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Further Experiments with B. Pertussis Filtrate.

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Toomey and McClelland[1] have described a substance found in cultures of veal brain broth during the growth of *H. pertussis*. The following is an additional report on the character of this filtrate, termed for convenience B.P.F.

The strain of *B. pertussis* used was number 778 of the American Type Culture Collection, originally isolated by Park. It was planted and grew luxuriantly in 50 Erlenmeyer flasks, each of which contained 50 cc. of bloodless veal brain broth medium.[1] For 50 successive days, the contents of a flask were treated each day as described previously.[1] The filtrate obtained was kept sterile by the addition of 0.2% of tricresol. The 50 specimens collected were divided into groups of 10 each.

One group of filtrates was injected intradermally into the arms and forearms of at least 6 individuals, in doses of 0.1 cc. There was no appreciable change in the toxicity of the filtrate obtained from these growths as evidenced by the reactions to skin tests until the filtrate of the twelfth day was harvested. The filtrates that were obtained after the twelfth day always gave local inflammatory reactions when injected intradermally in the skins of individuals; these reactions were still present 24 hours after the injection, although a positive reaction was sometimes obtained with those filtrates that had grown for from 8 to 12 days. The filtrates obtained after the twentieth day of growth, however, contained no greater amount of B.P.F. than those obtained from the twelfth to the twentieth day.

Cultures were taken of the growths preceding each daily filtration to make certain of purity and to note the length of time that organisms remained viable. The transplantation was made on solid veal brain agar. Beginning with the twentieth day, the transferred growth became more sparse. By the twenty-fifth day, little or none of the transplanted material had grown, and by the thirtieth day, all the organisms were dead since no further growth could be obtained

[1] Toomey, John A., McClelland, Joseph E., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 34.

on subculture. Demonstrable death of the organisms started about the twentieth day.

In the group of patients that was tested as described in the foregoing, the reaction itself and not its character or extent was noted. To test the difference in local reactions quantitatively, B.P.F was obtained from cultures which grew for 1, 8, 12, 20, 30, 40, and 50 days, respectively. One-tenth cc. of each of the 7 filtrates was injected into each of 24 individuals and the reactions were read in 6 and 24 hours. The first injection of B.P.F. obtained from the 24-hour growth frequently caused an immediate but fleeting local reaction which disappeared before 24 hours had elapsed. The reactions obtained after injections of filtrates that were harvested after the eighth and subsequent days were definite 6 hours after injection and were still present after 24 hours. The maximum reaction value was reached about the twelfth day.

In order to study the effect on B.P.F. production, batches of new media were made up with the various factors of the original media, alone or in combination. These solutions were as follows: No. 1. Sodium phosphate, 1.6 gm.; water, 98.4 cc: No. 2. Sodium phosphate, 1.6 gm.; sodium chloride, 1.6 gm.; water, 96.8 cc. No. 3. Sodium phosphate, 1.6 gm.; sodium chloride, 1.6 gm.; peptone, 3.3 gm.; water, 93.5 cc. No. 4. Sodium phosphate, 1.6 gm.; sodium chloride, 1.6 gm.; peptone, 3.3 gm.; veal solution, 93.5 cc. No. 5. Veal solution, 100 cc. No. 6. Sodium phosphate, 1.6 gm.; sodium chloride, 1.6 gm.; peptone, 3.3 gm.; veal brain solution, 93.5 cc.

Culture number 778 was planted in each flask of the 6 media solutions listed and incubated for 20 days. The growth was centrifuged, the supernatant fluid passed through a Mandler N and W filter, and the filtrate solutions from the 6 growths were tested out on 6 individuals with the 1/1 dilution, on 6 with the 1/25 and on 6 with the 1/50 dilution. The amount injected intradermally each time was 0.1 cc. The reactions were read after 6 and 24 hour intervals. There were no reactions to filtrates 1 and 2. Solution number 3 had a fair bacterial growth in the medium and only a moderate production of toxic filtrate as determined by skin tests. Number 5 medium, containing only veal solution, had a bacterial growth and a toxin production similar to number 3. Media combinations 4 and 6 were nearly alike in their growth promoting qualities, although the addition of brain infusion to the latter seemed to enrich it slightly. These conclusions were found by noting the comparative extent and character of the reaction and the length of time it persisted after each intradermal injection in test subjects.

