formed with the other ANSAI, and ibuprofen was the most effective drug in blocking directional movement when added in the chamber (figure 2B). The chemotactic activity was completely abolished starting from the lowest concentrations of ibuprofen, whereas the other drugs exhibited their effect in the range of 10^{-8}–10^{-4} M. This cell behaviour was confirmed by retesting PMN from the same individuals in separate blood samples. On the other hand, pre-incubation of PMN with this anti-inflammatory drug, which selectively inhibited the directional movement, did not interfere with random movement. The mechanism by which acidic non-steroidal anti-inflammatory agents inhibited PMN cellular functions are speculative. Since ANSAI have been shown to interfere specifically with PG synthetase system, which transforms the suitable fatty in prostaglandins[9-11], it is likely, therefore, that these agents may carry on their pharmacological effect on human PMN throughout such a mechanism. Most recently it has been reported that low concentration of indomethacin, as 10^{-8}–10^{-5} M, inhibited PG production in PMN from rat[12] and human eosinophil[13], respectively. The data obtained, added to those with endogen mediators of inflammation[14], suggested that it is possible to hypothesize that the 2 forms of locomotions, random motility and chemotaxis, are 2 dissociable processes.

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2 P.C. Wilkinson, Exp. Cell Res. *103*, 515 (1976).
3 P.C. Wilkinson, Exp. Cell Res. *93*, 420 (1975).
4 P.C. Wilkinson and McKay, Eur. J. Immunol. *2*, 570 (1972).
5 P.C. Wilkinson, in: Chemotaxis and Inflammation, p. 168. Churchill-Livingstone, Edinburgh 1974.
6 S.H. Zigmond and J.G. Hirsch, J. exp. Med. *137*, 387 (1973).
7 J.F. Borel, Experientia *29*, 676 (1973).
8 D.A. Norris, W.L. Weston and W.M. Sams Jr, J. Lab. clin. Med. *90*, 569 (1977).
9 J.R. Vane, in: Inflammation, p. 261. Ed. I.M. Lepow and P.A. Wald. Academic Press, New York 1972.
10 J.R. Walker, M.J.H. Smith and A.W. Ford-Hutchinson, Agents Actions 6, 602 (1976).
11 F.A. Kuehl, J.L. Humes, R.W. Egan, E.A. Ham, G.C. Beveridge and C.G. Van Arman, Nature *265*, 170 (1977).
12 F. Dray, E. McCall and L.J.F. Youlten, Int. Congr. Inflammation, Bologna 1978, Abstr. p. 140.
13 G. Marone, A.K. Sobotka and L.M. Liechtenstein, Int. Congr. Inflammation, Bologna 1978, Abstr. p. 139.
14 S. Spisani and S. Traniello, Proc. Soc. exp. Biol. Med., submitted for publication.

Protective effect of native levan on endotoxin toxicity in mice and rats[1]

M. Michowitz, Judith Leibovici and M. Wolman[2]

Departments of Surgery B, Ichilov Municipal-Governmental Medical Center and of Pathology, Tel-Aviv University Sackler Medical School, Tel-Aviv (Israel), 29 September 1978

Summary. High polymer levan administered to mice and rats before the injection of endotoxin partly protects the animals from the lethal effects of the LPS.

Endotoxic shock is a common cause of morbidity and mortality in patients suffering from a variety of disease processes[3]. It probably represents an important pathogenetic mechanism underlying tissue damage in sepsis, burns and hypovolemic shock. The mortality rate in endotoxic shock has been reported to amount to 50–70% of patients[4,5]. Native levan, a polyfructoside of an average mol. wt of 2×10^7, is known to inhibit the passage of proteins and cells across the endothelial barrier[6-8]. Coating of endothelial surfaces by levan might be the main reason for its inhibitory effect on processes such as acute inflammation[9,10] and graft rejection[11]. Endotoxin was shown to bind primarily to the endothelial surface of blood vessels[12] and to affect circulating cells[13].

The present paper describes experiments in which the effect of levan administration on the toxicity of endotoxin was tested on mice and rats.

