

THE RELATIONSHIP BETWEEN THE VASCULAR MANIFESTATIONS OF SHOCK PRODUCED BY ENDOTOXIN, TRAUMA, AND HEMORRHAGE

I. CERTAIN SIMILARITIES BETWEEN THE REACTIONS IN NORMAL AND ENDOTOXIN-TOLERANT RATS*

BY BENJAMIN W. ZWEIFACH† PH.D., AND LEWIS THOMAS, M.D.

WITH THE TECHNICAL ASSISTANCE OF P. VIERA AND C. MIKROS

(From the Department of Pathology, New York University-Bellevue Medical Center, New York)

(Received for publication, April 29, 1957)

The progression of the hemorrhagic shock syndrome toward a lethal outcome carries with it the potential of instigating and perpetuating changes which become increasingly difficult to correct by blood replacement measures. Although the evidence (1) strongly favors a derangement of the peripheral circulation as the ultimate defect leading to the "irreversible" breakdown of circulatory homeostasis, there is no agreement on the factors responsible for the progressive impairment of vascular integrity. Studies of the biological effects of the lipopolysaccharide endotoxins of Gram-negative bacteria (2, 3), particularly with respect to the vascular reactions to epinephrine, have indicated a close similarity between the conventional shock syndrome induced by hemorrhage or trauma and the circulatory collapse produced by lethal doses of endotoxin. Although Fine and Rutenburg (4) have postulated that the absorption of bacteria or their products into the blood is the cause of the collapse of the circulation during shock, the complexity of factors involved in the syndrome makes this hypothesis extraordinarily difficult to prove or disprove.

In the present study, certain points of similarity between the vascular reactions during hemorrhagic, traumatic, and endotoxin shock are presented. It is shown that animals rendered tolerant to endotoxin develop resistance to both traumatic and hemorrhagic shock, although the opposite kind of cross-tolerance could not be demonstrated. Moreover, small sublethal doses of endotoxin were proved capable of greatly enhancing the development of irreversible fatal shock during sublethal episodes of hemorrhage.

Good and Thomas (5) have reported that intravenous injections of colloidal

*Aided by grants from the National Heart Institute, United States Public Health Service (H-2267), the Life Insurance Medical Research Fund, the Research and Development Division, Office of the Surgeon General, Department of the Army, under Contract No. DA-49-007-MD-590, and the American Heart Association.

† Established Investigator of the American Heart Association.

materials taken up by the cells of the reticulo-endothelial system, such as thorotrast, trypan blue, or saccharated iron oxide, greatly increased the susceptibility of rabbits to the lethal action of bacterial endotoxin. Later, McKenna and Zweifach (6) demonstrated that the administration of similar colloidal substances undermined the capacity of both normal and drum-resistant rats to withstand shock. An extension of the observations indicating that the functioning of the reticulo-endothelial system may be of importance in determining resistance or susceptibility to both endotoxin and traumatic shock is contained in the paper which follows this one (7).

Materials and Methods

Shock was produced in rats by graded hemorrhage and by exposure to trauma in the Noble-Collip drum. A new strain of rats, Carworth Farm Nelson (CFN), specially bred to be resistant to upper respiratory infections (8), was used in the present study. Most of the animals were females of 120 to 150 gm. body weight. Bleedings were made by way of the carotid artery through a cannula which was connected to a self-regulating bleedout reservoir (9). The blood pressure was lowered by graded bleedings to a level of 60 to 65 mm. Hg for 1 hour and then to 35 to 40 mm. Hg for an additional 2 hours. At this point, the blood remaining in the reservoir system was returned, the incisions closed, and survival at 48 hours recorded. In all instances, rigid aseptic precautions, including sterilization of all glassware, tubing, instruments, and solutions, were maintained. Traumatic shock was induced by the rotating drum procedure (40 revolutions/minute), originally introduced by Noble and Collip (10).

A lipopolysaccharide endotoxin derived from *Escherichia coli*, prepared by the method of Landy and Johnson (11) by the Difco Laboratory, Detroit, was used in all experiments to be described. The material was dissolved in double-distilled, pyrogen-free water and freshly prepared for each experiment.

EXPERIMENTAL

Comparison of the Circulation in the Mesentery during Hemorrhagic and Endotoxin Shock

Hemorrhagic Shock.—Previous studies of the microcirculation during the development of irreversible hemorrhagic shock (12) have shown that the reaction passes through two distinct phases, an initial stage of compensatory adjustments, succeeded in turn by decompensatory sequelae of varying extent and severity. The altered behavior of the small blood vessels in each stage coincided with an associated set of changes in vascular reactivity of terminal arterioles and precapillary sphincters, and an orderly restriction of the capillary circulation to preferential channels. Decompensation was associated with the loss of spontaneous vasomotion, a progressive hyporeactivity, and an inability to maintain an effective venous outflow from the capillary bed.

Inasmuch as the Nelson strain of rat has not in the past been used for studies of the microcirculation during shock, a group of 20 of these animals were subjected to graded hemorrhage (65 mm. Hg for 1 hour, 35 mm. Hg for an additional 2 hours) as a basis for comparison with the endotoxin series. Particular attention was paid to the action of epinephrine in furthering the development of hyporeactivity and the subsequent deterioration of the capillary circulation.

