (Fig. 3). At the site where Colony B subsequently developed nothing was at this time to be seen, even with a magnifying glass; Colony B appeared during the second 24 hours.

The organisms in Colony B provided material for a study of the effect of gentian violet on single cell transplants that was above criticism. Not only had it come from a pure gentian-negative strain and been kept growing for weeks on gentian violet media, but the organisms in Colony B were all the descendants of a single cell which had survived and reproduced in the presence of the dye. One could feel certain that every individual in this culture was gentian violet-negative; one did not have to consider the possibility that failure of growth in single cell transplants might be a matter of chance and due to the presence of individuals susceptible to the dye, which happened to be picked up. As a matter of fact, this possibility of a chance picking up of susceptible organisms could hardly be seriously considered as an explanation of the results, if the large number of transplants made is borne in mind.

The pedigree of Colony B is shown in Text-fig. 1. The results of the inoculation of broth with this strain which was called Strain Z showed beyond doubt that there is a fundamental difference between the behavior of one cell and that of a small group of cells toward gentian violet. 80 per cent positives were obtained in the controls in which single cells were inoculated into plain broth; and almost 100 per cent positives were obtained when thirty or more cells were planted in gentian violet broth. Yet when single cells were planted in gentian violet broth, or when very small groups (two to eight organisms) were seeded, growth did not occur (Figs. 6 and 7). On the basis of 140 successive single cell transplants into gentian violet broth without growth in any instance, the conclusion seemed justified that single cells would never grow under these conditions. In a final series, however, of eight inoculations delayed growth was obtained in one tube in the second 24 hours, an occurrence so rare that it seems justifiable to regard it as the occasional and unexplained exception met with in the study of almost every biological phenomenon. These negative results with single cells were striking when compared with the almost absolute constancy with which growth occurred when groups of thirty or more cells were inoculated.

The term communal activity of bacteria is used to express the facts just detailed. This term is open to the objection that bacterial interreactions are implied, for which there is at present no rigorous evidence. It might be that thirty cells succeed in growing, merely because thirty cells are able to produce some antidye substance in an amount sufficient to destroy the bacteriostatic effect of the dye, while one cell fails to grow because it is unable to do this. Without



TEXT-FIG. 1. Pedigree of Strain Z. *A*, original culture of *B. coli*, containing gentian-negative and gentian-positive individuals; *B*, pure culture of gentian-negative strain, isolated from *A; C*, pure culture of gentian-positive strain isolated from *A; D*, single cell from Strain B; *E*, colony resulting from planting Strain D on agar.

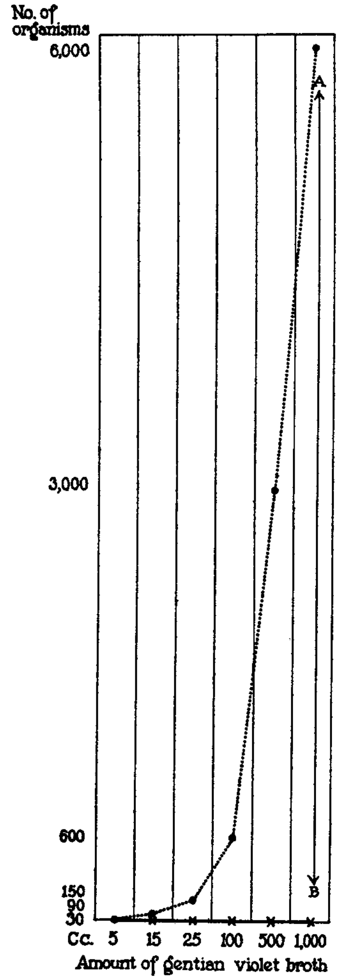
implying anything as to the underlying source of the phenomenon we use the term communal activity to describe it in order to indicate that in the contest between bacteria and bacteriostatic agents very small groups of organisms may be able to accomplish together what they could not accomplish if working singly. The significance of these facts for the study of chemotherapy and particularly for the study of the effect of bacteriostatic agents by the single cell method is clear. One cannot conclude from the behavior of a single cell in the presence of such a substance anything as to the behavior of a group of cells, even a very small group, under the same conditions.

