THE ANTAGONISM OF BACTERIA AND THEIR PRODUCTS TO OTHER BACTERIA.*†

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EVERYWHERE in nature we see numerous examples of two opposing biological activities. On the one hand, living organisms frequently derive mutual benefit by association; on the other, the presence of one organism, or its products, is inimical to the life and growth of certain other forms. These principles are not limited to animals and the higher forms of plant life, but occur also as low down in the scale as bacteria themselves. Nor do we wonder at this when we consider how numerous and unlike are the products of different bacteria.

Recent investigations show that certain types of bacteria thrive best when associated with each other; for example, the typhoid bacillus and streptococcus.¹ Again, there are numerous instances in which bacteria exert antagonistic action toward each other. It is with the latter theme that this paper will deal.

Lode² claims for a certain diplococcus, whose identity he was unable to establish, that it possessed antagonistic properties toward a number of well known bacteria; among them, *B. anthracis*, *Staphylococcus pyogenes aureus*, the bacillus of fowl cholera, the typhoid bacillus, and the cholera vibrio. In culture tubes and plates this diplococcus tended not only to check the growth of the above mentioned organisms, but also to kill them. Furthermore, culture fluids retained this property after filtration through a Berkefeld filter. Animal experimentation gave negative results, however.

According to Turro,³ subcutaneous injections of beer yeast protect a rabbit against fatal experimental streptococcus and

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¹ VINCENT: *Ann. de l'Inst. Pasteur*, 1893, 7, p. 141; and *Compt. rend. Soc. de Biol.*, 1892, 44, p. 597.

² *Centralbl. f. Bakt.*, 1903, 33, p. 196.

³ *Centralbl. f. Bakt.*, 1903, 34, p. 22.

staphylococcus infection. In fact, beer yeast has been employed as a remedy in cases of erysipelas and smallpox (the latter during the suppurative stage).

Much has been said and written about Emmerich and Löw's¹ "Pyocyanase-Immunproteïdine." A brief review of the work of these authors will not be out of place here. Emmerich and Löw showed that certain pathogenic organisms produce proteolytic enzymes both in artificial culture and in the animal body, which have the power of dissolving or digesting the bacteria that elaborate them; for example, the streptococcus of swine erysipelas and *B. anthracis*. Again, other microorganisms produce enzymes which are able to digest not only the organisms themselves, but possess this property in respect to other bacteria. The most interesting representative of this class is perhaps the pyocyanous bacillus.²

Emmerich and Löw have named the active "bacteriolytic enzyme" of *B. pyocyanus*, *pyocyanase*, and have separated it in a rather impure form from old broth cultures by filtration, concentration of the fluid to a small volume, dialyzation, and finally precipitation with alcohol and ether. Extremely small quantities of the pyocyanase prepared in the above manner destroyed millions of diphtheria, cholera, typhoid, and plague bacilli in but a few seconds of time, and rendered their poisonous products inactive. The pyocyanase showed marked antagonism also toward *Streptococcus pyogenes*, *Staphylococcus pyogenes aureus* and the anthrax bacillus.

Of special interest in the work of Emmerich and Löw is the application of the principle of bacterial antagonism to the protection of animals against fatal infection by the anthrax bacillus. At first varying quantities of pyocyanase (dissolved in water) were injected into rabbits at about the same time that they received a fatal dose of an anthrax culture. The results were far from satisfactory; and this the authors explained on the supposition that pyocyanase is not a very stable body, and that soon

¹ *Zeitschr. f. Hyg.*, 1899, 31, p. 1; *ibid.*, 1901, 36, p. 9. See also, EMMERICH: *Centralbl. f. Bakt.*, 1902, 32, p. 821; LÖW AND KORSCHUN: *ibid.*, 1902, 31, p. 1; EMMERICH AND TROMMSDORFF: *ibid.*, 1903, 33, p. 627.

² BLAGOVESTCHENSKY: *Ann. de l'Inst. Pasteur*, 1890, 4, p. 689.

after its introduction into an animal it is in a large measure destroyed by the animal tissues, before it can exert its protective function. It was conceived that if the pyocyanase could be converted into some more stable form, better results would be attained. Consequently, Emmerich and Löw prepared their so-called "Immunproteidine" by mixing the pyocyanase with blood or other animal tissues, in this way presumably bringing about its combination with certain of the tissue proteids. With the "Immunproteidine" they were able repeatedly to protect rabbits against fatal infection by anthrax. Although the work of Emmerich and Löw has received considerable criticism, and much remains to be desired from the practical standpoint, it has been substantiated, in a large measure at least, by a number of other investigators, particularly Vaerst,¹ Krause,² and Tavernari.³

Quite recently Dr. Maher,⁴ of New Haven, published the results of some work carried on by him on the subject of bacterial antagonism. He claims to have repeatedly cured tuberculosis in man by subcutaneous injections of a certain spore-bearing bacillus which he isolated originally from sour milk—in all probability *B. mycoides*. Kinghorn⁵ employed this same bacillus in connection with experimental tuberculosis in rabbits, but obtained negative results.