During the 1st hour after bleeding (hyperreactive phase), topical epinephrine in above-threshold amounts (2 to 4 $\mu\text{g./ml.}$) resulted in complete vasoconstriction and cessation of capillary flow which was unrelieved for periods up to 4 to 5 minutes, and after which the circulation did not return to control levels for at least another 3 to 5 minutes. During the 2nd hour of hypotension, the application of epinephrine at frequent intervals led to extensive capillary stasis. Another indication of the deleterious action of epinephrine was the early development of hyporeactivity in tissues exposed repeatedly to constrictor doses of this agent. Preparations, which were left undisturbed for several hours, or were exposed for study 1 to 2 hours after shock had been induced, showed a decline in reactivity only during the 3rd hour of shock or after blood replacement. In instances in which the vessels were challenged with epinephrine at regular intervals during the period of impaired circulation, they developed a decreasing reactivity as early as 90 to 120 minutes after the first bleedings. A similar regime of epinephrine tests in controls had no effect on vascular reactivity. In animals subjected to non-lethal hemorrhage (65 mm. Hg for 1 hour and 40 to 45 mm. Hg for 2 hours), epinephrine served only to accentuate the hyperreactive state and did not cause the vessels to become unreactive, as in fatal shock.

Note should also be made of the fact that the small blood vessels became extraordinarily sensitive to changes in temperature within 15 to 20 minutes after the onset of bleeding. Coincident with the appearance of an augmented reactivity to epinephrine, the muscular vessels showed an immediate and sustained closure when the temperature of the irrigating medium bathing the mesentery was dropped by as little as 1–2°C. This feature was in marked contrast to control animals in which the vessels showed little or no response to fluctuations in the temperature of the irrigation fluid of as much as 6–8°C.

Endotoxin Shock.—Emphasis in a previous paper (3) was placed on the augmented response to epinephrine as the basic defect underlying the pathological manifestations of bacterial endotoxins. It is evident that hyperreactivity by itself was not the explanation for the lethal action of these materials, since an equivalent epinephrine response was elicited by doses of endotoxin which were non-lethal and were not associated with extensive vascular pathology. Further investigation has indicated that rats given endotoxin show a number of associated derangements in the microcirculation which, either in conjunction with the altered attitude of the small blood vessels to epinephrine, or as a result of this situation, may account for the pathological changes involved.

The studies were conducted in 20 rats which received overwhelming doses of *E. coli* endotoxin (4 to 5 mg./100 gm. body weight, intravenously). This dose of endotoxin was lethal to 85 to 90 per cent of the animals, collapse and death developing in from 6 to 12 hours. The rats were prepared for microscopic examination of the mesocecum at various times after administration of endotoxin.

The earliest spontaneous disturbances were an erratic opening and closing of the small arterioles and precapillary sphincters during the first 15 to 20 minutes. This phase persisted for a comparatively short time and at 1 hour after the endotoxin had been administered, the capillary bed appeared normal. Thereafter, the capillary

circulation began to be slowed, particularly in the collecting venules, despite the absence of measurable changes in the caliber of the feeding arterioles or precapillaries. Spontaneous vasomotor movements became less evident and were completely absent by the third hour (Table I). Blood pressure measurements during the first 2 hours indicated no significant fall in arterial pressure. The hypotensive effect of lethal doses of endotoxin did not develop in the rat until between the 3rd and 4th hour after administration of the *E. coli* preparation, when the blood pressure fell progressively to 60 to 70 mm. Hg, and remained in this range until the terminal collapse.

TABLE I
Comparison of Vascular Changes during Reaction to Endotoxin and Hemorrhage

Animals	Vasomotion*	Reactivity to epinephrine‡		Stasis		Petechial hemorrhages
		Precapillary	Venule	Capillary	Venule	
Controls	+	0.5 ± 0.2	2.0 ± 0.4	0	0	0
<i>Phase 1</i>						
Endotoxin	+++	0.005 - 0.01	0.01 ± 0.005	+	+	+
Hemorrhage	+++	0.0025 - 0.01	1.0 ± 0.3	0	0	0
<i>Phase 2</i>						
Endotoxin	±	3 - 10	0.01 ± 0.004	++	++	++
Hemorrhage	±	5 - 10	1.0 ± 0.2	+	+	0

Data represent observations on 20 rats in each of the categories indicated.

±, indicates occasional, barely recognizable changes in caliber.

* Vasomotion refers to spontaneous cyclic changes in caliber of terminal arterioles and precapillary sphincters—graded arbitrarily according to frequency of the cycles.

‡ Figures refer to average concentration of epinephrine which produced threshold response in precapillaries and venules.

The contractile process *per se* showed a number of characteristic changes which offer some insight into the mechanism of action of bacterial endotoxins. In normal animals, the application of threshold concentrations of epinephrine to the mesentery elicited a short-lived closure of the vessels which then reopened gradually over a period of about 20 to 30 seconds. In animals given endotoxin, the contraction-relaxation sequence was strikingly changed, chiefly in the venules. It was noted that coincident with the heightened sensitivity to epinephrine previously recorded (3), the constrictor phase of each reaction became disproportionately prolonged. Thus, the vessels remained constricted with threshold concentrations of epinephrine for as long as 4 to 5 minutes, in contrast to 30 to 40 seconds for control animals. The subsequent relaxation phase was even more profoundly altered. The muscular venules (50 to 75 μ in diameter) which at first did not regain their original caliber for periods up to 8 to 10 minutes after a single epinephrine stimulus, with successive exposures to epinephrine, no longer relaxed and persisted in a partially narrowed state. These changes were associated with a drastic curtailment of capillary blood flow and the bypassing of large segments of the terminal vascular bed.