It may perhaps be again emphasized that the results obtained in these experiments were clearly not due to individual variants, susceptible to gentian violet, which happened to be picked up in making the single cell transplants. Not only was the strain used one which had proved its insusceptibility to gentian violet by growing in the presence of this dye, but the failure of growth in 140 consecutive single cell transplants into gentian violet broth eliminates, by the law of probabilities, such an explanation.

It should be clear that the fact here established is of a different order from the well known relation between growth and gross size of inoculum. The presence of large numbers of dead organisms in the ordinary culture and of organisms which, though living, are susceptible to the slightly unfavorable conditions of the new media into which they are transplanted makes it more probable that growth will follow the inoculation of media with 500,000,000 organisms than if only 500 are used. But in the experiments described here only living, motile organisms were used, and only individuals from a strain which had proved its ability to grow in the presence of the dye and did so constantly when inoculated in groups of thirty or more. It seems probable that some factor not hitherto recognized must be found to account for the difference in behavior between one cell and thirty cells and that it is a factor other than that which accounts for the difference in behavior between 500 cells and 500,000,000.

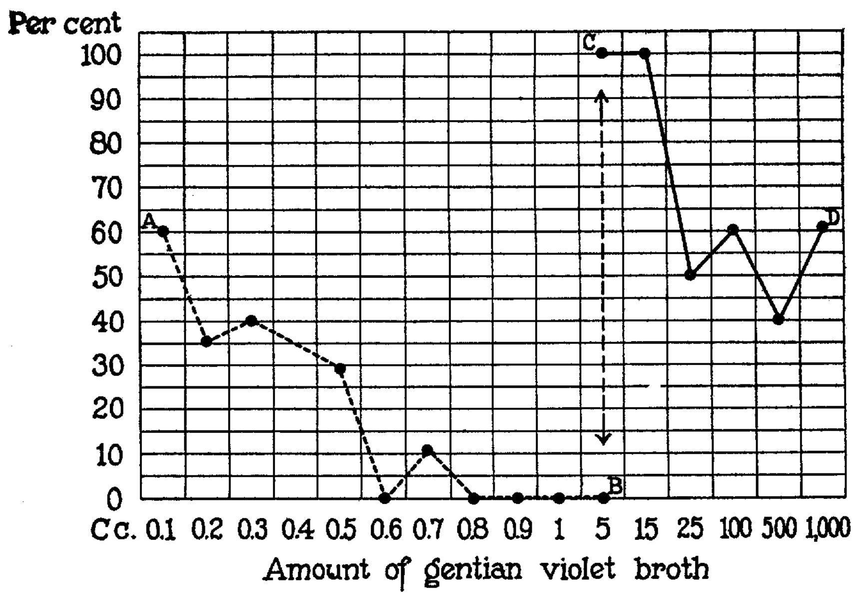
We have done a large number of experiments to determine whether the facts just detailed were to be explained simply by the relation of the number of transplanted organisms to the amount of available gentian violet; that is, whether the whole phenomenon is a purely quantitative one. Large inoculations of this gentian-negative strain grow in the presence of gentian violet without any apparent restraint; so, too, do inoculations of thirty cells; whereas single cells, under identical conditions, do not grow at all. This might be due to the fact that groups of cells, even small groups of thirty individuals, were able to make some change in the dye, gentian violet being assumed to offer a slightly unfavorable medium even for this gentian-negative strain, in spite of the absence of any apparent inhibition to the growth of groups of cells. Single cells might be unable to effect this change in dye in an amount sufficient to allow growth to take place. If this explanation were the correct one, it should be possible to demonstrate an approximate relation between the number of organisms and the amount of gentian violet broth in which a given group could grow. In the experiments thus far described the transplants were made into 5 cc. of 1: 100,000 gentian violet broth. Under these conditions one cell would not grow but thirty cells would. If it is assumed that this was because thirty cells could produce thirty times the amount of antidye substance produced by one cell, then by merely multiplying by thirty the amount of gentian violet broth into which the inoculations were made, that is by using for example 150 cc., the growth of thirty organisms should be prevented. As a matter of fact thirty organisms will grow, with a fair degree of constancy, not only in this amount of gentian violet broth, but in very much larger amounts. When inoculations of thirty organisms were made, for example, into a liter of gentian violet broth, growth occurred in 60 per cent of the flasks, though if the explanation of the phenomena described were purely quantitative it should be necessary to seed 6,000 organisms into this amount of gentian violet broth in order to obtain growth; that is, thirty organisms accomplish not thirty times what one organism accomplishes, but very much more than this. This discrepancy between the work actually accomplished by thirty organisms, and the work which they might be expected to accomplish on a purely quantitative basis is represented by the line *A–B* in Text-fig. 2.

