Altered Reactivity to *Escherichia coli* Endotoxin of Mice Subjected to Sub-Lethal Tourniquet Treatment. (25211)

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One group of investigators has reported enhanced susceptibility of rabbits following hemorrhagic shock to the lethal action of the endotoxins of gram negative bacteria(1,2). The significance of this observation with regard to the pathogenesis of the irreversible stage of hemorrhagic shock remains controversial(2,3,4). The mechanisms through which susceptibility to bacterial endotoxin is increased are not completely known; and the extent to which this phenomenon operates in other animals and in shock produced by means other than hemorrhage has not been clearly defined. This study demonstrates that significantly less endotoxin was required to elicit a lethal end point in mice subjected to sub-lethal tourniquet treatment, and that prophylactic isotonic saline administration significantly reduced 24-hour mortality in mice which received endotoxin after tourniquet treatment.

Materials and methods. Webster strain white swiss mice of either sex, weighing 18-20 g, were employed throughout. The basic experimental design employed 4 groups of mice. *Group I* was subjected only to the standard tourniquet procedure (*vide infra*). *Group II* was given a single predetermined intravenous dose of *Escherichia coli* endotoxin contained in 0.5 ml physiological saline. *Group III* was subjected to the standard tourniquet procedure employed in Group I and was then given the same amount of endotoxin as Group II one hour after tourniquet removal. *Group IV* was given 4 ml of sterile physiological saline (at room temperature) intraperitoneally 30 min. after the standard tourniquet procedure. Thirty minutes following saline these animals were given *E. coli* endotoxin as in Group II. All mortality data were based on a 24-hour observation period. The *Escherichia coli* endotoxin preparation was extracted by the procedure of Boivin *et al.*(5) from cells grown in fluid cultures under forced aeration. The LD_{50} of a single intravenous dose of this material contained in 0.5 ml, determined by the method of Reed and Muench(6) from titration of serial 2-fold dilutions in normal mice employing 8 mice per dilution, was 117 μg. Sub-lethal tourniquet "shock" in mice, based on results of preliminary experiments, was induced by application of a tightly wound rubber band placed as high as possible on the left lower extremity. Constriction was released after 2 hours. The quantity of edema fluid in the injured extremity was determined by a previously reported bisection technic(7). Gain in weight of injured over normal leg permitted an estimate of the amount of extravasated fluid.

Results. Tourniquet application to one hind limb of a mouse for 2 hours was followed by marked local limb changes. Under these conditions, however, mortality over a subsequent 96-hour period following tourniquet removal was negligible, as indicated by 100% survival of 20 mice so treated in a preliminary experiment. The severity of the "state of shock" in mice subjected to tourniquet treatment could not be quantitated. However, the severity of the local damage could be gauged roughly by amount of edema, as judged by increase in limb weight over that of the opposite untreated limb. *Table I* records paired limb weights in a series of animals 24 hours after tourniquet removal and demonstrates that, on the average, tourniquet treated limbs weighed 0.32 g more than the untreated members, a gain in weight presumably caused by extravascular fluid accumulation.

Mice subjected to such sub-lethal tourniquet treatment, nevertheless, succumbed to lower doses of *E. coli* endotoxin than did nor-

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TABLE I. Weight Increase in Bisected Hind Limbs 24 Hours after Tourniquet Removal in 10 Mice.*

	Limb wt (g)		
	Tourniquet side, left	Normal side, right	Difference (tourn. – norm.)
	.70	.47	.23
	.84	.53	.31
	.75	.46	.29
	1.00	.57	.43
	.84	.55	.29
	.78	.45	.33
	.82	.50	.32
	.94	.63	.31
	.95	.57	.38
	.90	.60	.30
Avg	.85	.53	.32

* Mean difference in wt of left hind limb over right hind limb of 10 normal mice was 0.05 g.

mal mice. This increased "susceptibility" was observed by 2 different experimental approaches. *Table II* shows, over a range of endotoxin dosages, increased death rate of tourniquet treated mice as compared to control mice. These results were observed regularly and are all statistically significant ($p < .04$). *Table III* demonstrates that only one-fourth as much endotoxin is required to elicit a 50% lethal end point in tourniquet treated mice as compared to controls. The results of Table III are reproducible and statistical analysis by the methods of Reed-Muench(6) and Pizzi(8) indicate $p < .01$.

Attempts were made to determine if the enhanced mortality of tourniquet treated mice receiving *E. coli* endotoxin could be prevented by prophylactic saline administration. Preliminary experiments indicated that 1-2 ml of physiological saline intraperitoneally did not prevent enhanced mortality. However, when 4 ml of physiological saline was given intraperitoneally 30 minutes after tourniquet removal and 30 minutes prior to 150 μg endotoxin, a protective effect was observed (Table II). These experiments were performed in triplicate with reproducible results and are statistically significant ($p < .01$). When 10 normal mice were pretreated with 4 ml. of physioligical saline 30 minutes prior to 150 μg endotoxin administration no protective effect was observed.