Materials and methods. Endotoxin (E.coli 0111:B4 lipopolysaccharide W Difco) was dissolved in pyrogen-free 0.85% NaCl solution. Animals were injected i.p. or i.v. with 0.2 ml of solution containing the desired amounts of endotoxin. *Aerobacter levanicum* levan was prepared according to Hestrin et al.[14] with consequent alkaline ethanol precipitation in order to free the levan from endotoxin[9]. A 5% levan solution was administered to mice i.p. in amounts of 10 or 25 mg per mouse and 50 mg per rat. As the batch of levan used in these experiments caused death in some animals, they were pretreated with small doses (5–10 mg) of levan on a number of alternate days before the endotoxin injection. Balb/c mice of both sexes, 20–25 g in weight, and Charles River female rats, 200–250 g in weight, were used. The rats were sensitized to endotoxin by i.p. injection of 10 mg of lead acetate dissolved in 0.2 ml of 0.85% NaCl solution together with the endotoxin[15]. 3 animals of each experimental group and 3 controls were killed by neck dislocation and their lungs and kidneys taken for histological examination.

Results. In repeated experiments on over 100 animals, levan injected together with or after the endotoxin had no beneficial effect on survival. The protective effect of levan was obvious, however, in animals started on levan *before* the injection of endotoxin.

Protective effect of levan on endotoxin toxicity in levan pretreated mice

Group	Endotoxin per 20-g mouse	Levan i.p.	Mortality within 20 h	Mortality within 24–26 h
1	0.8 mg	–	9/11	11/11
2	0.8 mg	10 mg	3/8	4/8
3	0.8 mg	25 mg	0/8	0/8
4	1.2 mg	–	10/10	10/10
5	1.2 mg	10 mg	4/10	7/10
6	1.2 mg	25 mg	4/10	9/10

In a typical experiment mice were injected with 0.55 mg of endotoxin per 20 g b. wt. 6 out of 12 animals died within 24 h (LD_{50}). Not 1 mouse died in a group of 12 treated i.p. on 7 alternate days before the test, and given 10 mg of levan 2 h before the endotoxin. In a 3 group of mice, 1 out of 13 died after a similar treatment in which the last 4 doses of levan were of 25 mg.

In other experiments, the protective effect of levan was tested on mice given endotoxin in different doses (table 1). In the control series, all 11 mice given 0.8 mg endotoxin per 20 g b. wt died within 24 h. Of 10 mice treated with 1.2 mg endotoxin, all were dead after 15 h. It can be seen in the table that in this experiment levan exerted a marked protective activity on animals given a dose of endotoxin which was approximately 1 MLD, and had some protective effect even on a 1.5 MLD dose. The effect consisted of decrease in mortality and in a somewhat prolonged survival time.

Histological study of the lungs of animals protected by levan showed normal structure. In the animals killed by endotoxin, the pulmonary septa appeared swollen with congestion and leukostasis. No contributory findings were observed in the kidneys of mice of either group.

In a typical experiment on rats, an i.p. dose of 0.1 mg endotoxin administered together with 5 mg of lead acetate per 100 g b. wt caused death in 8 of 10 animals. Administration of 50 mg of levan (after 3 preliminary doses of levan during the week preceding the test) protected all 16 animals. With i.v. injections of a similar dose of endotoxin, all 10 animals died within 24 h, while i.p. levan (50 mg) protected 3 of 7 rats.

Discussion. The experiments show that levan treatment has a protective effect on endotoxin toxicity in mice and rats. Administration of levan prior to the injection of endotoxin reduced its lethality in animals and prolonged life by a few h in animals which were not saved. It has been shown[7] that death in animals given endotoxin is due to both the direct effect of the injected endotoxin and to the effects of endogenous endotoxin derived from enteric bacteria. This might account for the observation that levan had a clear cut effect on lethality over a rather narrow range of endotoxin concentrations.

