# SYMPOSIUM ON MICROBIAL TOXINS[1]

SAMUEL J. AJL[2]
*Army Medical Service Graduate School, Washington, D. C.*

WILLIAM BURROWS
*University of Chicago, Chicago, Illinois*

C. A. STETSON
*New York University School of Medicine, New York, New York*

W. J. CROMARTIE
*University of North Carolina, Chapel Hill, North Carolina*

C. L. WISSEMAN, JR.
*University of Maryland School of Medicine, Baltimore, Maryland*

A. C. BRAUN AND D. W. WOOLLEY
*Rockefeller Institute for Medical Research, New York, New York*

W. E. VAN HEYNINGEN
*Sir William Dunn School of Pathology, Oxford, England*

In arranging the symposium on microbial toxins, an attempt was made to stress those aspects in this general field which have not been discussed frequently. It is for this reason that emphasis was placed on the so-called endotoxins, and such varied topics as rickettsial and phytopathogenic toxins were included. To avoid omission of the rather well known and often discussed exotoxins, Dr. W. E. van Heyningen was asked to cover some aspects dealing with these as well.

The discussions were divided roughly into two parts. Dr. Burrows and Dr. Stetson covered some phases of the recent work on endotoxins, which was followed by a discussion led by Dr. Cromartie. Following this, plague, rickettsial, and phytopathogenic toxins were discussed, and Dr. van Heyningen gave his report.

Dr. Burrows stressed the fact that bacterial endotoxins are of substantially the same nature, regardless of origin, and that they consist of a substance of relatively low molecular weight which occurs in combination with protein and polypeptide, polysaccharide, and lipid. The most precise information supporting this belief has been obtained in studies of dysentery bacilli, the Shiga bacillus by British workers, and the Flexner bacillus by Goebel in this country. Less definite information concerning the endotoxins of microorganisms such as the cholera vibrio, *Brucella spp.*, a number of species of enteric bacilli, and certain of the gram-negative cocci is in essential agreement. Dr. Burrows indicated further that some evidence exists that suggests that the cell substance of the toxic bacteria contains more than one species of toxicity, but it is possible, or even probable, that this is an artifact. Of the components of endotoxin as it exists in the cell, it would appear that the protein is irrelevant to toxicity and possibly to antigenicity and occurs in loose combination, since yields of apparently intact endotoxin may be increased as much as twofold by preliminary digestion with proteolytic enzymes. It is also obvious, the speaker indicated, that the lipid component is unrelated to toxicity and antigenicity. The polypeptide fraction, however, appears to be essential to complete antigenicity and the polysaccharide may function as a hapten, but the low molecular weight component has not been prepared in active form except in combination with one or another of these sub-

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[2] Convenor.

stances. Since the toxic activity can be prepared in combination with differing molecules, it is probable, according to Dr. Burrows, that the discrepancies in the biochemical properties associated with the activity as reported in the literature are artifacts arising from varied methods of preparation, particularly the preliminary extraction. This has been found to hold true with cholera endotoxin in studies carried out in Dr. Burrows' own laboratory where the endotoxin has been prepared in at least two forms, differing in solubility and elementary chemical analysis.

Dr. Stetson's discussion dealt primarily with the possible mechanisms of action of endotoxins *in vivo*. The classical effects of endotoxin administration are described as local, focal or systemic, depending on the route of administration. In nature and in degree, the reactions to endotoxin, according to Dr. Stetson, resemble those of the specifically sensitized animal to antigen. The Shwartzman phenomenon and the pyrogenic and other effects of endotoxin can be reproduced in the hypersensitive animal by injections of antigen. These observations suggest to the speaker that endotoxins may not be true toxins, but may possess biologic activity in the same sense that tuberculin is biologically active in hypersensitive animals. This concept implies the existence of some "natural" hypersensitivity in supposedly "normal" experimental animals or suggests the existence in normal animals of a reactive mechanism to these bacterial antigens, which is closely allied to the mechanism operating in bacterial allergy.

After some remarks by Dr. Cromartie which dealt primarily with the relationship of the experimental findings just discussed with those encountered during the disease, Dr. Ajl presented a summary of his recent work dealing with plague which was done in conjunction with Dr. Joel Warren and several graduate students. These included: (a) the purification and characterization of plague toxin; (b) the immunological properties of the purified material; and (c) a possible mechanism of its action at an enzymatic level *in vitro*.

Utilizing a number of chemical fractionations combined with paper electrophoresis a protein toxin was obtained from *Pasteurella pestis* which appears to be homogeneous, free of envelope substance and immunogenic. The toxin contains about 15 per cent nitrogen, essentially no phosphorus or carbohydrate but significant amounts of sulphur. The amino acid content, after acid hydrolysis was similar to that of most proteins. The protein hydrolysate contained materials which either absorb or fluoresce in ultraviolet light.

Antitoxin to the purified material was readily produced in rabbits provided the latter were injected with toxin-adjuvant mixtures. The antiserum thus obtained flocculated, hemagglutinated and fixed complement with toxin and toxoid. Immunization with formalin treated toxin protected mice against 60 to 80 $LD_{50}$'s of toxin when the animals were challenged intraperitoneally. However, only a few $LD_{50}$'s of toxin were neutralized when toxin-antitoxin mixtures are administered intraveneously to mice.

