

ANTAGONISM ON *B. FLUORESCENS* AND *B. TYPHOSUS* IN CULTURE.

P. A. TETRAULT.

It is a fact long established that when two organisms live together in close relationship, the association will be one of tolerance, of mutual benefit or of one-sided injury. The term antagonism as used in this paper has more the meaning of one-sided injury. The phenomenon, for the bacteria, was recognized as far back as 1888 when Freudenreich and Garre, working independently, demonstrated specific antagonisms between given bacterial forms. The last named worked especially with *B. typhosus*. It was found that the typhoid organism did not thrive in a medium where certain other bacteria had previously grown; in other words, the cell secretions were toxic for *B. typhosus*.

W. D. Frost,* working on this same problem, discusses a number of theories advanced to account for this phenomenon.

One theory is that of the exhaustion of the food supply. All the available food has been extracted from the medium by the first organism growing on it. This was controverted by Olitzky by demonstrating that *Micrococcus aureus* would grow on a medium which had nourished a previous crop of bacteria but which did not permit the growth of *B. typhosus*.

Another theory was that of enzyme action. This, Frost says, could not hold in this case because enzymes are colloidal in nature and could not pass through a collodion membrane.

A history and comparison of the different cultures used in my work is given below.

All the cultures came from The Museum of Natural History, New York. No. 29 was obtained originally from the University of Chicago and was isolated from the swimming pool. No. 469 came from the Kral laboratories, Germany. No. 31 also came from the University of Chicago,

*The Antagonism Exhibited by Certain Saprophytic Bacteria against the *B. typhosus Gaffky*. Jour. of Inf. Diseases. Nov. 5, 1914.

was isolated from the Mississippi River and was labeled *B. fluorescens liquefaciens*.* No. 502 came from the University of Vermont and was labeled *B. fluorescens tenuis*.

When grown on various media these cultures gave the following reactions:

TABLE I.

Media.	No. 29.	No. 469.	No. 502.	No. 31.
L. milk	Digested.	Digested.	No reaction.	No reaction.
Lac. broth	No gas.	No gas.	No gas.	20%.
Nitrate broth.	Nitrates reduced.	Nitrates reduced.	Nitrates reduced.	Nitrates reduced.
Pep. broth	No indol.	No indol.	No indol.	No indol.
Gelatin.	Liquefied.	Liquefied.	Not liquefied.	Not liquefied. †

The only appreciable differences between these two groups of cultures lie in the litmus milk and gelatin reactions. This would suggest that the process was one of digestion, but by direct microscopic methods it could not be determined. When *B. typhosus* was mounted in some of the sterilized *B. fluorescens* filtrate and examined under the microscope, no agglutination was observed.

When the two organisms *B. fluorescens* and *B. typhosus*, are grown in parallel streaks on solid media, it is found that there always remains a zone between the two where no growth occurs, *B. fluorescens* gives off a pigment which facilitates the study of this phenomenon by microscopic methods. *B. typhosus* never trespasses over the green border line put up by *B. fluorescens*. This suggested a further study of the two organisms in liquid media. The method was practically the same as that used by W. D. Frost in his work on "The Antagonism Exhibited by Certain Saprophytic Bacteria against the *B. Typhosus Gaffky*" and described in his article on "Collodion Saes." †

A gelatin capsule is fastened onto the end of a glass tube by heating the tube slightly before applying the capsule. The capsule and part of the tube are then dipped into collodion and allowed to harden. After a few dippings the sac is strong enough to stand without the aid of the gelatin. The gelatin is dissolved by means of hot water and the sac is ready for use. The sac is filled with nutrient broth and inserted into a flask con-

*Although labeled *B. f. liquefaciens*, No. 31 failed to liquefy gelatin.

†Reports and Papers of the Am. Pub. Health Assn. Vol. 28.

taining the same kind of medium. After sterilization in the autoclav, the sac and flask are inoculated with the different cultures to be studied. The sac prevents the bacteria from mingling but, being permeable, permits the diffusible products of metabolism to distribute themselves uniformly throughout the liquid medium. By taking samples from both tube and flask and plating, it becomes a rather simple matter to determine whether the life or the growth of either organism is affected by the manufactured products or wastes of the other.

