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Pertussis Toxin or Antigen.

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Mishulow, Mowry and Scott[1] demonstrated that exotoxic filtrates could be obtained from whooping cough cultures. They planted their organisms on chocolate agar over which they had poured a small amount of horse serum-beef heart broth. The small amount of diluent made the harvest of toxin necessarily small. On the other hand, Craster and Smith[2] mentioned that there was a signal absence of toxin formation in planted cultures, and Riesenfeld[3] stated that there was no proof that this bacillus produced a toxin in ascitic broth. Besredka[4] isolated toxic substances termed endotoxins from the dried and powdered organisms.

In 1928, we found that pertussis bacillus produced a substance in culture growth, the exact nature of which was problematic. With the report of Mishulow, Mowry and Scott, we felt that further work should be done along this line. Since large amounts of toxin could not be practically produced on solid preparations, various liquid media were tried. We found that Pelouze's and Viteri's gonococcous medium slightly modified by omission of the agar could be used to advantage. The formula was as follows: 250 cc. of veal infusion, 80 cc. of brain infusion, 3.3 gm. of peptone, 1.6 gm. of NaCl, and 1.6 gm. of sodium phosphate. The medium had a pH of 7.2.

There are numerous protein factors in this medium, and when we tested out the unplanted control filtrates by intradermal injection of amounts of 0.1 cc., we found that humans gave positive reactions in a little less than 1% of the individuals tested and that in all instances veal was the reacting factor.

Of the 4 strains of the American Type Culture Collection experimented with, No. 778, a strain isolated by W. H. Park, gave us best results and was used in our subsequent work.

The bacillus was planted and grown arbitrarily for 10 to 14 days

[1] Mishulow, Lucy, Mowry, Isabelle W., and Scott, Eleanor B., *J. Immunol.*, 1930, **10**, 227.

[2] Craster, Chas. V., and Smith, Ellis, *J. Med. Soc. New Jersey*, 1931, **28**, 236.

[3] Riesenfeld, Edwin A., *J. Am. Med. Assn.*, 1923, **80**, 158.

[4] Teissier, P., Reilly, J., Rivalier, E., and Cambessédès, H., *J. Physiol. et Path. Gen.*, 1929, **27**, 549.

in the medium described. There was a rapid growth on the bottom of the flask after the fourth to the fifth day. By the ninth day, this growth had extended along the bottom and up the sides as a thick, tenaceous, white film. Colonies connected with the bottom of the flask by a thick, ropey strand grew on the surface of the media. After 14 days, the culture was a thick, stringy, mucilagenous mass which adhered firmly to the smooth glass and was delivered only as large masses of semi-gelatinous consistency.

After having been grown for an average of about 19 days, the material was passed through a few layers of gauze to separate the sticky part from the filtrate since direct passing through a Berkefeld filter was impracticable. The filtrate was then filtered through coarse paper to separate it from any remaining stringy filament. Either this procedure was followed or the mass was centrifuged at high speed for from 10 to 20 minutes and the supernatant fluid poured off. Even with high speed centrifugation over a long time, there was difficulty in throwing down all of the sticky mass. The filtrate obtained in either case was first passed through an N or a V Berkefeld filter, then through a W filter, tested for sterility, 0.2% tricresol was added and the material was then bottled. The medium takes on a peculiarly distinctive but undescribable odor. The pH did not change much, averaging 7.4 in 10 specimens.

Six hundred and eighty individuals were injected intradermally with 0.1 cc. of the filtrate. There was always a local skin reaction in 6 hours. In 502 of the individuals tested, the local reaction persisted for 24 hours, ranging from ½ to 2 inches in diameter and from faint to deep redness with induration. The highest dilution which gave a positive reaction was 0.1 cc. of a 1/50 dilution in saline obtained after 14 days of growth. This would indicate that there were approximately 500 skin test units of a toxic or antigenic element in each cubic centimeter.

Conclusion. Whooping cough bacillus when grown in the liquid medium as described, produced a substance which in the dosages used caused a local skin reaction in either 6 or 24 hours after injection in all the 680 individuals tested. Whether there is a specific fraction for whooping cough in this material has not been determined.