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

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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.

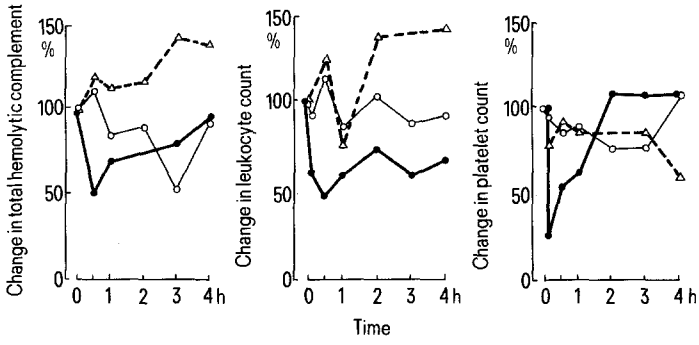
Materials and method. Muscle extract was prepared from muscle dissected from cold saline perfused (via abdominal aorta) hind limbs of anaesthetized 400-g hooded rats after vena caval effluent was clear. A nonheated muscle homogenate in 0.15 M saline was made and the resultant suspension was centrifuged 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

Mean arterial pressures in muscle extract, myoglobin and saline infused rats

Time after infusion	Muscle extract		Myoglobin		Saline	
	Systolic	Diastolic	Systolic	Diastolic	Systolic	Diastolic
0 min	103	78	102	75	98	70
15 min	82	60	110	82	107	85
30 min	100	73	104	78	100	80
60 min	109	91	114	86	100	75
2 h	124	99	110	88	101	78
4 h	130	101	118	78	103	80
5 h	125	102	116	84	104	78



Percentage in total hemolytic complement, leucocyte count and platelet counts in rats following muscle extract (●——●), myoglobin (○——○) or physiologic saline (△----△) infusion. Each point represents the mean value of 6 studies.

by an indirect immunofluorescent technique using rabbit antiserum to rat muscle extract and fluorescein isothiocyanate labelled goat antirabbit 7S globulin.

The same parameters were studied in control groups (6 animals each). 1 control group was infused with 2 ml normal saline and the other control group was studied with myoglobin infusion (10 mg protein/100 g in 2 ml) (horse myoglobin type 1, Sigma Chemical Co., St. Louis). Blood pressure was monitored in 6 other experimental and 3 saline and 3 myoglobin infused animals via femoral artery catheters (PE10) connected to a transducer (Statham, Puerto Rico) and A-V monitor (Med. Science, St. Louis).

Results. After the muscle extract infusion, there was a precipitous fall in total white blood cell and platelet counts and in CH_{50} with a return towards normal values by about 4 h; at 24 h values were above pre-infusion levels (figure). Myoglobin injection was associated with a leukocytosis in the 1st 4 h with leukopenia in both control groups at 24 h. No precipitous drop in CH_{50} levels was noted in either of the control groups. CH_{50} of all animals studied was at a pre-infusion level by the end of the 24-h period. A mild vaso-depressor effect of the muscle extract was noted 15 min after infusion (figure). In 5 control animals induction of hypotension, by venous blood withdrawl of 70 mm Hg maintained for 30 min did not produce oliguria greater than 1 h.

A variable degree of oligoanuria (duration 2–3 h) was induced in the experimental group. Microscopic examination of the first voided urines were as follow; numerous granular casts, 2–5 red blood cells/hpf; a dipstick urine test indicated a neutral pH, 50–100 mg% protein, and large amounts of glucose and blood. A consistent form of tubular damage, localized in the proximal tubule and consisting of vacuolization of the cells, disruption of the brush border, and proteinaceous material within the tubular lumina was observed. There were no significant glomerular changes. Immunopathologic studies were negative. The control groups had only transient myoglobinuria in the myoglobin injected rats. Light and immunofluorescent studies of control animal tissues were negative. Storage of $-70\,^{\circ}C$ was required to maintain toxicity of supernatant.

Discussion. A precipitous drop in leukocytes, platelets and hemolytic complement was noted in experimental animals shortly following muscle extract infusion. By the 24th h, no difference in the complement level was noted among the 3 groups of animals. Leukopenia was present in both control groups at 24 h. We have no explanation for this observation. The mild vaso-depressor effect of the extract infusion was not sufficient to induce renal shutdown.

All previous experimental models of myoglobin nephropathy have used supplementary manipulations, e.g. dehydration, induction of hypovolemia or administration of toxic chemicals[8]. These manipulations themselves are potentially nephrotoxic. In clinical reports urinary myoglobin may be detectable only by immunoassay[9]. The observations indicate that myoglobin is not nephrotoxic.

Instead of applying supplementary manipulations, we examined the possibility that other muscle constitutents released during muscle injury, in addition to myoglobin, might be nephrotoxic. Our study with crude muscle extract infusion versus saline or myoglobin in rats, supports this hypothesis. Although we have not yet isolated the nephrotoxic fraction(s) we conclude that other muscle consistents are responsible for the renal and systemic changes observed and that pathogenesis of the crush syndrome. The lability of this nephrotoxic fraction and the requirement of storage at $-70\,^{\circ}C$ to maintain toxicity suggests that it undergoes enzymatic digestion unless deep frozen.

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