In our previous report it was shown that extensive hemorrhagic necrosis of the skin was produced by epinephrine in endotoxin-treated rabbits (2). Lesions occurred after an intradermal injection of epinephrine in animals given endotoxin by vein or when mixtures of epinephrine and endotoxin were injected locally. The mesenteric vessels of the rat were observed to undergo comparable destructive changes, when large doses of epinephrine were tested in endotoxin-treated animals. In the present study, 5 to 10 μg . of epinephrine were applied at about 5 minute intervals onto the mesentery of control and endotoxin-treated rats. After 5 to 6 applications, the arterioles and precapillaries in controls remained constricted for periods of 3 to 4 minutes, but rarely beyond this time. Prolonged spasm of venules did not develop, nor did petechial hemorrhages occur in the affected area. Repeated applications of epinephrine in the endotoxin-treated rat brought about complete vasoconstriction involving both arterioles and venules. Cessation of blood flow persisted for periods as long as 15 minutes; and even after reopening of the arterioles, the circulation remained markedly slowed for 1 hour or longer. In such preparations, petechiae appeared throughout the area, particularly around collecting venules and postcapillaries.

Continued irrigation of the tissue with epinephrine for 60 minutes (10 μg ./ml./minute) produced a similar sequence of changes but with this additional development: after irrigation with epinephrine had been stopped, the arterioles dilated almost maximally and were now hyporeactive, failing to constrict even after 20 to 30 μg . epinephrine. The venules, on the other hand, remained comparatively unaffected, giving rise to a situation in which the normal gradient of vessel reactivity was completely reversed. Thus the capillary circulation was inordinately slowed in a bed where the arterioles were dilated and refractory to epinephrine, and the venules narrowed and unduly reactive.

In endotoxin-treated animals which had been left undisturbed for about 2-3 hours before the mesoecum was exteriorized for study, the capillary circulation was sparse and ischemic. During the ensuing 2 hours, there was a progressive reopening of the capillary circulation, together with the appearance of petechial hemorrhages in the postcapillaries and collecting venules. Spontaneous rupture of vessels and petechial hemorrhages occurred without the application of epinephrine, as early as 1 hour in animals given the largest doses of endotoxin (200 μg . or greater). With small doses of endotoxin, petechiae did not appear unless the tissue was exposed to repeated applications of epinephrine.

As in the case of rats subjected to hemorrhagic shock, the small blood vessels became unusually sensitive to temperature. During the shock reaction induced in anesthetized animals with *E. coli* endotoxin, the body temperature fell to levels as low as 28-32°C. Under these conditions, when the exteriorized mesoecum was artificially maintained at 38°C., the capillary bed continued to show an active circulation despite a considerable rise in vascular activity. When the temperature of the irrigating fluid was lowered in accord with that of the body, vasoconstriction and capillary ischemia were considerably accentuated during the hyperreactive stage. After hyporeactivity had been developed, fluctuations in temperature had almost no effect on the capillary circulation.

The Reaction of Endotoxin-Tolerant Rats to Hemorrhage and Trauma

As is well known, rabbits, rats, and mice can be made to tolerate lethal amounts of endotoxin by exposing them to a series of injections of increasing dosage (13, 14). Development of tolerance did not seem to depend on the formation of specific antibody, since animals resistant to endotoxin from one bacterial species are tolerant to endotoxin from immunologically unrelated microorganisms. Tolerance is usually of brief duration, but can be maintained for long periods by repeated injections of endotoxins.

In the present experiments, tolerance to *E. coli* endotoxins was induced by the following arbitrarily selected dosage schedules. Successive daily intraperitoneal injections for 5 days, respectively, of 50, 100, 150, 200 μg . of *E. coli* endotoxin resulted in tolerance to test doses of 4.0 mg., given 72 to 96 hours after final injection. Rats, in which endotoxin tolerance had been induced by this method, were then tested for their capacity to withstand lethal episodes of hemorrhagic or drum trauma.

The results of the tests indicated a high degree of protection against both forms of shock, as is shown in the following sections.

Hemorrhagic Shock.

Paired control and endotoxin-tolerant rats were subjected to graded bleedings so as to maintain the blood pressure at 65 mm. Hg for 1 hour and then at 35 mm. Hg for an additional 2 hours. Blood replacement brought about recovery of only 33 per cent (5/15) of the controls, whereas 80 per cent (12/15) of the endotoxin-tolerant rats survived.

Examination of representative protocols indicated that the protective action was associated with an increased output of blood during the hemorrhage, a negligible uptake from the blood reservoir, and a rapid restoration of the blood pressure to control levels with blood replacement. At autopsy, the viscera (particularly the liver, small intestines, and adrenals) showed no evidence of the congestion and stasis characteristic of irreversible shock in control animals. The small intestines were contracted and pale, in contrast to the atonic, purplish appearance in controls subjected to shock.