From these experiments, it seems that the addition of veal or brain infusion brings about a better growth of the organisms and an increase in the production of B.P.F. units. The corollary seems to hold true that the production of B.P.F. depends on the healthy growth of the organisms.

Culture number 778 was again planted in Erlenmeyer flasks, each containing 50 cc. of veal brain broth medium. After 20 days of growth, the organisms were separated from the filtrate and the latter was passed through Mandler N and W filters as in other experiments. The organisms remaining at the bottom of the centrifuge tube were killed and the whole was brought up to the original volume with normal saline. This we called B.P. vaccine. The B.P.F. and the killed B.P. vaccine were tested out on 82 individuals. It was found that more people reacted with the B.P.F than with the B.P. vaccine solution made as described, the local reactions being greater with the filtrate injections in 54 of the 82 cases.

Twelve day B.P.F. units in dilutions of 1/1, 1/10, 1/25 and 1/50 in doses of 0.1 cc. were injected intradermally in 35 babies about 12 months old. After an interval of 6 hours, there were good reactions in practically all instances, the inflammatory reaction sometimes becoming as large as 5/8 of an inch (1.54 cm.) in diameter with induration. After 24 hours these reactions had become negative or had materially lessened in size.

When 23 mothers and their newborn babies were simultaneously tested, the local reactions at the end of 6 and 24 hours were about equal.

Children who had whooping cough in an inactive stage were tested intradermally with 0.1 cc. doses of B.P.F. and usually showed good reactions which persisted for more than 24 hours. When they were tested again on an average of from 6 to 12 days later, an enhancement of the reaction occurred.

Boiling B.P.F. for 10, 30, and 90 minutes did not decrease the reaction value of the mixture.

Culture number 778 was planted in brain veal broth medium to which 20% placental blood was added to determine whether the toxic value of the filtrate would be enhanced by blood. It was found that the number of skin test units obtained with this preparation was not greater than that obtained on veal brain broth medium.

Combinations of equal parts of B.P.F and convalescent pertussis serum in various dilutions were injected intradermally in humans. There was no consistent neutralization of the toxin; in fact, in

many instances, there was obvious enhancement of the local response.

The striking thing about the growth of *H. pertussis* culture number 778 in liquid veal brain broth media was the formation of a thick tenacious substance. The resemblance between this material and the thick stringy sputum coughed up by children after an attack of whooping cough was obvious.

Culture number 778 was also planted on many types of solid media, 1½% agar with broth, Pelouze's gonococcus and Bordet-Gengou media. On the broth agar and Pelouze's media, the growth was rapid and in about 5 to 6 days the surface began to appear dully glazed. After this time, the growth stuck tenaciously to the medium so that separation was not possible with the ordinary platinum loop. In marked contrast, however, there was never any stickiness to the growth that appeared on the surface of the Bordet-Gengou media.

Growths of culture number 778 on Bordet-Gengou, as well as on veal brain broth media, were blown, dropped and syringed into the nasal passages of 5 *Macacus rhesus* monkeys weighing on an average of 6 pounds. In no instance was there a response that suggested whooping cough, either clinically or hematologically. This may be explained by the fact that culture number 778 was old and probably had lost its pathogenicity.

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Further Studies on the Specific Carbohydrates of Vibrio Cholerae and Related Organisms.*

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In a previous note[1] on the specific carbohydrates of cholera and cholera-like vibrios some preliminary results were indicated. Since then our work has been considerably broadened, and we are able to extend our conclusions.

The method of extracting the carbohydrates used at first con-

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[1] Linton, Richard W., and Shrivastava, D. L., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 600.