These results suggest the possibility that serum from animals reacting to endotoxin might show a similar protective effect against a wide variety of infections. We also consider that the protective substance may be present in the blood serum of animals reacting to toxic agents other than endotoxin, by analogy with the idea that endogenous pyrogen is produced by tissues damaged by diverse pyrogenic agents. These possibilities and the nature of the protective substance are being studied.

A. W. Gledhill

National Institute for Medical Research,
Mill Hill, London, N.W.7.
Nov. 12.

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Isolation of a Lipopolysaccharide from *Vibrio fetus*

Vibrio fetus infection is an important cause of infertility in cattle throughout the world. Little is known of the pathogenesis of this disease, partly because of the difficulty of obtaining massive growth of the vibrio *in vitro*. The recent development in this laboratory of a method of obtaining satisfactory yields of cells[1] has enabled fractionation studies on *Vibrio fetus* to be undertaken.

A toxic lipopolysaccharide has been isolated by the phenol extraction method of Westphal *et al.*[2]. Before extraction, flagella were detached by shaking the cells in sterile saline for 1 hr.[3], and surface antigens removed by treatment with 0·5 *M* potassium thiocyanate for 72 hr. at 37° C.[4]. The lipopolysaccharide was dialysed at 0° C. to remove all traces of phenol and gave a viscid, opalescent solution. An apparently identical lipopolysaccharide has been recovered from both smooth and rough forms of *Vibrio fetus*.

The substance conforms to the general characteristics of the lipopolysaccharides of Gram-negative bacteria in being toxic, pyrogenic and antigenic. Examination of a sample of the lipopolysaccharide by Dische's sulphuric acid – cysteine method[5] revealed the presence of an aldoheptose component. The absorption spectrum in a Hilger 'Uvispek' showed a curve suggestive of a lipopolysaccharide with a minimum contamination with nucleic acid. Hydrolysis with 1 per cent (v/v) acetic acid at 100° C. for 3 hr. yielded a small amount of a chloroform-soluble lipid.

The toxicity and pyrogenicity is greatly enhanced by heating the solution at 80° C. for 5 min. Rabbits are particularly susceptible, and deaths have occurred at the dose-level of 400 μgm./kgm. administered intravenously. The lethal dose is reduced to 40 μgm./kgm. rabbit by pre-heating. Nevertheless, rabbits withstand intramuscular injections of 10 mgm. of unheated lipopolysaccharide solution without apparent ill-effects. Mice are less susceptible, and the lethal dose is in the vicinity of 2 mgm. administered intravenously.

A febrile response in rabbits may be elicited with as little as 4 μgm./kgm., and the pyrogenic dose is reduced by heat treatment to as low as 0·005 μgm./kgm. Increases in temperature of 3–7·9° F. have been recorded. The temperature rises sharply within the first hour, reaches a peak in the majority of cases in 3 hr. and slowly falls to normal. The return to normal may take more than 24 hr.

Lipopolysaccharides, from both smooth and rough variants, are adsorbed on to human 'O' erythrocytes and give hæmagglutination when tested against homologous *Vibrio fetus* whole-cell rabbit antisera. The use of *Vibrio fetus* lipopolysaccharide in such a test may be of value in providing earlier and more accurate diagnosis of the disease in cattle. The lipopolysaccharide is a suitable antigen in the 50 per cent-hæmolysis-unit complement-fixation test using *Vibrio* antisera from rabbits. Further serological studies with this lipopolysaccharide are being undertaken.

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S. M. Dennis

Department of Veterinary Pathology,
University of Sydney.
Nov. 11.

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Effect of Lytic Enzyme from *Bacillus circulans* and Chitinase from *Streptomyces* sp. on *Aspergillus oryzae*

The enzyme contained in a culture fluid of *Bacillus circulans* has been shown to exert lytic activity towards *Aspergillus oryzae* and that a polymer of melibiose was liberated from the cell wall. However, the cell wall was not completely lysed by the enzyme owing to the large amount of chitin present. During the search for enzymes causing lysis of fungal cells, it was found that a mixed preparation of chitinase and lytic enzyme exerted strong lytic activity on the cell wall of *A. oryzae*.

In this communication some observations pertaining to the action of chitinase and lytic enzyme, and to the fine structure of the cell wall are given.

The cell wall and the lytic enzyme were prepared by methods previously described. The crude chitinase was used as a chitinase preparation[2]. The mycelia of *A. oryzae* which had been previously shaken in the peptone–starch medium for 12 hr. at 30° C. was used as intact cells. Washed living mycelia and the cell wall were suspended in the various enzyme solutions using *tris*-buffer *M*/50, *p*H 6·8 and were incubated at 37° C. Turbidity of the suspension was determined by the Itô Beckman type spectrophotometer at 10-min. intervals over a period of 1½ hr.

As shown in Fig. 1, the lysis progressed more rapidly when the lytic enzyme was added together with the chitinase than when added alone. Moreover, after 2 hr. had elapsed scarcely any cell wall and intact cells could be detected by either a phase-contrast microscope or an electron microscope.

40 mgm. of the cell wall were suspended in a solution consisting of 20 ml. of the lytic enzyme solution, 20 ml. of the chitinase solution or 20 ml. of the