Traumatic Shock.

Rats can be rendered highly resistant to drum shock by repeated daily exposure to sublethal episodes of drum trauma (15). A similar degree of resistance to trauma was demonstrated in the animals in which tolerance to endotoxin had been induced. These observations are summarized in Table II.

The dosage and timing of endotoxin injections were found to be crucial features in the development of the resistance to trauma, as is illustrated by the experiment cited: Several groups of rats were given daily doses of endotoxin in the following order: 50, 100, 200, 400, 800, and 1500 μg ., administered intraperitoneally at 2-day intervals. These animals failed to exhibit resistance to trauma when tested at 72 and 96 hours after the last dose. Tolerance to endotoxin was also demonstrably less at this time.

The day on which the endotoxin-treated rats were tested with trauma was also important. When drum shock was induced at 24 to 48 hours after the final injection of endotoxin, instead of 72 hours, no resistance was demonstrated.

About 10 to 15 per cent of the animals tested were unable to tolerate hemorrhagic or drum trauma. In fact, these animals, after a course of endotoxin, appeared to be more susceptible than untreated controls to shock. In the case of hemorrhage, they they invariably died during the procedure. It is therefore possible that these animals were not rendered tolerant by the endotoxin regime, but on the contrary were less tolerant than normal rats.

TABLE II
Endotoxin Tolerance and Drum Trauma

Category	Survival*
Controls	3/20 (15%)
Endotoxin-tolerant	9/10 } 9/10 } (87%) 8/10 }
Drum-resistant	18/20 (90%)

* 850 turns in Noble-Collip drum.

The Production of Endotoxin Tolerance in Adrenalectomized Animals

In view of the known vulnerability of adrenalectomized animals (16) and the protective action of cortisone against endotoxin shock (17), experiments were undertaken to determine whether tolerance to endotoxin or trauma could be induced in adrenalectomized rats.

Rats were subjected to bilateral adrenalectomy in one stage and maintained on 1 per cent salt in their drinking water for several weeks for recovery from the operative procedure. The adrenalectomized animals were highly susceptible to the lethal action of *E. coli* endotoxin. Thus, whereas 2 to 3 mg. of this preparation were required to produce a consistent lethal effect in controls, the adrenalectomized rats were killed by as little as 250 μ g.

It was found that tolerance to endotoxin could be readily established in adrenalectomized rats by two procedures. In one set of experiments, the adaptive regime was begun with small doses of *E. coli* endotoxin (10 μ g.), and higher levels built up by 10 μ g. increments over a period of several weeks (group A). By this means, adrenalectomized rats were made tolerant to 1 mg. of endotoxin (8/10 survived as compared with 1/10 in controls). In another set of adrenalectomized rats, a small dose of cortisone (2 mg./100 gm. body weight) was administered at each step of the endotoxin schedule. When tolerance had been established by giving 10, 20, 40, 80, and 160 μ g. of *E. coli* endotoxin intraperitoneally on 5 successive days, the cortisone was dis-

continued and tolerance retained by two doses, each of 150 μ g. of endotoxin, spaced at 72-hour intervals (group B).

Previous studies (15) have shown that adrenalectomized rats can be made resistant to drum trauma by exposure to sublethal drummings, with a schedule which began with 50 rotations and was increased at 2-day intervals by an additional 50 rotations in the drum. By this method the adrenalectomized rats were able to tolerate 650 rotations in the drum (11/12 survived as compared with 0/12 adrenalectomized controls).

Adrenalectomized rats, rendered tolerant to endotoxin, were found to withstand greater amounts of trauma than did untreated adrenalectomized animals (Table III).

TABLE III
Effect of Adrenalectomy on Cross-Tolerance to Drum Trauma

Category	Survival	
Adrenalectomized controls	6/20	(30%)*
Adrenalectomized endotoxin-tolerant		
Group A	19/20	(95%)*
Group B	8/10	
	9/12	(75%)‡
	10/12	
Adrenalectomized drum-resistant	18/20	(90%)‡
	8/10	(80%)‡

* 500 turns in Noble-Collip drum.

‡ 650 turns in Noble-Collip drum.

Group A, initial dose 10 μ g., successive increments of 10 μ g. on alternate days until 100 μ g. is given.

Group B, received 5 mg. cortisone throughout period of endotoxin regime, until tolerance established. No cortisone for 1 week prior to drum challenge.

Thus, 500 rotations represented an LD₇₅₋₈₀ dose in conventional adrenalectomized rats. With the development of endotoxin tolerance, rats in group A tolerated exposure to 500 rotations in the drum without lethal outcome (19/20 survivors). Animals in group B withstood 500 rotations with no deaths (20/20 survivors). These animals also withstood 650 rotations in the drum, an LD₁₀₀ dose level, with only 20 to 25 per cent deaths (8/10; 9/12; 10/12 survivors in three separate runs). However, they could not withstand 850 rotations in the drum, an amount successfully handled by normal rats with acquired resistance to endotoxin (3/12 survivors in adrenalectomized rats; 11/12 in conventional series).

