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Tetanus Toxin Production on a Simplified Medium.

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The routine preparation of tetanus toxin for the purpose of antitoxin production, or for conversion into toxoid, appears to offer difficulties similar to those formerly encountered in the case of diphtheria toxin, although perhaps in lesser degree. Active immunization against tetanus by means of toxoid, now coming into general use, demands that an attempt be made to place the manufacture of this important biological product on as satisfactory a basis as it has now been possible to reach in the case of diphtheria toxin.[1, 2] The traditional explanation for failures and irregularities with the latter, namely the types of peptones and meat infusions used in the medium, has been shown to be completely erroneous, although still held to be applicable in dealing with tetanus. In this country, Witte, or the very similar Berna peptone is generally held to be indispensable, together with veal infusion. Elsewhere, enzymatic digests are employed, often with the addition of even more complex materials.

The writers have undertaken a study of the nutritional requirements of the tetanus bacillus and a parallel study of factors influencing the production of its toxin. Since an exact definition of the substances essential to growth may well prove to be a time-consuming matter, it seems worth while to report at this time the production of potent toxin on a simplified, but by no means chemically defined medium, and to indicate that the concentration of iron in the medium, as in the case of diphtheria, is at least one of the controlling factors.

As a basis for experiment we have employed the casein acid hydrolysate medium described by one of us[2] for the production of diphtheria toxin. This medium itself fails to support growth of any of the tetanus strains investigated, as was to be expected, but when it is enriched by the addition of tryptophane, glucose and 1% of a commercial liver extract, growth occurs with all the strains which have been tried. The liver extract employed is the crude 95% alco-

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1 Pappenheimer, A. M., Jr., and Johnson, S. J., *Brit. J. Exp. Path.*, 1936, **17**, 335.

2 Mueller, J. H., *J. Immunol.*, 1939, **37**, 103.

hol precipitate fraction used in the treatment of pernicious anemia, and the writers are indebted to the Lederle Laboratories, Inc., and to Dr. Y. Subbarow of the Department of Biochemistry, Harvard University Medical School, for a supply of the material.

Using a culture obtained from the New York State Department of Health at Albany, through the courtesy of Dr. Harold W. Lyall, we have regularly obtained toxin of considerable potency on the medium. Only small experimental lots have so far been produced. These have been tested after the addition of 0.5% phenol and prolonged centrifuging, but have not been filtered because of the likelihood of considerable loss through adsorption. We realize that this procedure is not beyond criticism.

Toxin killing mice in 4 days in a quantity of 0.000,01 cc has been obtained (about 100,000 M.L.D.). Tested on guinea pigs in quantities of 0.000,2, 0.000,1, 0.000,05 and 0.000,025 cc the same material caused death in 39, 50, 72, and 76 hours respectively, indicating approximately 50,000 M.L.D. for this species. Addition of $FeSO_4$ to the medium before inoculation (iron having first been removed as completely as possible by adsorption[1] on a calcium phosphate precipitate), resulted in progressively weaker toxin as the concentration was increased up to 0.3 mg Fe per liter. The effect of the iron was more pronounced when cultures were grown in well-filled Erlenmeyer flasks exposed to the air, than when strictly anaerobic conditions were employed.

The work is being continued in an effort to identify the components of the liver extract essential to growth of the tetanus bacillus, and to define the optimal conditions for toxin formation.