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The mechanism of shortening of the lag period in bacterial cultures containing certain food accessory substances.

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Avery and Morgan[1] have recently demonstrated that fresh potato tissue, which was shown to contain a powerful peroxidase as well as certain food accessory substances, is able to promote the growth of pneumococcus in culture media. In these studies they noticed that a piece of potato when added to a broth culture of pneumococcus is able to abolish the lag period in the growth of this micro-organism, which enters, immediately after inoculation, into the logarithmic prase of growth.

In the author's experiments, the influence of tomato extract on the lag period in *B. Shiga* cultures was studied. It was found that this extract is able to shorten the lag period in cultures of this micro-organism if the original inoculum is sufficiently large.

The object of this investigation was to find an explanation of the mechanism of the shortening of the lag period by tomato extract. For this purpose a clear understanding of the function of the lag period in general is required. This seems to have been given by Sherman and Albus.[2] These authors believe that during the lag period the old cells of the inoculum undergo a biological rejuvenescence which fits them for reproduction. As a matter of fact, they showed that, just before the end of the lag period when no measureable increase in growth had as yet taken place, the inoculated old bacterial cells assumed certain characteristics which belong to young and actively multiplying bacteria. This was shown in the fact that old bacteria inoculated into broth remained resistant to the action of a 5 percent NaCl solution for a period of about one hour and became susceptible, *i. e.*, were killed by the NaCl after 1½ hours of inoculation, or just before the end of the lag period. This increased susceptibility

[1] Avery, O. T., and Morgan, H. J., *J. Exp. Med.*, 1924, xxxix, 275 and 289.
[2] Sherman, J. M., and Albus, Wm. R., *J. Bact.*, 1924, ix.

of the bacterial cells to 5 percent NaCl, they take as evidence of rejuvenescence.

Since the lag period seems to be strictly associated with the time required for the bacterial cells to assume the characteristics of young cells, it may be assumed, *a priori,* that any factors which are able to induce a prompt "rejuvenescence' of bacterial cells, in terms of susceptibility to 5 percent NaCl, should bring about a shortening of the lag period. As has been stated above, the author found that the tomato extract is able to shorten the lag period. He concluded that the food accessory substances of this extract are able to "rejuvenate" the bacterial cells in a very short time, make them fit for reproduction much quicker than would occur in plain broth, and bring about the shortening of the lag period.

The following experiments were carried out:

1. The resistance to hypertonic salt solution of *B. Shiga* when exposed to the influence of tomato extract for various intervals of time, was determined, to discover whether a prompt "rejuvenescence" actually occurs.

2. A study was made to determine whether this "rejuvenescence" occurs at the time when no increase in the amount of cells had as yet taken place.

3. An investigation was made to determine whether the same factors, which are responsible for the shortening of the lag period, are also responsible for the "rejuvenescence" of the bacteria.

The technique of testing the resistance of bacterial cells to the action of 5 percent salt solution was as follows:

A four day old culture of *B. Shiga* was inoculated into media of known hydrogen ion concentration. Immediately after seeding, and at 15 minute intervals thereafter, 0.1 cc. of the culture was removed, mixed with 9.9 cc. of salt solution, and plates inoculated for counts of viable micro-organisms. The salt solution suspensions were then kept at room temperature for fifteen minutes and plates again inoculated for counts of viable organisms. The percentage of micro-organisms left alive after treatment with this solution for fifteen minutes was considered as the *index of resistance.*

In preliminary experiments it was found that an inoculum of *B. Shiga* derived from a four day old culture, and freshly

seeded in *plain* broth, reached an index of resistance of 38-40 in *one hour and five minutes* of incubation. This is coincident with the end of the lag period.

Inoculations were now made with tomato extract broth of the same pH as was used in the plain broth (pH 7.0), and it was found that the index of resistance 38-40 was reached in this medium in *15 minutes.* (Fig. 1.) This index, as is seen from

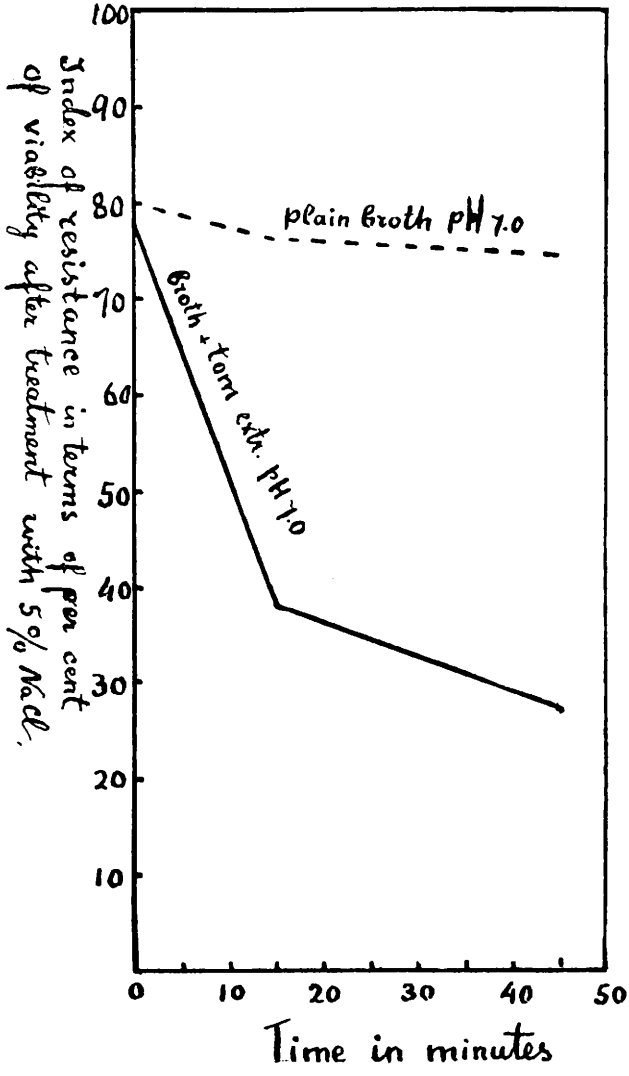

FIG. I.