It might be objected that bacteria ought not to be thought of as units exactly equal to one another in efficiency. A given group of thirty might be able to do only fifteen times the work of one very vigorous organism, while another group of thirty might be able to do 60 times the work of a relatively weak organism. If, however, there was any very marked individual variation in the ability of individual organisms to cope with gentian violet, this would have shown itself in occasional growths of vigorous cells when single cell transplants were made. Furthermore, such a variation would hardly explain why thirty cells can grow in a liter of gentian violet broth, and thus do what not less than 6,000 might be expected to do.



TEXT-FIG. 2. The solid line at the bottom of the chart represents the curve of growth of thirty organisms. These grow even in a liter of gentian violet broth. The dotted line represents the curve that would be expected if the growth of groups of organisms depended entirely on the quantity of available gentian violet; in a liter of gentian violet broth 6,000 organisms would have to be seeded to produce growth. The line *A–B* represents the discrepancy between what actually occurs and what would be expected to occur from a quantitative theory of the difference between the behavior of one cell and that of a group of cells.

That the amount of available gentian violet does, within very narrow limits, play some part in the prevention of growth of single cells is shown in Text-fig. 3. The dotted line A–B represents the results of single cell inoculations into gentian violet broth. It will be seen that single cells never grow in more than 0.8 cc., but that in amounts smaller than this a fair number of positives occur. When 0.1 cc. was used 60 per cent positives were obtained. The corresponding amount of gentian violet broth for thirty organisms would



TEXT-FIG. 3. *A–B* is the curve of growth for single cells—0 per cent when 5 cc. of broth were used, 60 per cent when 0.1 cc. was used. *C–D* is the curve of growth for thirty cells, 100 per cent when 5 cc. of broth were used, 60 per cent when 1 liter was used.

be thirty times 0.1 cc., or 3 cc.; yet as a matter of fact 60 per cent positives were obtained with thirty organisms in over 300 times this amount of gentian violet broth; that is, in a liter (Text-fig. 3, line *C–D*). It is important to observe that the amount of broth used for the experiments, independent of the amount of gentian violet, did not play a part in the results, for in experiments made to determine this point, 75 per cent positives were obtained when single cells were inoculated into a liter of plain broth.

It seems clear, therefore, that thirty cells, instead of being able to accomplish thirty times what one cell can accomplish, are able to accomplish very much more than this. To this discrepancy between the work that thirty cells can do and the work they might be expected to do, on a purely quantitative basis, the term communal activity has been applied. For the present its nature cannot be more accurately defined.

SUMMARY.

1. The behavior of a single bacterial cell toward gentian violet differs fundamentally from that of a small group of cells (thirty).

2. The explanation of this phenomenon is not purely quantitative; thirty cells accomplish much more than thirty times what one cell can accomplish.

EXPLANATION OF PLATES.

PLATE 78.

Fig. 1. Single unstained cells planted on gentian violet agar; no growth.