For some time I have been engaged in a study of the chemical and physiological properties of *B. prodigiosus*, more particularly its products. Among other things, I soon found that dead prodigiosus bacilli and their products had a distinctly protective action against infection by the anthrax bacillus. Subcutaneous or peritoneal injections of prepared "prodigiosus powder," which were made at the same time and at certain intervals after inoculation with an ordinarily fatal dose of anthrax bacilli, protracted the life of the guinea-pigs from 14 to 72 hours, and in one instance entirely prevented the death of the animal. The method of investigation was as follows:

¹ *Centralbl. f. Bakt.*, 1902, 31, p. 293.

² *Ibid.*, p. 673.

³ *Ibid.*, p. 786.

⁴ *New York Med. Jour. and Phila. Med. Jour.*, 1904, 79, p. 163.

⁵ *Jour. of Med. Res.*, 1904, 10, p. 249.

The prodigious powder was prepared from potato growths, which were four to five days old, by scraping the growths with a sterile section lifter, thoroughly macerating in a mortar under chloroform at certain intervals covering a period of 24 hours, and completely drying in an exhaust desiccator. Finally, the dried mass was ground to a fine powder. The entire process was carried on under aseptic conditions.

A prodigious powder prepared in the above manner possesses the following properties: Its color varies from a pink to a deep red. It is sterile. It coagulates milk within a few hours, and redissolves the coagulum. It rapidly liquefies gelatin, and has a marked proteolytic action in general. It inverts cane sugar. Finally, it possesses pronounced toxic action when injected into guinea-pigs and rabbits. The chief effects of its toxic action are fever, soreness of the eyes, loss of appetite, emaciation, and frequently diarrhea. Marked necrosis usually occurs at the point of injection.

Since the powder possessed such toxic properties, it was necessary to inject very small quantities. For this purpose definite amounts (usually 0.05 to 0.1 gram) of the sterile powder were mixed in a test tube with 10 c.c. physiological salt solution. After filtration through loosely packed absorbent cotton, one or two cubic centimeter portions (5 to 20 mg.) were injected by means of a hypodermic syringe. The injections were usually made near the site of the anthrax inoculation. Owing to the minute quantities of powder employed, the injections were, as a rule, repeated several times at more or less regular intervals.

The anthrax material was prepared as follows: A "standard" platinum loopful of a 24 hour agar growth of the anthrax bacillus was thoroughly mixed with 10 c.c. physiological salt solution (sterile). One cubic centimeter of this suspension was introduced into a second tube (10 c.c. salt solution); and from this again one cubic centimeter was brought into a third tube. Definite portions of the third and last dilution were injected into the animals by means of a syringe. Bacterial counts were made by the usual method, and in this way the number of bacilli injected was approximately ascertained.

EXPERIMENT I.

a. Guinea-pig, weight 452 grams. Subcutaneous injections of prodigiosus powder: Feb. 24, 3 P. M. (50 mg.); Feb. 25, 3 P. M. (10 mg.); Feb. 26, 9 A. M. (10 mg.). Inoculation with 10,000 anthrax bacilli (subcutaneous), Feb. 24, 3:30 P. M. Died in 60 hours.

b. Control pig, weight 450 grams. Similar inoculation with anthrax bacilli. Died in 36 hours.

Difference in length of life = 24 hours.

EXPERIMENT II.

a. Guinea-pig, weight 425 grams. Subcutaneous injections of prodigiosus powder: Mar. 1, 4 P. M. (10 mg.); Mar. 2, 10 A. M. (10 mg.); Mar. 3, 10 A. M. (10 mg.) and 3 P. M. (5 mg.); Mar. 4, 10 A. M. (5 mg.) and 5 P. M. (5 mg.). Inoculation with 9,000 anthrax bacilli (subcutaneous), Mar. 3, 10 A. M. Died in 50 hours.

b. Control pig, weight 475 grams. Died in 36 hours.

Difference = 14 hours.

EXPERIMENT III.

a. Guinea-pig, weight 600 grams. Subcutaneous injections of prodigiosus powder: Mar. 15, 11 A. M. (25 mg.) and 4 P. M. (10 mg.); Mar. 16, 10 A. M. (10 mg.) and 4 P. M. (10 mg.). Inoculation with 5,000 anthrax bacilli (subcutaneous), Mar. 15, 4 P. M. Died in 120 hours.

b. Control pig, weight 560 grams. Died in 48 hours.

Difference in length of life = 72 hours.

Organs of prodigiosus pig apparently normal and sterile.

Anthrax bacilli in large numbers in organs of control pig.

EXPERIMENT IV.

a. Guinea-pig, 400 grams. Subcutaneous injections of prodigiosus material: March 24, 11 A. M. (25 mg.); March 25, 10 A. M. (10 mg.). Inoculation with 800 anthrax bacilli (subcutaneous), March 24, 11 A. M. Died in 72 hours.

b. Control pig, 500 grams. Died in 46 hours.