The Reaction to Endotoxin in Trauma-Resistant Rats

It has already been established that resistance to trauma can be engendered, either by a regime of sublethal trauma or by a schedule of endotoxin as de-

scribed above. The question was then raised whether trauma-resistant animals were also tolerant of normally lethal doses of endotoxin.

Drum-resistant rats were found to show some carry-over with respect to their capacity to tolerate bacterial endotoxin, but this was neither as consistent nor as pronounced as the tolerance achieved by repeated exposure to *E. coli* preparations (see Table IV). Thus, when a series of 50 trauma-adapted animals was injected with lethal doses of endotoxin (3 to 4 mg.) two of the groups showed a clear cut tolerance to *E. coli* material (8/10; 9/10), while the three groups showed no significant differences from controls (3/10; 2/10; 4/10).

Drum-resistant rats showed an increased capacity to withstand traumatic shock for at least 12 to 15 days following completion of their training regime. Thereafter, the tolerance to shock declines progressively unless the rats receive maintenance or

TABLE IV
Lethal Effects of Bacterial Endotoxins

	Survival*	
Controls	2/20	(10%)
Endotoxin-tolerant	9/10	(90%)
	11/12	(91%)
Drum-resistant		
Group A	8/10	} (85%)
Group B	9/10	
Group C	2/10	
Group D	4/10	} (36%)
Group E	3/10	

* 4.0 mg. *E. coli* extract, intraperitoneally.

booster doses of trauma. Cross-tolerance to endotoxin in these animals could not be demonstrated when they were challenged with bacterial lipopolysaccharides at 8, 10, or 12 days after trauma-resistance had been established.

Inconstant results were also obtained at different times of the year, and with various modifications of the drum training regime. It was not possible to determine the factor responsible for the variable results. The fact that cross-tolerance was obtained in some of the groups which were made trauma-resistant may be of greater significance than the negative findings in this regard.

Evidence for some cross-tolerance to endotoxin was obtained in a series of 24 trauma-resistant rats pretreated with 100 to 250 μ g. of *E. coli* endotoxin 1 to 2 hours before exposure to trauma, in which only 4 animals succumbed to 850 rotations in the Noble-Collip drum. It will be recalled that conventional controls, treated with endotoxin prior to exposure to normally non-lethal shock, became more susceptible and showed a survival of only 40 per cent compared with 90 per cent in untreated controls.

Further support for a common factor in the phenomena of drum resistance and endotoxin tolerance is derived from experiments dealing with the maintenance of

tolerance for protracted periods of time. Trauma-adapted rats lost their resistance unless they were exposed at about 7 to 10 day intervals to maintenance episodes of drum trauma (300 rotations in the drum was usually sufficient). When, instead of drum trauma as the challenge, resistant rats were given weekly injections of bacterial endotoxins (100 μ g. of *E. coli* material intraperitoneally), the animals retained their resistance to lethal drum trauma for at least 60 days (14/15 survived). In the analogous situation, that of acquired tolerance to endotoxin, a similar set of circumstances prevailed. Ordinarily, tolerance to lethal doses of endotoxin could be retained by an injection of from 100 to 200 μ g. of *E. coli* endotoxin once or twice weekly. It was possible to substitute exposure to 300 rotations in the drum as the stimulus for maintenance and thereby to retain tolerance to 4 mg. of endotoxin for periods of at least 6 to 7 weeks (15/15).

The Enhancement of Hemorrhagic or Traumatic Shock by Simultaneously Administered Endotoxin

It was anticipated that the introduction of lipopolysaccharide extracts of Gram-negative bacteria at different stages of the shock experiment might provide an indication of their relative importance in the syndrome. For this purpose, *E. coli* endotoxin was administered during the course of a non-lethal episode of hemorrhagic shock (65 mm. Hg for 1 hour and 40 mm. Hg for 2 hours). Several contingencies were examined separately; endotoxin was given either before the induction of shock, during the shock episode, or during the post-transfusion recovery period. It was soon found that the dose of endotoxin and the mode of administration were important determinants of the subsequent effect. Doses which were lethal by themselves or which made the rats obviously ill were not used. In the majority of experiments, 100 to 250 μ g. of the *E. coli* preparation were used, since these amounts had been shown to elicit changes in the microcirculation of the mesentery without outward evidence of toxicity.

The exacerbating effects of a single injection of 250 μ g. of bacterial endotoxin are indicated in Table V for hemorrhagic shock and in Table VI for drum trauma. The most striking effects were obtained with pretreatment which consistently undermined the capacity to tolerate shock. When the endotoxin was given 1 to 2 hours in advance of drum trauma, the collapse following trauma developed with unusual rapidity, many of the animals dying during the bleeding procedure. With graded hemorrhage in pretreated animals, the compensatory phase appeared only in abbreviated form, while the decompensatory uptake of blood from the reservoir became more prominent, the rats taking up as much as 50 to 60 per cent of the volume lost.

Experiments were also conducted to demonstrate whether bacterial endotoxin would either counteract already existing compensatory readjustments or would accelerate the shift into a decompensatory pattern of behavior. Doses of 100 to 250 μ g. of endotoxin, injected at 30, 60, 90, 120, and 180 minutes after the induction of hemorrhagic hypotension (65 mm. Hg for 1 hour, 40 mm. Hg for an additional 2 hours), were all without effect. As was the case with untreated controls, there was no spontaneous uptake of blood and the animals were readily reversible by blood replacement measures. Endotoxin added to the final blood infusion, did not alter the recovery phase nor affect the eventual outcome, unless given in large amounts (500 μ g. or greater).