TABLE II. Mortality of Tourniquet Treated Mice Receiving *E. coli* Endotoxin.

		24 hr mortality after endotoxin dose*			
Group	Treatment	150 μg	100 μg	50 μg	12.5 μg
I	Tourniquet only	0/16†	0/16	0/16	0/16
II	Endotoxin "	8/16	5/16	4/16	2/16
III	Tourniquet and endotoxin	16/16	16/16	10/16	8/16
IV	Tourniquet saline‡ and endotoxin	9/16			

* Single intrav. dose of endotoxin 1 hr after removal of 2 hr tourniquet from 1 hind limb.

† Deaths/total.

‡ 4 cc of physiological saline given IP 30 min. after removal of 2 hr tourniquet from 1 hind limb.

Discussion. Significantly less endotoxin was required to elicit a lethal end point in tourniquet treated than in normal mice. The cause(s) of enhanced lethality in such mice, though unknown, might be associated with several factors, e.g.; (1) plasma loss, (2) passage of bacteria or their products across an intestinal membrane made increasingly permeable by ischemia, (3) release of toxic substances from the ischemic limb, (4) activation of the gas gangrene bacillus, (5) reticuloendothelial system injury.

TABLE III. LD_{50} of *E. coli* Endotoxin for Normal and Tourniquet Treated Mice.*

Group	LD_{50}† (μg)
Normal	117
Tourniquet treated	31

* Determined by single intrav. inj. of 2-fold endotoxin serial dilutions into groups of 8 mice/dilution.

† Calculated by method of Reed and Muench(6).

If mouse plasma volume be gauged from known mammalian plasma volumes(9), then roughly 25-35% of the original plasma volume of about 1 ml is lost into the tourniquet limb. Such mice experiencing a depletion of original plasma volume which in itself does not cause death, nevertheless, exhibit an enhanced lethality with *E. coli* endotoxin. However, if such mice are given large volumes of physiological saline (room temperature) intraperitoneally, no enhanced lethality is observed. Simple administration of large volumes of physiological saline has been shown (10) to reduce mortality in mice in which tourniquet treatment of both hind limbs would ordinarily lead to a fatal outcome. This amelioration of lethal effect acts presumably through a restoration of circulating blood vol-

ume. Indeed if lethal quantities of endotoxin diminish peripheral vessel reactivity to physiological regulators of vascular tone as described by Zweifach *et al.*(11) in an animal whose plasma volume is already reduced, a lethal outcome might result from cumulation of these two effects alone. However, since the protective effect of saline was obtained when administered early in the course of tourniquet shock and prior to endotoxin challenge, it is uncertain as to whether the protective effect was related to plasma volume replacement *per se*, and /or to inhibition of some train of events secondary to plasma volume depletion. such as increased absorption of bacterial endotoxin from the gastrointestinal tract.

Time sequence of introduction of bacterial endotoxin is probably important in susceptibility during shock. Zweifach and Thomas (12) have observed that rats treated with endotoxin *prior* to induction of non-lethal hemorrhagic or drum shock had a higher mortality than rats receiving endotoxin *after* such shock induction. It is emphasized that the 4-fold increase in susceptibility of tourniquet treated mice reported in this study is based on observations limited to a sequence of administration of endotoxin one hour *after* tourniquet removal.

The need for large amounts of saline in excess of estimated plasma loss to protect tourniquet treated mice receiving bacterial endotoxin may be attributed to the rapid loss of the administered saline into the injured area and adjacent tissue(13) and the slow peritoneal absorption. The observation that large amounts of saline failed to protect normal mice receiving endotoxin suggests that the saline does not act by simple endotoxin dilution and supports the concept that plasma volume loss constitutes the major factor in initiating the enhanced lethality of tourniquet treated mice receiving endotoxin.

Summary. Significantly less *E. coli* endotoxin (administered as single intravenous injection) was required to elicit a lethal end point in tourniquet treated mice than normal mice. Early intraperitoneal administration of large volumes of physiological saline significantly negated the effects of tourniquet treatment as regards lethal outcome with a given *E. coli* endotoxin dose. Such saline therapy failed to prevent death in normal animals given endotoxin. These observations plus the estimation of 25-35% original plasma volume loss to the traumatized area suggests that reduction of plasma volume is the major factor in initiating enhanced mortality of tourniquet treated mice receiving *E. coli* endotoxin.

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