1 This study summarizes a thesis in partial fulfilment of the requirements for the degree of Master of Medical Sciences in the School of Continuing Medical Education of the Tel-Aviv University Sackler Medical School.
2 Established Investigator of the Bureau of the Chief Scientist, Ministry of Health, Israel.
3 D.P. Fossard and V.V. Kakkar, Br. J. Surg. *61*, 798 (1974).
4 J.H. Duff, G. Malave, D.I. Peretz, H.M. Scott and L.D. MacLean, Surgery *58*, 174 (1965).
5 L.S. Hershey, in: «Shock», p.43, 129, 160, 227. Ed. Little. Brown, Boston 1964.
6 A. Behar and M. Shilo, Am. J. Path. *57*, 383 (1969).
7 D.T. Caridis, M. Ishiyama, P.W.H. Woodruff and J. Fine, J. Reticuloendoth. Soc. *14*, 513 (1973).
8 A.M. Davies, M. Shilo and S. Hestrin, Br. J. exp. Path. *36*, 500 (1955).
9 M. Shilo and B. Wolman, Br. J. exp. Path. *39*, 652 (1958).
10 M. Shilo, M. Wolman and B. Wolman, Br. J. exp. Path. *37*, 219 (1956).
11 J. Leibovici, I. Bleiberg and M. Wolman, Proc. Soc. exp. Biol. Med. *149*, 348 (1975).
12 H.S. Rubenstein, J. Fine and A.H. Coons, Proc. Soc. exp. Biol. Med. *111*, 458 (1962).
13 D.G. McKay, W. Margaretten and I. Csavossy, Lab. Invest. *15*, 1815 (1966).
14 S. Hestrin, M. Shilo and D.S. Feingold, Br. J. exp. Path. *35*, 107 (1954).
15 H. Selye, B. Tuchweber and L. Bertok, J. Bacteriol. *91*, 884 (1966).

Renal injury after muscle extract infusion in rats: Absence of toxicity with myoglobin[1]

S. O'Regan, J.S.C. Fong and K.N. Drummond

Department of Nephrology, McGill University, Montreal Children's, Hospital Research Institute, 2300 Tupper Street, Montreal (Quebec, Canada B3H 1P3), 14 April 1978

Summary. A crude muscle extract infused into rats produced oliguria, a precipitous drop in total hemolytic complement, and in circulating white cell and platelets counts. A mild vaso-depressor effects was noted. These changes were not produced by myoglobin or saline infusion. Muscle constituents other than myoglobin are responsible for the systemic and renal nephrotoxic effects observed.

Animal[2] and human studies[3,4] of the crush syndrome suggest to us that muscle constituents other than myoglobin could be responsible for the renal tubular damage.

Materials and method. Muscle extract was prepared from muscle dissected from cold saline perfused (via abdominal aorta) hind limbs of anaesthetized 400-g hooded rats after vena caval effluent was clear. A nonheated muscle homogenate in 0.15 M saline was made and the resultant suspension was centrifuged at 7800 × g for 10 min at 4 °C, the supernate (protein conc. 28 mg/ml) rapidly frozen in 3 ml aliquots and stored at − 70 °C until used. Immediately before each experiment the required volume was rapidly thawed in a water bath at 30 °C and diluted with cold saline to 2 ml.

Experimental group awake male rats were infused with the 2-ml saline diluted muscle extract (10 mg protein/100 g) over 20 min via a right external jugular vein cutdown. Rats were immobilized on a metabolic frame which allowed sequential uncontaminated urine collection; freely flowing blood was taken from the tail. Sequential peripheral white blood cell and differential counts were done using a Fisher Autocytometer 2 (Fisher Scientific Co., Ltd) and Wright stained blood smears. Platelet counts were done using a Neubauer hemacytometer. Total hemolytic (CH_{50}) complement activity was also studied[5]. Each of these studies was done prior to extract infusion, and at 5 min, 30 min, 1, 2, 3, 4 and 24 h.

The period between muscle extract infusion and the 1 urine voided was noted; urinalysis was done on this specimen. Kidney tissue was studied 1, 3, 5 and 24 h following the infusion for histopathologic and immunopathologic findings. Cryostat sections were cut at − 20 °C from snap frozen kidney tissue and stained for muscle extract proteins