

It is evident from the above mentioned observation that the logarithmic phase of growth of culture inoculated at initial pH 7.0 is shorter than that of initial pH 8.2. The logarithmic phase of cultures of initial pH 5.8 is again much shorter than that of initial pH 7.0, whereas it is completely abolished in cultures of initial pH 5.4. Hence the lower the initial pH, the greater the period during which the growth promoting properties of the tomato extract medium exert their influence.

To find more experimental proof for the above mentioned explanation of the influence of initial hydrogen ion concentration of media on growth promoting effect of tomato extract, a micro-organism with a narrower zone of optimum pH range than that of *B. Shiga*, such as Cholera V, was chosen.

The critical point for this organism in tomato extract medium was found to be pH 6.6-7.0. The critical point is higher than that of *B. Shiga*, hence the initial pH for the growth promoting effect of the tomato extract should be higher than that of *B. Shiga*, if the explanation given is correct. This in fact was found to be the case. It was observed that the rate of growth in tomato extract cultures of initial pH 8.2-9.0 showed considerable growth promotion, while these of initial pH 6.6-7.8 actually produced inhibition because as is seen, the cultures are in a pH where the constant increase in hydrogen ion concentration very quickly brings it to the unfavorable critical point.

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Food accessory substances in bacterial growth. III. Their fate in bacterial cultures.

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Since the previous observations have shown that bacterial cultures containing tomato extract undergo a rapid change in hydrogen ion concentration leading to a considerable increase in pH, it was decided to determine whether the pH of surrounding fluid

would have anything to do with the fate of growth promoting factors in bacterial cultures.

Preliminary experiments had shown that during the first 24 hours of multiplication of *B. Shiga* in *plain broth* of various initial pH no growth promoting nor growth inhibiting substances can be detected in the surrounding fluid which could vitiate the interpretation of the following results.

Cultures of *B. Shiga* containing *tomato extract* and of initial pH 5.4, 7.0 and 8.6 and control cultures of the same pH in plain broth were centrifuged at various intervals of incubation time for a period of 24 hours, the pH of these samples recorded, adjusted to pH 8.2 and inoculated with *B. Shiga*. These experiments demonstrated that the growth promoting factors are present in fluids derived from cultures of initial pH 5.8 and 8.2 for the period of 24 hours of observation, whereas they completely disappear from tomato extract broth cultures with initial pH 7.0 as soon as the latter reaches 5.2 (10 to 14 hours). It may be pointed out that the culture of initial pH 8.2 does not reach a pH higher than 5.8 during 24 hours of incubation, while that of initial pH 5.8 naturally reaches pH 5.2 very quickly (2 to 3 hours).

What, then, are the conditions that lead to the disappearance of growth promoting factors from bacterial cultures with initial pH 7.0?

1. The phenomenon of disappearance of growth-promoting factors does not depend on *the amount* of bacteria in these cultures, since these factors persist in tomato extract cultures of initial pH 8.2 which, as was shown before,¹ contain a more abundant growth than the cultures under discussion.

2. *The length of time* during which the growth-promoting factors are in contact with living bacterial cells, does not influence the phenomenon, as they persist in cultures of initial pH 8.2 for 24 hours of observation and disappear from cultures pH 7.0 in 10 to 14 hours.

3. *An increase in the hydrogen ion concentration* of cultures containing tomato extract to pH 5.2 seems to be a necessary condition for disappearance of these factors from cultures pH 7.0.

As the pH 5.2 seems to be the only condition with which the disappearance of growth-promoting factors is associated, it

seemed strange that no disappearance of these factors occurred in cultures of initial pH 5.8 which reach the pH 5.2 very quickly.

But since the initial pH 5.8, even of cultures containing tomato extract, is not favorable for the growth of *B. Shiga*, it was thought that the amount of bacterial cells is too small to bring about the disappearance of growth-promoting factors from this culture. An experiment, in which culture-medium containing tomato extract and of initial pH 5.4 was inoculated with 2 cc. of a heavy emulsion of *B. Shiga* (about 700,000,000 organisms) demonstrated that the growth promoting factors do not disappear even under these conditions.

Three possible explanations for the disappearance of growth-promoting factors from cultures of initial pH 7.0 when they reach pH 5.2, and for the persistence of these factors in cultures of initial pH 5.4-5.2 even in heavily inoculated media presented themselves, namely :

1. That although the medium pH 5.4 was seeded with a heavy inoculum, the bulk of the microorganisms may have died out,² and that it is necessary to have *living* bacterial cells at pH 5.2 in order to cause the disappearance of the growth promoting factors.

2. That the heavy inoculum at pH 5.2 does not give rise to as many *dead* bacterial cells as would be produced eventually by a culture seeded into broth of initial pH 7.0 and kept until pH 5.2 is reached.³

3. That it is necessary to have the process of *active multiplication* such as could be produced by the growth of bacteria in tomato extract culture of initial pH 7.0, and that this culture must be eventually changed to pH 5.2 in order to bring about the disappearance of growth promoting factors from bacterial cultures.

An experiment, in which a large amount of bacterial cells killed by heating to 60° for ½ hour was brought in contact with tomato extract of various pH, definitely demonstrated that the growth promoting factors cannot be removed by this process.

On the other hand a tremendous amount of *living* bacterial

² See 18 (2541).

³ In 18 (2541) it was shown that there is a pronounced death of bacterial cells when a tomato extract culture pH 7.0 reaches pH 5.2.

cells such as washings of 21 agar slants, when left in contact with $\frac{1}{2}$ cc. of tomato extract of pH 5.2 for 24 hours on ice did not bring about the disappearance of the growth promoting factors from this extract. Nevertheless, the content of living cells in this experiment must have exceeded many times that produced in tomato extract cultures of pH 7.0 receiving a small inoculum, because at initial pH 5.2 the rate of death of bacteria should leave approximately two-thirds of the original number alive in a period of 24 hours, as previous experiments had shown.

Since there remains only the third possibility it was decided to determine whether the actively growing bacterial cultures containing tomato extract and reaching pH 5.2 produce any substances which would be responsible for the phenomenon under discussion.

An experiment in which the fluid derived from a 24 hours old tomato extract culture of initial pH 7.0 and of final pH 5.2 was mixed with fresh tomato extract in proportion 1:10 and then, after being adjusted to pH 8.2, was inoculated with *B. Shiga*, demonstrated that this fluid contains certain factors which are able to destroy or inactivate a new supply of food accessory substances when brought in contact with the latter at pH 5.2. Furthermore, fluid containing inactivating substances is not able to effect the growth promoting factors if it is previously adjusted to pH 8.2 and only then brought in contact with the tomato extract.

Evidently the disappearance of food accessory substances from *B. Shiga* tomato extract cultures of initial pH 7.0 is due to the formation of unknown factors by actively multiplying bacterial cells, the actual process of inactivation being favored by pH 5.2. A study of these factors is now under way.

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