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Toxicity of Acid-Soluble Typhoid Toxin for Laboratory Animals.

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The reported data¹ indicate that the toxins characteristic of the typhoid bacillus are identified with the bacterial cell and are present in culture-filtrate only as the result of extraction or lysis of the cell. More recently Shwartzman and his colleagues have studied the "skin reactivity" of rabbits to washings and filtrates of typhoid cultures. Burnet² demonstrated the "Shwartzman activity" of typhoid "endotoxin" prepared by the method of Besredka. Boivin *et al.*,³ and Topley *et al.*⁴ have reported recently that certain chemically isolated components of *Eberthella typhosa* were highly toxic for rabbits and mice, and stimulated the formation of somatic "O" agglutinins when given in sublethal doses.

The present paper summarizes our observations on the toxicity of N/4 trichloroacetic-acid extracts of defatted typhoid bacilli. The extracts were further purified by repeated fractional precipitation with alcohol. The purified toxin gave the following analytical data (average of 6 preparations) : N 3.1%, P 1.5%, no reducing sugar before hydrolysis, 30-32% reducing sugar (as glucose) after hydrolysis; the biuret reaction was usually negative, the Molisch reaction strongly positive, and osazones were readily formed. The toxic material appears to be a complex mixture of polysaccharides, free of native protein.

Rabbits are highly susceptible to the toxic somatic antigen-complex of *E. typhosa*, mice are less susceptible, guinea pigs are relatively resistant, while rats show little or no effects, when the toxin is injected intravenously or intra-abdominally in doses as small as 1 to 2 mg. There is, usually, a latent period of about 45 minutes. With onset of symptoms there is rapidly developing dyspnea, irregular heart rate, vascular engorgement, flaccid paralysis of the posterior extremities, and diarrhea. Death usually occurs in

¹ For summary of the literature see Topley, W. W. C., and Wilson, G. S., *The Principles of Bacteriology and Immunity*, 2nd Edition, 1938, Edward Arnold and Co., London.

² Burnet, F. M., *J. Path. and Bact.*, 1931, **34**, 45.

³ Boivin, A., and Mesrobian, L., *Rev. d'immunol.*, 1936, **2**, 113.

⁴ Topley, W. W. C., Raistrick, H., Wilson, J., Stacey, M., Challinor, S. W., and Clark, R. O. J., *Lancet*, 1937, **1**, 252.

18-36 hours (90%), but may occur as early as 1 hour, or as late as the 5th day after injection.

Necropsy findings: Animals dying within 8 hours showed little but generalized hyperemia. In several animals that died shortly after injection, death was due to massive hemorrhage into the pericardial cavity. Microscopically there was moderate to severe capillary hemorrhage in all tissues, including the brain and spinal cord. Animals dying later than 12 hours after injection showed severe hyperemia and numerous punctate hemorrhages in all tissues; the lungs usually showed severe multiple infarction; the small intestine was filled with a bloody matrix of mucus and desquamated epithelium; the Peyer's patches were swollen, hyperemic, and showed punctate hemorrhages. Microscopically the principal features were: generalized capillary hemorrhage with consequent foci of necrosis, fragmentation of the heart muscle, and loss of follicular arrangement, due to edema, in the lymph-nodes. The adrenals showed rapid loss of chromaffin material 3 hours after injection.

The toxic somatic antigen-complex is highly active in preparing for and eliciting the Shwartzman phenomenon. There is a reciprocal relationship between the size of the preparing and eliciting doses. One of our preparations (Lot 17/A) prepared the skin of susceptible rabbits in a dose of 0.00025 mg when 5 mg of the same fraction was given intravenously 22 hours later. The reaction was neutralized by specific homologous antitoxic sera as well as by specific antibacterial antiserum. The neutralizing capacity of a serum was paralleled by its somatic "O" antibody-content. The highest doses of the toxin used for preparing the skin produced hyperemia and petechial hemorrhage at the site of injection within 18 hours.

Conclusion: The fraction containing the somatic antigen-complex of the typhoid-bacillus is highly toxic. The macroscopic changes observed are those attributed to the so-called endotoxin.⁵ The histopathological changes observed indicate that the effect of the toxin is primarily on the blood vessels, damage to which results in degenerative changes in the tissues supplied. A monocytic response is not a component of the tissue-response to the toxin.

⁵ Arima, R., *Centralbl. f. Bakt.*, I Abt. Original, 1912, **63**, 424.