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V. Bacterial Endotoxins

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Endotoxins are the toxic principles of the cell wall of many pathogenic and non-pathogenic bacteria,[1] such as typhoid, dysentery, colibacilli and others, most of them belonging to the big class of *Enterobacteriaceæ*. Many of the non-pathogenic species are normal inhabitants of the digestive tract. Under the conditions of infection, these toxic substances are set free and, depending on their concentration, may cause a series of typical, more or less toxic manifestations: production of fever,[2] characteristic changes in the white blood picture (leukocytosis, etc.), stimulation of various hormone and enzyme systems[3, 4]—especially of proteolysis (fibrinolysis)[5]—and activation of many cellular systems mainly of the reticulo-endothelial system (RES).[6] These and further responses of the higher animal to *small doses* of endotoxins can be taken as an acute mobilization of the body's defence mechanisms against infection.[1, 6, 7, 8] Higher doses of endotoxin, however, may cause a marked fall of the number of circulating white blood cells, a decrease of defence mechanisms, severe cellular injury (of lungs, kidneys, the digestive tract, etc.) and haemorrhagic reactions.[3] Most of these acute endotoxic reactions are non-specific; endotoxins from many different bacilli cause the same type of reactions, and the stimulation of defence mechanisms by small amounts of endotoxin leads to protection against many different microorganisms.[1, 6, 7, 8]

Endotoxins are also antigens; they cause the production of antibodies, which can be demonstrated in the sera (immune sera) of infected or immunized organisms after a few days. These antibodies are specifically directed against the bacterial species from which the endotoxin has been derived. The production of antibodies can be taken as the second phase of the body's defence, leading to a more specific protection only against the infecting

micro-organism. Although these antibodies do not significantly reduce the toxic effects of isolated endotoxins,[9] they nevertheless are of great importance for the host, because they facilitate the incorporation of living bacteria into the white cells (phagocytosis) and increase the bacterial activity inside these cells.[10]

The study of the defence mechanisms of higher animals, especially man, against infections and other irritations gained new interest when bacterial endotoxins became available in highly purified and dosable form. The possibility of stimulating defence mechanisms, not only against microbial infections,[7, 8] but also against some viral diseases[11] and even against radiation injury,[12] by using purified endotoxins,[8, 11–13] has caused a great increase of interest on the part of many clinical research groups. One of the problems, with respect to possible therapeutic applications of these bacterial products, is to reduce their undesired side effects, such as fever, but at the same time to retain their therapeutic effectiveness.

During the past 30 years procedures have been worked out by chemists and microbiologists of various countries to extract endotoxins from suitable bacteria grown in mass cultures. It has been possible to purify these substances to a high degree and to analyse them with the aid of modern chemical and physico-chemical techniques.[1, 14]

The most active endotoxins were found to be water-soluble colloids, mainly carbohydrate in nature. They are high-molecular-weight complex polysaccharides, composed of various sugars. Besides the polysaccharide moiety, a lipoidic component was identified and, in fact, the most potent endotoxin preparations are lipopolysaccharides.[1, 14] Less than 0·1 μg of purified bacterial endotoxin, injected intravenously into man, produces a fever peak of 1–2° within a few hours.[13, 15] The threshold dose (i.v.) of lipopolysaccharides with respect to systemic reactions in higher animals and man was found to be of the order of 0·0005 to 0·005 μg/kg.

The acute reactions to an endotoxin injection are only transient; powerful inactivating enzymes, discovered in 1952 by Hegemann,[16] exist in the blood and tissue fluids of higher animals, including man, and these are now being studied in more detail by various groups.[17] Endotoxins are most potent after intravenous injection,

much less so after subcutanous or intramuscular routes. If highly diluted endotoxin solutions are sprayed, absorption through the lungs may cause typical systemic reactions (fever, etc.). In some Swedish factories where bibles are printed on India paper and air has to be kept at high humidity by spraying warm water, the so-called bible printer's disease was very common. The disease is caused by the absorption of small droplets of water, contaminated with endotoxin-producing bacteria.[18]

The lethal dose of endotoxins depends largely on the species and to an even greater extent on the hormonal state of the individual. Adrenalectomized animals may be a hundred to a thousand times more sensitive towards the toxic effects of endotoxin than normal animals.[3] On the other hand, glands stimulated by trophic hormones of the hypophysis show a much greater tendency to develop haemorrhagic reactions after endotoxin, as demonstrated by Tonutti.[19] Rabbits of a sensitive strain will die after the intravenous injection of ~ 100 μg/kg, showing very marked leukopenia and often severe diarrhoea before death.[20] Clinical experience indicates that the human being may be relatively sensitive to endotoxins; an increase of the threshold dose of about $0 \cdot 05$–$0 \cdot 1$ μg to about 1 μg[21] will cause very marked side effects, high fever, marked leukopenia, and very often provocation of viral diseases (herpes) or of latent focal bacterial infections. Recently it was shown[22] that the susceptibility of mice to the lethal effects of endotoxins is largely dependent upon their bacteriological state. Animals maintained free of ordinary bacterial pathogens were found to be much more resistant than normally grown animals. The authors concluded that the pathological effects of endotoxins involve at least two unrelated mechanisms: a primary toxicity, and an immunological reaction manifested only in animals presensitized to endotoxins by prior exposure to Gram-negative bacteria.

