PHYSIOLOGY

Composition of and Physiopathology produced by Plague Endotoxins

Early attempts to isolate an endotoxin from *Pasteurella pestis* by most classical methods have failed. Recently Davies[1] isolated a lipopolysaccharide containing a heptose and phospholipid by extraction in hot phenol water. Cocking *et al.*[2] produced a similar toxin by the use of ultra-sound and purification by column chromatography. These workers also reported briefly on the pathology produced by their toxins.

The purpose of the present investigation was to explore further the chemical composition of plague endotoxin and to observe its physiological and pathological effects in laboratory animals.

Endotoxins were prepared from acetone-dried *P. pestis* either by extraction in hot phenol[1] or by grinding in a ball mill followed by enzymatic digestion with 'Pronase' (California Corporation for Biochemical Research) and by ethyl-ether extraction to remove non-essential proteins and lipids. Phenol extracts were further purified by alcohol precipitation and enzymatic digestion to remove the bulk of non-essential protein.

Both preparations showed the presence of one or two antigens in Ouchterlony gel diffusion plates using Lederle's or Squibb's antiplague serum against whole organisms. Neither reacted with antibody to plague protein exotoxin in gel plates. The $L.D._{50}$ of the phenol-extracted endotoxin was 3 mg for mice and 25 mg for guinea-pigs. The $L.D._{50}$ of ball-mill endotoxin for mice was 20 mg, which was later improved to 0·75 mg. This has not yet been tested in guinea-pigs.

Endotoxins were prepared from three different plague strains, 195/*P*, *EV*-76 and *TRU*. A 7-carbon sugar showing peak absorption at 510 mμ was detected in all three endotoxins by the sulphuric acid–cysteine method of Dische[3].

Lipids from sixteen different plague strains were obtained by simple overnight extraction in chloroform methanol (50/50). All extracts on silica gel *G* thin-layer chromatoplates showed at least three lipid-like substances. Two of these migrated at rates similar to lecithin and cephalin controls prepared from egg yolk. After hydrolysis in hot sodium hydroxide, the three endotoxins already mentioned showed a single cephalin-like lipid migrating at a rate similar to phosphatidyl ethanolamine.

Ball-mill endotoxin, in doses of half the $L.D._{50}$, was injected into mice which were then bled and killed at 3-h intervals for 21 h. During the first 12 h three physiopathological trends occurred—a decrease in liver glycogen, a decrease in plasma glucose, and an increase in blood urea nitrogen (Fig. 1).

Phenol-extracted endotoxin injected into mice and guinea-pigs, and ball-mill endotoxin injected into mice, led to major signs of peripheral vascular collapse at autopsy. There were petechiate haemorrhages, haemoglobin imbibition and enlarged congested capillaries or vessels in various organs and tissues. In mice, brain tissue, the heart and liver were most frequently involved. In guinea-pigs the intestinal tract, subcutaneous tissues, peritoneal wall and adrenals were most frequently congested or haemorrhagic. Microscopical examination of kidney sections also showed acute renal tubular necrosis in both mice and guinea-pigs. Sections of the liver showed vacuolization of parenchymal cells resembling acute fatty metamorphosis. These changes were most striking in mice killed 48 h after inoculation.

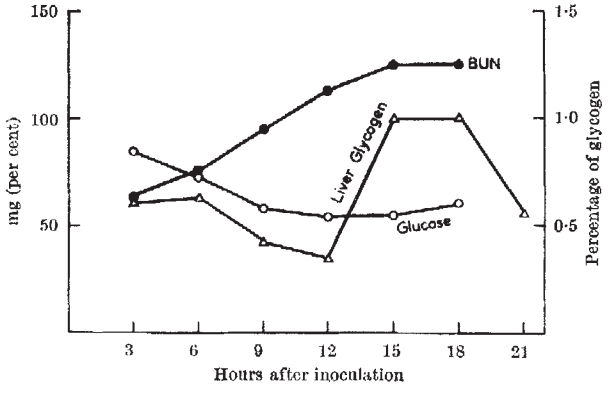

Fig. 1. Changes in blood glucose, blood urea nitrogen (*BUN*) and liver glycogen after intraperitoneal injection of 10 mg of *P. pestis* 195/*P* endotoxin

These results show further that plague organisms do have an endotoxin similar in structure and physiological or pathological effects to endotoxins of other Gram-negative organisms.

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[1] Davies, D. A. L., *Biochem. J.*, **63**, 105 (1956).
[2] Cocking, E. C., Keppie, J., Witt, K., and Smith, H., *Brit. J. Exp. Pathol.*, **61**, 460 (1960).
[3] Dische, Z., *J. Biol. Chem.*, **204**, 983 (1953).

Variable Diastolic Ventricular Compliance : a General Property of Mammalian Cardiac Muscle

Studies of the extensibility of quiescent cardiac muscle have been the cause of recurrent controversy. Starling's law of the heart relates the energy of contraction to the length the muscle fibres attain just prior to contraction. In studies of the regulation of cardiac contraction the assumption is made that measurement of pressure within the ventricle during diastole provides an accurate index of ventricular volume and that changes in pressure thus predict changes in fibre length[1]. This assumption requires that the compliance of ventricular muscle, that is, the change in length for a given change in force, remain constant throughout diastole in spite of changing conditions. Although there have been indications in the older literature that the compliance or 'tone' of the heart may change during diastole as a dynamic variable[2], present interpretations of cardiac performance are based on the existence of constant diastolic extensibility[3].

Recently[4,5] we demonstrated that the diastolic compliance of the *in situ* dog ventricle changes in a predictable manner when the force of contraction is altered by changing the pattern of stimulation. In these experiments pairs of stimuli (PS) were employed to cause a premature contraction after each regular contraction; prior to and concomitant with the resulting postextrasystolic potentiation of contraction (PESP) there was a change in diastolic volume at constant pressure[5]. It also was possible to demonstrate differences in pressure at constant volume in the right heart by-pass preparation with a sutured tricuspid valve[5]. Sonnenblick *et al.* specifically looked for such changes in compliance and concluded: "As in all of our previous studies on isolated heart muscle, there was no evidence that a change in extensibility of the muscle had been produced by paired stimulation"[6]. For this reason, as well as to extend the results of our own *in vivo* findings, we have studied the diastolic compliance of the cat papillary muscle *in vitro*. In the investigations reported in this article, the sensitivity of the recorder was up to 40 times greater than that used by previous workers[6]. The records obtained show clear evidence that PESP and other experimental interventions alter diastolic compliance. The changed compliance is manifested as either a decrease