Fig. 2. Groups of unstained cells of a gentian-negative strain of *B. coli* planted on gentian violet agar. The smaller groups did not grow. The number of cells in the group planted is indicated on each tube.

PLATE 79.

FIG. 3. Transplantation of single cells on plain agar for control. In Divisions 1 and 2 two separate plants (*a* and *b*, *c*, and *d*) were made, in the other divisions only one. Six growths were obtained out of seven plants.

FIG. 4. Transplantation of single cells of *B. coli* on gentian violet agar. One cell was planted in each of the ten divisions. No growth occurred.

FIG. 5. Transplantation of single cells of *B. coli* on gentian violet agar. A single cell was planted in each division. *A*, barely visible, *B*, invisible at the end of 24 hours. These two colonies are the only two growths which have ever occurred from single cells in gentian violet media.

PLATE 80.

FIG. 6. Single cell transplantations into broth. Strain Z, a gentian-negative strain from a single cell, was used for the seeding. Growth is represented by shading. 80 per cent positives were obtained in the control series (upper row), no positives in the gentian violet broth series (lower row).

FIG. 7. Group transplantations of a gentian-negative strain of *B. coli* (Strain Z) into gentian violet broth. The number of cells seeded is indicated on each tube. Groups below twenty-seven did not grow. Compare the growth of the larger groups with the failure of single cells to grow (see Fig. 6).

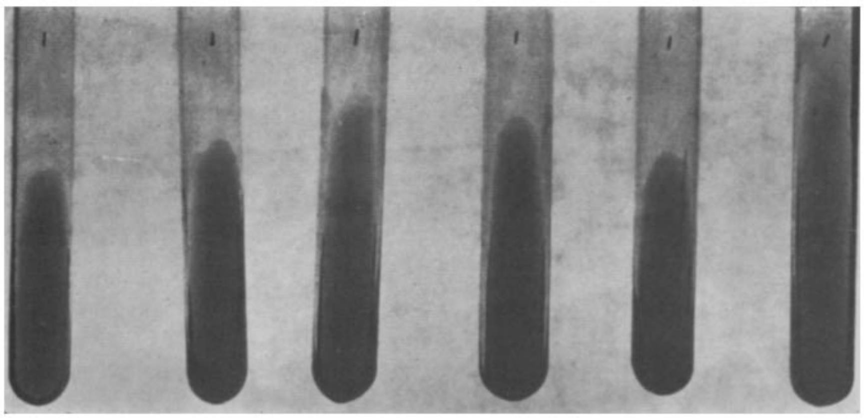


FIG. 1.

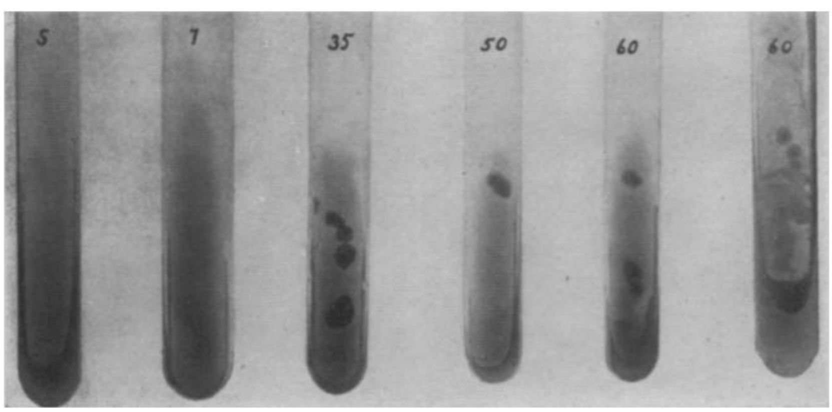


FIG. 2.

(Churchman and Kahn: Communal activity of bacteria.)