The above experiments could be criticized on the grounds that a single injection of endotoxin was probably removed from the bloodstream within a few minutes. A closer approximation of the spontaneous course of events was attempted by infusing the bacterial preparation slowly with a motor-driven microburette which could be adjusted to deliver from 0.01 to 0.03 ml./minute so that a total of 50 to 250 μg . in 1 ml. was injected during a 30 to 60 minute period.

TABLE V
Effect of Single Dose of E. coli Extract on Non-Lethal Episode of Hemorrhagic Shock

Time of endotoxin* administration	Survival
None given	7/8 (87%)
Prior to bleedings	2/7 (28%)
After 1 hr. at 65 mm. Hg	2/3 (67%)
After 1 hr at 65 mm. Hg and 1 hr. at 40 mm. Hg	4/5 (80%)
After 1 hr. at 65 mm. Hg and 2 hrs. at 40 mm. Hg	1/4 (25%)
1 hr. post-transfusion	4/8 (50%)

* 250 μg . of *E. coli* endotoxin given intravenously.

TABLE VI
Effect of Bacterial Endotoxin on Drum Shock

Time of administration	Dose (i.p.)	Survival*
Controls	—	13/20 (65%)
2 hrs., pretrauma	150 μg . †	1/10 (10%)
1 hr., pretrauma	150 μg .	2/10 (20%)
0 time	250 μg .	8/15 (53%)
1 hr., post-trauma	250 μg .	6/15 (40%)
3 hrs., post-trauma	150 μg .	4/15 (27%)

* 650 turns in Noble-Collip drum.

† *E. coli* extract, intraperitoneally.

The slow intravenous infusion of endotoxin had a considerable effect on the counter-shock reaction following hemorrhage. The accompanying Table VII lists the effects observed when endotoxin was administered for 1 hour during different phases of the hemorrhage reaction. In each instance, there was no direct effect but after an interval of from 60 to 75 minutes, decompensatory uptake of blood from the reservoir developed. Thus, the earlier in the syndrome the *E. coli* endotoxin was infused, the more rapid was the development of decompensation. The number of deaths were significantly higher in each of the categories listed.

It was possible with infusions of endotoxin to bring on the development of decompensatory uptake, as early as 1 hour after hemorrhage had been instituted, in a syndrome which normally did not show any of the decompensatory stigmata of shock. Blood loss was poorly tolerated, maximum output being reduced from control levels of 3.9 to 4.2 per cent of body weight to 1.5 to 2.0 per cent; blood replacement failed

to restore the blood pressure to levels above 70 mm. Hg in contrast to the 95 to 120 mm. Hg achieved in controls; and the lethal outcome was 60 per cent (6/10 deaths) as compared with 20 per cent (2/10 deaths) in control experiments.

Effect of Endotoxin on Microcirculation during Hemorrhagic Shock

A series of studies on the microcirculation in the mesentery was conducted in 10 rats to determine whether the deleterious effect of endotoxin on the reaction to hemorrhage was associated with changes in the response of the small blood vessels to epinephrine. In these experiments, the animals were bled in graded steps to sustain the blood pressure at 65 mm. Hg for 1 hour and then at 40 mm. Hg for an additional 2

TABLE VII
Influence of Bacterial Endotoxin on Response to Graded Hemorrhage

	Blood reservoir		Survival†
	Output	Uptake	
	<i>per cent body wt.</i>	<i>per cent body wt.</i>	
Untreated	3.7	0.15	8/10
1 hr. preshock*	1.93	0.2	0/4
0 time	3.1	0.35	0/4
1 hr. 65 mm. Hg	3.5	0.2	2/4
1 hr. 65 mm. Hg	3.2	0.45	0/4
1 hr. 40 mm. Hg			
1 hr. 65 mm. Hg			
2 hrs. 40 mm. Hg	3.1	0.5	1/4
1st hour post-transfusion	3.6	0.2	1/4

* 250 $\mu\text{g.}$ *E. coli* extract infused intravenously at rate of 5 $\mu\text{g./minute}$ during 30 minute period.

† 65 mm. Hg for 1 hr. and 40 mm. Hg for additional 2 hrs., followed by blood replacement.

hours. In untreated controls, the typical hyperreactivity to epinephrine developed in the terminal arterioles and precapillaries (epinephrine threshold in controls 0.5 to 1.0 $\mu\text{g./ml.}$). Reactivity levels returned to normal within 15 minutes after completion of the blood replacement.

The systemic administration of bacterial endotoxin during the 1st hour of the hypotensive period, by slow infusion in amounts varying from 2.0 to 4.0 $\mu\text{g.}$ *E. coli* extract/minute, was sufficient to attenuate the progressive development of hyperreactivity to epinephrine. Instead of thresholds of 0.01–0.05 $\mu\text{g./ml.}$ of epinephrine, levels of 0.02–0.05 $\mu\text{g./ml.}$ were attained (a 5- to 10-fold change as contrasted with the 50- to 100-fold change in controls). This phase was then followed by an abrupt decline in reactivity to subnormal levels. Bacterial endotoxin, infused after hyperreactivity to epinephrine had already reached its peak (2nd to 3rd hour), produced no observable effect for at least 20 to 30 minutes, when a rapid decline in reactivity developed. In all the experiments in which *E. coli* endotoxin was given during the course of the shock reaction, blood replacement failed to restore epinephrine reactivity to control levels.