The experiments were run in series. Each series consisted of five flasks. Four of these contained *B. typhosus* in the sac and one of the cultures of *B. fluorescens* in the flask. The fifth was used as a control and contained only *B. typhosus*. Four of these series were run simultaneously. The temperature was 37 degrees Centigrade. The experiments ran through a period of twelve weeks.

Of the strains of *B. fluorescens* used, cultures No. 29 and No. 469 imparted a very deep color to the medium after growing for twenty-four hours; No. 31 and No. 502 imparted very little color.

The next table shows a certain correlation between the elimination of *B. typhosus* by *B. fluorescens* cultures secreting a deep colored pigment as compared with those cultures secreting very little pigment.

TABLE II.

After twenty-four hours incubation.

Flask-containing <i>B. fluorescens</i> .	Sac containing <i>B. typhosus</i> .
No. 29	Growth.
No. 469	Growth.
No. 502	Growth.
No. 31	Growth.

After forty-eight hours incubation.

No. 29	No growth.
No. 469	No growth.
No. 502	Growth.
No. 31	Growth.

After seventy-two hours incubation.

No. 29	No growth.
No. 469	No growth.
No. 502	Growth.
No. 31	Growth.

The above table shows that the secretions of *B. fluorescens* in the flask penetrate the collodion sac containing the typhoid culture. There takes place, then, not merely an inhibitory action, but an actual bactericidal one. The secretions have to be of a certain concentration before this action takes place. It is found that the above is not true for the cultures showing slight chromogenesis.

In the next experiment, *B. fluorescens* was planted and allowed to grow before *B. typhosus* was introduced into the sac. The following table shows the results obtained.

TABLE III.

After growing *B. fluorescens* for twenty-four hours, inoculating the sac with *B. typhosus*, and again incubating for twenty-four hours:

<i>B. fluorescens.</i>	<i>B. typhosus growth.</i>
No. 29	Absent.
No. 469	Absent.
No. 502	Present.
No. 31	Present.

After growing *B. fluorescens* for forty-eight hours, inoculating the sac with *B. typhosus*, and again incubating for twenty-four hours:

<i>B. fluorescens.</i>	<i>B. typhosus growth.</i>
No. 29	Absent.
No. 469	Absent.
No. 502	Present.
No. 31	Present.

This table shows that once the toxic substances are produced in sufficient quantities and time enough is given for them to penetrate the sac, the typhoid organisms will not grow.

In the next experiments the fluorescens organisms were grown for ten days, then filtered and the filtrate sterilized for ten minutes at a pressure of fifteen pounds in the autoclav. The sterilized filtrate was then inoculated with *B. typhosus* and at the end of twenty-four hours samples were plated with plain agar and incubated.

The results are shown in the following table:

TABLE IV.

<i>B. fluorescens.</i>	<i>B. typhosus</i> growth.
No. 29	Absent.
No. 469	Absent.
No. 502	Present.
No. 31	Present.

The active substance is not destroyed at the temperature of live steam under fifteen pounds pressure for fifteen minutes.

CONCLUSIONS.

The specific antagonism of *B. fluorescens* for *B. typhosus* is due to a substance secreted by the first named organism.

This antagonism is not characteristic of all *B. fluorescens* cultures. There seems to be a correlation between the intensity of the color and this property.

The action is bactericidal.

B. fluorescens cultures with slight pigment production do not prevent the growth of *B. typhosus*.

The metabolic substances must reach a certain concentration before they become effective.

The toxic substances secreted by *B. fluorescens* have the following properties:

1. They are thermo-stabile.
2. They are diffusible through a collodion sac.

Although the growth of *B. fluorescens* in milk would suggest a digestive process, the typhoid bacilli are not agglutinated when grown in a sterilized filtrate of the first named organism.