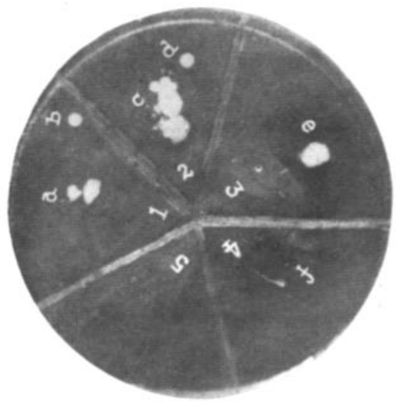


FIG. 3.

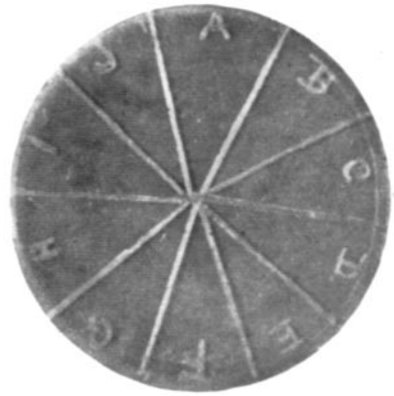


FIG. 4.

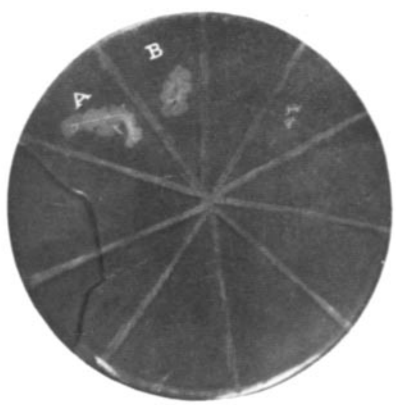


FIG. 5.

(Churchman and Kahn: Communal activity of bacteria.)

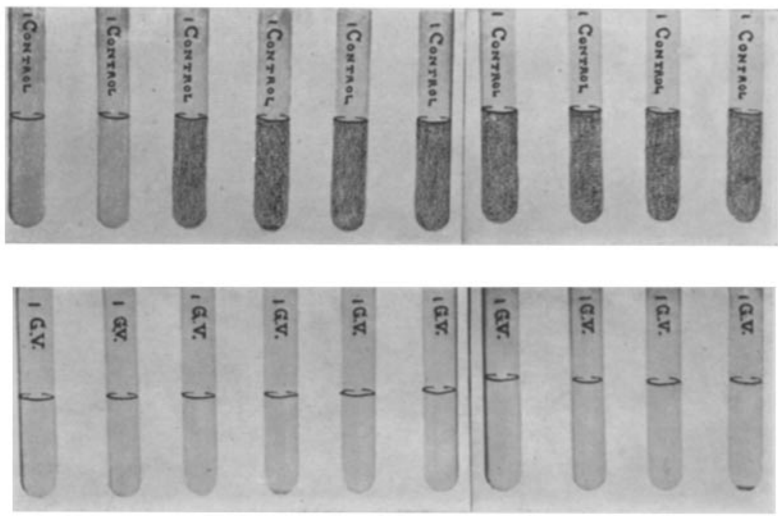


FIG. 6.

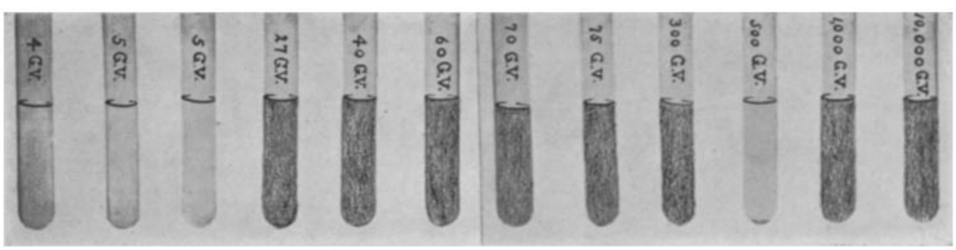


FIG. 7.

(Churchman and Kahn: Communal activity of bacteria.)