Dr. Ajl also discussed attempts to learn what the purified plague toxin molecule does at an enzymatic level. He reported that toxin inhibited the oxidation of both pyruvate and $\alpha$-ketoglutarate by either cell-free extracts of *Escherichia coli* or crude mouse liver homogenates. Diphosphopyridine nucleotide (DPN) reversed this inhibition, thus suggesting that the toxin molecule exerted its effect on DPN and not directly on the enzymes themselves which were dependent on the presence of DPN for activity. The idea that plague toxin kills mice by binding the available DPN of the animal could not be supported as yet since heated, hydrolyzed and formolized toxins which are not lethal for mice were still capable of inhibitory keto acid oxidation *in vitro*.

The present status of the rickettsial toxin was discussed by Dr. Wisseman. According to the speaker, among the toxic actions of the rickettsiae, the mouse lethal toxin and the hemolysin have received greatest attention. Suspensions of typhus rickettsiae, injected intraperitoneally or intravenously into mice, are lethal within a few hours. This toxic action is proportional to the rickettsial count, inseparable to date from rickettsial organisms, heat labile, partially dissociable from infectivity by ultraviolet irradiation, and neutralized by specific hyperimmune serum. Fatal toxemia in the mouse is characterized by a latent period followed by hemoconcentration, generalized plasma transudation into the tissues, diminution of peripheral blood flow and vasoconstriction, all progressing until the

animal dies. Histochemical studies of selected tissue enzymes have not revealed any specific effects. Compensating for differences in body weight, rats are about as susceptible as mice to epidemic typhus toxin but are more resistant to murine typhus toxin, whereas guinea pigs are highly resistant to murine toxin. No consistent correlation appears between toxicity and pathogenicity. The rickettsiae of murine and epidemic typhus, several members of the spotted fever group, and one strain of scrub typhus rickettsiae possess a mouse lethal toxin.

The hemolysin, reported in typhus and Rocky Mountain spotted fever rickettsiae, appears intimately associated with rickettsial bodies. *In vitro*, it is enhanced by glutamate and certain divalent metal ions and is inhibited by low temperatures and several enzyme inhibitors. The erythrocytes of rabbits and sheep are susceptible but those of rats, mice, and guinea pigs are resistant. Rapid intravascular hemolysis, which may be fatal within a few minutes, follows intravenous injection of murine typhus rickettsial suspensions into rabbits.

A series of beautiful experiments performed jointly by Drs. Braun and Woolley were described by Dr. Braun. The investigators attempted to define in mechanistic terms the toxemia associated with a bacterial disease of tobacco known as wildfire. Experiments in this study utilized the alga *Chlorella vulgaris*. The toxin inhibited growth of *Chlorella*, and this inhibition was reversed competitively with L-methionine. Of the known structural analogues of methionine tested, one, methionine sulfoximine, reproduced perfectly the toxic manifestations of the disease in tobacco. Like the toxin, methionine sulfoximine inhibited growth of *Chlorella* and the inhibition was reversed competitively with L-methionine. Mutants of *Chlorella* selected for resistance to toxin were equally resistant to comparable concentrations of methionine sulfoximine. The reverse was also true. Attempts to reverse with L-methionine the chlorotic lesions in tobacco produced by application of either compound have thus far failed.

The wildfire toxin has been found to be a lactone of $\alpha$-lactylamino-$\beta$-hydroxy-$\epsilon$-amino pimelic acid. The relationship between methionine and the toxin can be clearly seen by comparing the structure of these compounds with that of the synthetic analogue, methionine sulfoximine. In the toxin the sulphur of both methionine and methionine sulfoximine has been replaced by two carbon atoms. One of the carbon atoms of the toxin bears the oxygen and the other the nitrogen originally attached to the sulphur of the sulfoximine. In addition, in the toxin the oxygen and nitrogen bonds have been reduced and the methyl group has been oxidized to a lactone grouping.

The wildfire toxin appears to be, therefore, an antimetabolite specifically directed against a vital constituent of the host cell. Damage in the tissues of the host is caused by creation of a unique type of deficiency disease.

Dr. van Heyningen reviewed the effect of iron on the production of bacterial toxins. The production of at least 5 bacterial toxins (scarlet fever, tetanus, *Clostridium welchii alpha*, diphtheria, and shiga) is inhibited when iron is present in the culture medium of the parent organism. With scarlet fever toxin, the observation has been made in the course of large-scale production and has not yet been followed in detail. With some strains of *Clostridium tetani*, toxin production is greatest only at very low concentrations of iron, but with others iron has no inhibitory effect and indeed is added to promote bacterial growth. The iron content of the medium affects both toxin production and metabolic behavior of *C. welchii*, but these two effects do not appear to be connected. It has been suggested that diphtheria toxin may be related to the protein moiety of the bacterial cytochrome b, and that it is secreted as a waste product when there is not enough iron present for the synthesis of the cytochrome to be completed. Toxin production thus takes place at the expense of cytochrome production. With *Shigella shigae* the reverse appears to be true; as long as the iron is quantitatively converted into bacterial haems it does not inhibit toxin production; when the organism contains iron that is not in the form of haems there is inhibition of toxin production.