IMMUNOLOGY

Free Endotoxin

THE term "endotoxin" is used to denote the lipopolysaccharides of high molecular weight (1 million or above) which are considered to be integral structural units of the cell walls of Gram negative bacteria[1]. The conventional method of preparing endotoxin is by chemical extraction from intact cells (or cell walls) using, for instance, trichloroacetic acid at 4° C (ref. 2), 45 per cent aqueous phenol at 65°–68° C (ref. 3), or mixtures of ether and water at 6°–12° C (ref. 4). Heterogeneous extracts are obtained which may require extensive purification before they yield the final homogeneous product[5].

Endotoxins extracted from different sources have similar biological properties. These include toxicity, pyrogenicity, the ability to induce non-specific resistance to infection, diarrhoea, tumour necrosis and the local and general Schwartzman reactions. In certain growth conditions, we have found that an appreciable amount of toxic material indistinguishable biologically from endotoxin is liberated into the culture supernatants of a wide variety of Gram negative organisms. Preliminary chemical analysis of this material has shown the presence of lipopolysaccharide and ultracentrifugation has indicated a molecular weight of about 1 million. We propose to call this material "free endotoxin".

In addition to its other biological properties, when injected parenterally into mice in small doses, it induced hypothermia as did chemically extracted endotoxin. This has also been described in guinea-pigs[6] and rabbits[1].

Maximum yields of free endotoxin are obtained when bacteria are grown, with vigorous aeration, in a diffusible medium consisting of hydrolysed casein, sucrose and yeast extract at *p*H 6·5. *Vibrio*, *Salmonella*, *Escherichia* and *Serratia* strains grow to high bacterial densities (10^{10}–10^{11} organisms/ml.) and secrete free endotoxin in these conditions.

To investigate whether free endotoxin was produced by cell lysis, endotoxin contents were determined for cells grown on solid and in liquid media and for the culture supernatant from cells grown in liquid. Endotoxin content was assayed by the induction of hypothermia and diarrhoea in mice, a method we have found to be quick, reliable, and almost quantitative. Cells grown on solid and in liquid media possessed approximately equal amounts of endotoxin; the culture fluid of the latter contained in addition as much free endotoxin as could be extracted from the parent cells. These results suggest that free endotoxin is released because of excess production of cellular material during vigorous growth of the bacteria rather than as a result of cell lysis.

We have investigated in detail free endotoxin from *Escherichia coli* 078*K*80. A crude preparation containing free endotoxin was isolated from culture supernatants by precipitation with ammonium sulphate at a concentration of 65 per cent w/v. The yield of solid non-dialysable material from culture fluid endotoxin was about 0·1 per cent w/v. It had physical and biological properties

Table 1. BIOLOGICAL PROPERTIES OF FREE AND CHEMICALLY EXTRACTED ENDOTOXINS

Biological activity	Free endotoxin	Conventional cellular endotoxins Phenol extract	Ether extract
Effect on mouse temperature after parenteral injection	Hypothermic	Hypothermic	Hypothermic
Mouse lethality* (LD_{50}: μg)	370	> 600	550
Non-specific resistance to infection (ED_{50} : μg)	0·016	0·1	0·04
Rabbit pyrogenecity (FI_{40}: μg)†	17	31·7	25
Tumour necrosis in mice (Sarcoma 180)*	+	+	+
Schwartzman reactions			
(*a*) Local†	+	+	+
(*b*) General	+	+	+

* See ref. 9.
† FI_{40}: The dose producing an area of 40 cm² under a fever curve[10].
‡ Determined in mice[11].

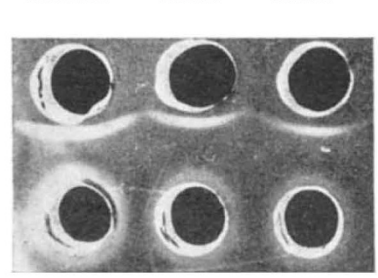

Fig. 1. Comparison of free and conventional endotoxins by immunodiffusion. Antiserum was prepared by immunizing rabbits against free endotoxin.

identical to those of crude endotoxin extracts of the intact parent cells[7]. Crude preparations of free and conventional endotoxins were examined by the Ouchterlony gel diffusion technique[8], and they were found to be immunologically indistinguishable (Fig. 1).

Our method of preparation is probably the mildest yet described and the least likely to lead to any degradation of endotoxin. Experiments have shown that the crude precipitated extracellular material can be further fractionated by column chromatography, yielding endotoxin and a non-toxic fraction. This latter substance has some of the immunological, but none of the toxic, properties of endotoxin and has a much smaller molecular weight[7].

M. J. CRUTCHLEY
D. G. MARSH
J. CAMERON

Bacteriology Department,
Wellcome Research Laboratories,
Langley Court,
Beckenham, Kent.

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Characterization of Antilymphocytic Antibody

ANTILYMPHOCYTIC serum (ALS) is now well known to have the power of prolonging the life of homografts on the animals into which it is injected[1–3]. All who have studied ALS agree that a high proportion of its immunosuppressive power resides in the 7*S* globulin fraction[2]. The experiments described here represent a formal analysis of the power of different fractions of ALS raised in rabbits and horses to prolong the life of A-strain tail skin homografts on CBA mice. Rabbit antisera against mouse thymocytes were prepared by the method of Levey and Medawar[3]. The horse antiserum, made by a scaled-up variant of the same technique, was kindly supplied to us by the Wellcome Foundation. The serum fractions were normally reconstituted to the concentrations at which they were originally present in whole serum, and assayed by the subcutaneous injection of 0·5 ml. on the second and again on the fifth days after skin grafting[3].

All sera were inactivated at 56° C for 30 min before further treatment. Separation into the 19*S*, 7*S*, 4·5*S*