The injection of large doses of endotoxin (500 $\mu\text{g.}$ to 750 $\mu\text{g.}$) during the 2nd or 3rd hour of hypotension, had a more immediate and drastic effect on the microcirculation. Hyporeactivity to epinephrine developed with extraordinary rapidity (within 15 minutes) in conjunction with extensive capillary and venular stasis which was not overcome by blood replacement measures.

DISCUSSION

The circulatory collapse, produced by trauma, hemorrhage, or bacterial endotoxins, show in common an almost identical derangement of the response of the small blood vessels to epinephrine. The strikingly similar course of these reactions raises the question whether we are dealing in all three cases with the same basic entity—endotoxemia (18)—or whether each of these contingencies ultimately interferes with the same set of homeostatic mechanisms and is related to the others only in so far as the end-result is concerned.

Three sets of findings in the present study lend support to the fundamental similarity of the reaction induced by hemorrhage, trauma, and bacterial endotoxins: (a) the near identity of the alterations in the microcirculation, particularly the altered response to epinephrine, culminating in a profound hyporeactivity; (b) the increased resistance to hemorrhagic and traumatic shock in animals rendered tolerant to bacterial endotoxins; (c) the conversion of a non-lethal episode of hemorrhagic shock into a typical "irreversible" syndrome by the administration of small doses of endotoxin.

Obviously, the close resemblance of the microcirculatory events does not by itself constitute sufficient proof that the ultimate collapse in each of the various forms of shock is mediated through the same basic pathway, particularly since it has been shown (19) that comparable effects on vascular behavior can be induced by a variety of humoral agents and tissue factors. On the other hand, when the vascular findings are considered, in conjunction with the experiments on tolerant animals, the endotoxin evidence becomes highly suggestive of an etiological relationship.

Alterations in the functional status of vascular smooth muscle during the conventional shock syndrome have in the past been measured on the basis of the response to topical epinephrine or norepinephrine (12). There was no direct means of establishing whether the vascular dysfunction operative during the syndrome was in fact concerned with epinephrine *per se*. In the case of shock produced by *E. coli* extracts, it could be demonstrated that the altered reactivity of the small blood vessels was exhibited only in response to epinephrine and norepinephrine and not to constrictor agents such as 5-hydroxytryptamine, pitressin, neosynephrine, and angiotonin (2). The exacerbating action of epinephrine on the development of hyporeactivity in all three forms of shock further substantiates the implied importance of this hormone in the breakdown of smooth muscle homeostasis.

Studies on the microcirculation during the irreversible phase of hemorrhagic shock (20) have indicated that the inhibition of vascular smooth muscle developed to the greatest extent in visceral structures, such as the omentum, mesentery, intestinal wall, and to a lesser degree in vessels of the skin or skeletal muscle. The vascular effects in the present experiments with endotoxin shock have been recorded exclusively in the microcirculation of the mesentery. Studies on the circulatory reactions in other tissues are needed to substantiate the systemic implications of the endotoxin-epinephrine relationship.

In view of the emphasis placed on the close resemblance of the vascular sequelae produced by bacterial endotoxins and by graded hemorrhage, note should be made of some of the differences which were encountered. Under normal circumstances, the arterioles, metarterioles, and precapillary sphincters are the most sensitive elements of the terminal vascular bed to both constrictor and dilator stimuli. In shock produced by lethal doses of the *E. coli* extract, the arterial to venous gradient of reactivity in the mesentery is reversed and the venous vessels become the most reactive components of the terminal vascular bed. This feature by itself may be responsible for the reduced capillary flow and impaired venous return observed during the development of endotoxin shock. The circulatory collapse produced by overwhelming doses of endotoxin is consistently associated with widespread injury to the capillary vessels. Neither of these stigmata develops to any significant extent in hemorrhagic shock. It is possible that this difference is a matter of degree, since in the case of shock produced by endotoxin, vasotoxic manifestations are maximally developed, whereas in hemorrhagic or traumatic shock, numerous ancillary factors initiate the hypotensive state and endotoxemia may be involved only secondarily.

The most convincing evidence in favor of a bacterial factor in the evolution of irreversible shock derives from experiments which show that animals, made tolerant to bacterial endotoxins, are also resistant to traumatic and hemorrhagic shock. Although the cross-over is neither as complete, nor as effective, as is the case when the test challenge is the same as the agency used to induce tolerance, the findings clearly indicate that both forms of resistance effectively circumvent vascular hyporeactivity following normally lethal episodes of shock. The endotoxin-tolerant animal is not completely indifferent to the lipopolysaccharide extract. Thus, 4.0 mg. of *E. coli* endotoxin, which are normally lethal and induce profound vascular hyporeactivity in the mesenteric bed, produce in tolerant rats an increased reactivity to epinephrine but none of the associated capillary stasis or petechial hemorrhages. When endotoxin tolerant rats are subjected to hemorrhage, they likewise show only hyperreactive sequelae in the microcirculation, with no evidence of hyporeactivity or vascular stasis.