the above experiment, seems to be necessary in order that the bacteria may enter the phase of logarithmic growth.

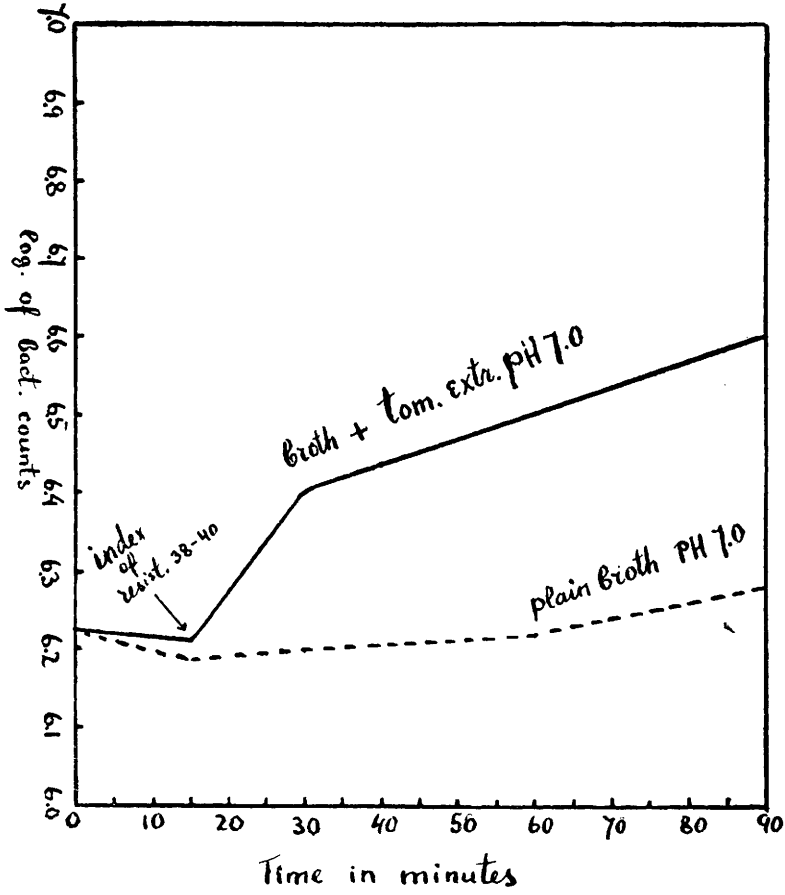

FIG. II.

Fig. II shows the rate of growth of the same inoculum which was employed for the above experiment. This curve again demonstrates that the lag period in the culture containing tomato extract has been shortened to 15 minutes. Moreover, a comparison of Figures I and II brings out the fact that the tomato extract cultures of *B. Shiga* reached the index 38-40 just before an appreciable increase in the numbers of viable micro-organisms.

Tomato extract broth culture from which the food accessory substances were removed by a method described elsewhere[3] were inoculated with *B. Shiga*. This experiment demonstrated that in

the absence of growth promoting factors there is no change in the resistance of *B. Shiga* to 5 percent salt solution for a period of 45 minutes of incubation.

To sum up, the food accessory substances of tomato extract are able promptly to "rejuvenate" *B. Shiga,* and thus make it possible for this micro-organism to enter the phase of active multiplication very quickly after inoculation.

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Acetylation as a physiologic reaction.

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After the ingestion of para-amino phenyl acetic acid by human beings it was found that this substance was detoxicated by acetylation of the amino group. When fed to rabbits, acetic acid was also employed as the detoxicating agent and p-acetyl amino-phenyl acetic acid was excreted in the urine. When the p-amino phenyl-acetic acid was fed to dogs, however, the amino group remained free and the detoxication of the compound was accomplished by combining with glycocoll.

Ortho, meta and para amino benzoic acids were ingested by human beings then fed to dogs and rabbits. All three compounds were excreted unchanged in the urine of the dog. The ortho amino benzoic was excreted unchanged by the human being and rabbit, but the meta and para amino benzoic were acetylated by the human being as well as by the rabbit. Ortho acetyl amino benzoic acid was prepared and fed to the dog and rabbit as well as taken by a human subject. The acetyl derivative was found to be non-toxic, and was in each case rapidly excreted unchanged. There was in no case a glycocoll compound formed as reported by Salkowski[1] after feeding meta amino benzoic acid to a human being, dog and rabbit.

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[3] Shwartzman, G., PROC. SOC. EXP. BIOL. AND MED., 1924, xxii, 44.

[1] Salkowski, *Zeit. Physiol. Chem.*, 1882-83, vii, 93.