Difference in length of life = 26 hours. Comparatively few anthrax bacilli in organs of prodigiosus pig.

EXPERIMENT V.

a. Guinea-pig, 575 grams. Subcutaneous injections of prodigiosus powder: April 14, 10 A. M. (30 mg.) and 5 P. M. (15 mg.); April 15, 5 P. M. (15 mg.); April 16, 12 M. (15 mg.). Inoculation with 150 anthrax bacilli (subcutaneous), April 14, 10 A. M. Died in 56 hours. No anthrax bacilli found in organs of dead pig. Death probably due to prodigiosus action.

b. Control pig, 600 grams. Lived.

EXPERIMENT VI.

a. Guinea-pig, 500 grams. Injections of prodigiosus powder: May 3, 10 A. M. (10 mg. subcut.); May 4, 10 A. M. (10 mg. subcut.); May 5, 9 A. M. (5 mg.

intraper.); 4 P. M. (5 mg. subcut.); May 6, 9 A. M. (5 mg. intraper.); 5 P. M. (5 mg. subcut.); May 7, 12 M. (5 mg. subcut.). Inoculation with 1200 anthrax bacilli (subcutaneous), May 3, 10 A. M. Died in 120 hours.

b. Control pig, 475 grams. Died in 48 hours.

Difference in length of life = 72 hours.

EXPERIMENT VII.

Both of the guinea-pigs lived after injection of 45 anthrax bacilli.

EXPERIMENT VIII.

a. Guinea-pig, 600 grams. Subcutaneous injection of prodigiosus powder, May 18, 12 M. (8 mg.). Intraperitoneal injections: May 18, 4 P. M. (5 mg.); May 19, 11 A. M. (5 mg.); May 19, 5 P. M. (3 mg.); May 20, 10 A. M. (4 mg.); May 21, 10 A. M. (5 mg.); May 22, 11 A. M. (5 mg.). Inoculation with 1600 anthrax bacilli, May 18, 12 M. Animal lived.

b. Control pig, 600 grams. Died in 47 hours after anthrax injection.

EXPERIMENT IX.

a. Guinea-pig, 510 grams. Injection of prodigiosus powder: June 22, 11 A. M. (5 mg. intraper.); 8:30 P. M. (4 mg. subcut.); June 23, 10 A. M. (5 mg. subcut.); 9 P. M. (5 mg. subcut.); June 24, 3 P. M. (5 mg. intraper.); June 25, 9 A. M. (3 mg. intraper.); 8 P. M. (2 mg. subcut.). Inoculation with 1000 anthrax bacilli (subcutaneous). Died in 90 hours. Comparatively few anthrax bacilli in organs of pig.

b. Control pig, 500 grams. Died in 65 hours.

Difference in length of life = 25 hours.

Let us briefly summarize the results of the nine experiments recorded here. In six of the experiments the life of the guinea-pigs was prolonged 14, 24, 25, 26, 72, and 72 hours respectively; while in one instance the animal quite recovered. It should be added, also, that in every case but one, microscopic examination revealed the anthrax bacilli to be far less numerous in the organs of the prodigiosus animals than in those of the control pigs. In fact, no bacilli could be found in one of the guinea-pigs (Exp. III). Here death was due, in all probability, to the toxic action of the prodigiosus material itself, and not to anthrax. In Experiment V it is likely, also, that the animal died in consequence of excessive dosage with the prodigiosus powder, as the organs proved to be sterile, and the control animal lived. In Experiment VII both animals lived after an injection of only 45 anthrax bacilli.

Besides the series of experiments recorded above, others were tried on rabbits and guinea-pigs. In three of these experiments, a prodigiosus powder was employed which was not sterile, and

the animals died from prodigiousus septicemia. In spite of the fact that culture tubes showed no growths when inoculated with small quantities of the powder, there were still enough of the living bacilli present to cause fatal infection. This experience fully accords with that of Bertarelli¹ and others, who found that subcutaneous and peritoneal injections of comparatively few living prodigiousus bacilli rapidly cause fatal septicemia in the lower animals.

In the course of two other experiments (rabbits), the work was suddenly cut short by a fatal septicemia which seized the animals, and which proved to be caused by a variety of the colon bacillus.

CONCLUSIONS.

The experiments tried up to the present time clearly indicate that in the animal body, at least, the products of *B. prodigiousus* exert a marked antagonistic action towards the anthrax bacillus. Such antagonism may even go so far as entirely to protect the animal against fatal anthrax infection. The successful employment, however, of prodigiousus powder in immunization work meets with a serious obstacle at the present time. The powder exerts such a degree of toxic action, when injected, that only very small quantities can be used without serious consequence to the animal. Attempts thus far made to destroy the toxic properties without lessening the protective action have not given the desired results.

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¹ *Centralbl. f. Bakt.*, 1903, 34, pp. 193 and 312.