It should be noted that some bacteria produce toxins of much greater toxicity as expressed in the lethal dose, for example the exotoxins of diphtheria, tetanus or botulinus. But their mode of action, as well as their chemistry, is completely different from that of bacterial endotoxins.

By short acid hydrolysis, endotoxic lipopolysaccharides may be dissociated into polysaccharide, lipid (lipid A) and free fatty

acids.[14, 23] The lipid moiety appears to be essential for non-specific endotoxic activities.[1, 24]

In 1945 W. F. Goebel *et al.* demonstrated that a toxic component exists in endotoxins which they termed 'T'.[25, 26] This component appears to be closely related to the lipid A moiety of endotoxic complexes. Lipid A is a characteristic component of the cell wall of many Gram-negative bacteria. Its chemistry is still incompletely known, but the main constituents are D-glucosamine phosphoric acid ester and long-chain fatty acids, including a large proportion of β-hydroxymyristic acid.[1, 23] Analytical data are in agreement with the assumption of lipid A as being a poly-(glucosamine phosphate)[1] to which long-chain fatty acids are bound by ester and amide linkages. If dispersions of the purified lipid material in water are injected into animals or man, many typical endotoxic reactions can be elicited, such as fever and changes in the white blood count.[24] The minimal pyrogenic dose (MPD), however, largely depends on the degree of dispersion (*loc. cit.*, [15] p. 115). Until now, the most active dispersions—lipid A from *E. coli* in 0·5 per cent low-molecular-weight dextran—showed about one fifth to one tenth the activity of the original lipopolysaccharide. Such lipid dispersions, injected into animals, also cause a marked increase in resistance to various experimental infections.[1, 24, 27, 28] The special chemical structures or combination of lipophilic–lyophilic groups responsible for non-specific endotoxic activities are still to be established. Various findings indicate that acute toxicity and pyrogenicity, and probably also resistance-enhancing activity, may be due to different characteristics of the complex bacterial lipopolysaccharides.[29]

The lipid-free polysaccharide component of endotoxins is devoid of any endotoxic activity and species-specific. It constitutes the *O*-antigen of the species. A large number of polysaccharides have been analysed.[14, 31, 33, 36] In our studies with Kauffmann, analysis of the sugar constituents of many *Salmonella*-,[31] *E. coli*-[34] and cross-reacting[32] *O*-antigens gave a close correlation of serotypes and chemotypes. For instance, the *O*-antigens of species belonging to one and the same *Salmonella* serogroup of the Kauffmann–White scheme[35] (A, B, C, D, E, etc.) are of the same chemotype, i.e. the specific polysaccharides of one serogroup were always found to be built up of the same series of sugar constituents. These highly

branched bacterial polysaccharides[37, 38] are sometimes composed of as many as 6 different monosaccharide constituents, including hexosamines, heptoses, hexoses, pentoses, 6-deoxyhexoses, and 3,6-dideoxyhexoses.[36, 39, 40, 41]

Quantitative studies on the inhibition of precipitation of polysaccharide-antibody systems by single sugar constituents demonstrated the role of terminal monosaccharides for the specificity of these polysaccharides,[30, 38, 42, 43] particularly of 3,6-dideoxyhexoses[41] which are linked terminally in the *O*-antigens of many clinically important enterobacterial strains. Inhibition studies with simple oligosaccharides, derived from the polysaccharides by partial hydrolysis, led to the elaboration of structures responsible for immunologic specificity.[38, 42–46]

The role of 3,6-dideoxyhexoses as immunologically determinant end-groups was confirmed by the synthesis of artificial antigens.[47] Colitose (3-deoxy-L-fucose),[48] the determinant terminal sugar in the endotoxic antigen of a *Coli* strain (*E. coli 0 111*), highly pathogenic for new-born infants and young children, and of cross-reacting *Salmonella 0 35* species (*S. adelaide*, *S. monschaui*) and *Arizona 0 20* (see [32]) was chemically coupled to an otherwise inert protein carrier (serum albumin). Injection of the artificial colitose protein complex into animals (goats) led to the production of antibodies which not only reacted with the artificial colitose antigen, but also cross-reacted with *E. coli 0 111* and serologically related strains. The artificial antigen is devoid of toxicity, because the groups responsible for endotoxic activities are absent. This is the first example of an antiserum against certain Gram-negative bacteria obtained after immunization with an artificial antigen containing only one sugar, colitose, which in the respective microbial polysaccharide antigen(s) is present as the immunologically determinant end-group. Further artificial antigens with bacterial sugars as the determinant groups are being synthesized and the properties of antibodies produced after immunization will be studied.

Another aspect of these investigations is the study of the many variations of bacterial species — including S→R variation,[49, 50] lysogenic transformation[51] (see also [46]) or crossing of different serotypes,[52]— reflected in the biosynthesis of numerous specific polysaccharide antigens. For instance, several hundred different

Salmonella serotypes have already been classified in the Kauffmann–White scheme[35] (see [31, 32, 38, 42, 43]). An equal number of different polysaccharide antigens exists; they can be extracted in larger amounts from any given bacterial serotype, further purified, and analysed with the goal of defining chemically the underlying structural species differences.

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