The converse situation, the tolerance of trauma-resistant rats to the lethal

effects of bacterial endotoxins, is much less consistent. Exceptions were encountered with different batches of rats treated as a group at various times throughout the year. In those instances in which endotoxins were lethal to drum-resistant animals, shock was accompanied by massive hemorrhages in the wall and lumen of the atonic intestines, the liver was enlarged, mottled, and congested, the lungs showed patchy areas of consolidation, and lymph nodes were uniformly filled with blood. In contrast, when protection against lethal doses of endotoxin was present, the gut remained pale and tonically contracted, the liver was firm and reddish, and the lymph nodes appeared colorless and inconspicuous.

The demonstration that adrenalectomized animals can be made tolerant to endotoxin and thereby resistant to drum shock is in accord with previous findings (15) that adaptation to drum trauma *per se* could be established following adrenalectomy. Adaptation was more difficult to achieve both for endotoxin and drum tolerance because of the unusual sensitivity of adrenally insufficient animals to all forms of stress, but was made possible through the use of comparatively small doses over a protracted period.

The development of tolerance to bacterial endotoxins is known not to depend on specific antibodies (21). Thus, the demonstration that acquired tolerance to *E. coli* lipopolysaccharides confers a reciprocal tolerance to traumatic and hemorrhagic shock, does not by itself imply that these animals are protected against any of the toxic manifestations of shock by virtue of a specific antagonism to *E. coli* endotoxins *per se*. It is even possible that the protective modality may not reside in the tolerance to bacterial endotoxins but in some related adaptive mechanism. This is not pure speculation, since the subsequent paper (7) shows that the development of tolerance to endotoxins is accompanied by a significant stimulation of the phagocytic activity of the reticulo-endothelial system, a feature which by itself may be related to the increased resistance to shock.

The experiments dealing with extracts of *E. coli*, administered coincident with hemorrhagic and traumatic shock, provide a number of pertinent facts concerning the mode of action of bacterial endotoxins. The observation that endotoxins increase the lethal tendency in shock is not as important as is the association of this effect with an exacerbation of the decompensatory stigmata of irreversible hemorrhagic shock—hyporeactivity and stasis in the terminal vascular bed, and congestion of the liver and intestinal tract. The depression of vascular reactivity appears to be indirect, since a latent period of at least 60 to 90 minutes elapses before decompensation developed. In cases in which the animals were treated with bacterial endotoxin before the induction of hemorrhage, vascular reactivity did not become significantly elevated and remained low throughout.

Persuasive as the endotoxin evidence is, there are a number of reservations

that make one hesitant about accepting the thesis at its face value. It is well known that shock is accompanied by changes in almost every important homeostatic function, many of which have been suggested as plausible explanations for the vascular sequelae of the syndrome. Thus, the development of irreversibility following hemorrhage is regularly associated with changes in properdin levels (22), in ferritin metabolism (23), and in the phagocytic activity of the reticulo-endothelial system (24). The available evidence does not make it possible to establish whether these are separate or related manifestations of the shock reaction to endotoxin.

What is even more crucial, is the existence of species differences with regard to bacterial involvement in shock in the rat and dog, for example, despite the fact that an identical pattern of vascular reactivity and irreversibility can be produced in both animals with shock-producing agencies (25, 26). Recent evidence with germ-free rats subjected to graded hemorrhage (27) indicates that irreversible shock can be established in the complete absence of bacterial elements. There were no significant differences in either the response pattern, or the lethal outcome, when germ-free and conventional rats were handled under comparable experimental conditions.

We are therefore left with a considerable body of circumstantial evidence which is highly suggestive, but in no way conclusive, of a common etiology in the development of shock following hemorrhage, trauma, and bacterial endotoxin. Until more specific information is available, particularly with regard to the mechanism whereby bacterial endotoxins affect vascular reactivity, and the reasons for the predilection to pathology in organs such as liver and intestinal tract during shock, care must be taken to avoid unqualified generalizations concerning the basic identity or lack of identity of these complex syndromes.

SUMMARY

The vascular effects of lethal doses of *E. coli* endotoxin, as observed in the mesentery of the rat, resemble the reactions of traumatic and hemorrhagic shock in the following respects: a profound inhibition of arteriolar and pre-capillary reactivity to topical epinephrine occurs after an initial stage of hyper-reactivity; the small veins show failure to relax completely following constrictor doses of epinephrine; and the terminal vessels develop an unusual sensitivity to fluctuations in temperature of the fluid irrigating the tissue.

Rats in which tolerance to bacterial endotoxin is induced, by repeated doses given daily, become highly resistant to the lethal effects of both drum trauma and hemorrhagic shock.

However, rats in which the adaptation to traumatic shock is produced by repeated exposure to drum trauma, do not develop a significant degree of tolerance to lethal doses of endotoxin.

The injection of small non-lethal doses of bacterial endotoxin during non-

lethal episodes of trauma or hemorrhage, leads to the development of irreversible shock and death.

The bearing of these findings on the problem of the relationship between endotoxin and traumatic shock is discussed.

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