THE ROLE OF EPINEPHRINE IN THE REACTIONS PRODUCED BY THE ENDOTOXINS OF GRAM-NEGATIVE BACTERIA

II. The Changes Produced by Endotoxin in the Vascular Reactivity to Epinephrine, in the Rat Mesoappendix and the Isolated, Perfused Rabbit Ear*

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In the preceding paper (1), it was shown that epinephrine becomes a potent necrotizing agent in the presence of Gram-negative bacterial endotoxin. Extensive lesions of hemorrhagic necrosis were produced by epinephrine (and norepinephrine) in the skin of rabbits which had previously received an intravenous injection of endotoxin, and comparable lesions occurred after local injection of mixtures of endotoxin and epinephrine.

On the basis of these observations, it has been proposed, as a working hypothesis, that the seemingly unrelated systemic effects of endotoxin may have, as a common etiological mechanism, a primary action on the reactivity of vessels of the peripheral circulation to epinephrine or norepinephrine.

Previous experience (2) has indicated that the behavior of small blood vessels can be profitably studied *in vivo* by microscopic visualization of suitable exteriorized tissues, in particular, the rat mesoappendix. This approach has proved useful in elucidating the nature of the vascular lesion during shock (3), various forms of experimental hypertension (4), and in adrenal insufficiency (5). In the present paper, the results of an application of this technique to the endotoxin problem will be presented.

Although rats are not as susceptible to endotoxin as are rabbits, they were used in a major portion of this study because of the considerable body of available information concerning the behavior of small blood vessels in this species. To supplement the microcirculatory experiments, the vessels of the isolated rabbit ear were studied by

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an *in vitro* perfusion method, employing a technique which has been extensively used for the bioassay of various vasoactive materials.

The combined *in vivo* and *in vitro* approaches have provided evidence that endotoxin has the property of altering to a striking degree the vasoconstrictor response of peripheral blood vessels to epinephrine. Small doses of endotoxin bring about a state of greatly augmented responsiveness, so that prolonged, intense vasoconstriction can be evoked by amounts of epinephrine which have little or no effect on normal vessels. Large doses of endotoxin cause a reversal of this effect, so that the terminal vessels in the mesoappendix lose their responsiveness to epinephrine and those in the isolated ear undergo vasodilation.

Materials and Methods

Mesocecum of Rat.—The *in vivo* studies were carried out by the rat mesoappendix procedure of Zweifach and Chambers as described in reference 6. A total of 125 rats of the CF Wistar strain of both sexes, weighing between 125 and 170 gm., were used. Although the animals in this weight range were not fully mature, they showed an exceptionally uniform pattern of response to vasoactive agents. The microcirculation was observed in the sheet of mesentery between the terminal ileum and cecal appendage of rats anesthetized with sodium pentobarbital (35 mg./kg.). Rigid precautions were taken to maintain the exteriorized tissues at body temperature by irrigation with a Ringer-gelatin solution. In order to rule out any nonspecific effect of the anesthetic agent on the observed changes, a series of 15 rats were prepared for *in vivo* study of the circulation while under urethane anesthesia (200 mg./100 gm.). Since there were no essential differences between the endotoxin reaction in the pentobarbital or urethane animals, pentobarbital anesthesia was used as routine during the experiments herein reported. The constrictor response of the terminal arterioles and precapillaries to the topical application of epinephrine or norepinephrine has been shown to represent a reliable index of the functional status of these vessels. For this purpose, the minimal effective or threshold concentration of epinephrine, *i.e.*, the smallest amount required to narrow the precapillaries and bring to a halt the blood flow in the capillaries, served as a measure of the state of reactivity. Observations were also recorded on the occurrence of spontaneous vasomotor changes (vasomotion), the extent and distribution of blood flow through the blood capillaries, and the caliber of feeding arterioles and collecting venules.

The endotoxin preparations were injected by the intravenous or intraperitoneal routes. No differences in the observed effects were demonstrable between the two routes of injection.

In Vitro Perfusion.—The *in vitro* experiments were carried out according to the method of Katz (7). The isolated ear of the rabbit was perfused with Tyrode solution at room temperature. The perfusion system consisted of an elevated reservoir from which the perfusate was introduced by a fine needle into an upright 25 cc. volumetric pipette calibrated in 0.1 cc. markings. The delivery end of the pipette was attached by a rubber tube to a 22 gauge needle which served as the arterial cannula. The rate of flow through the preparation was adjusted to 2 to 4 cc. per minute, and the perfusion fluid was kept at a given level in the pipette by constant flow from the reservoir. Test samples were introduced by way of a fine polyethylene catheter inserted through a glass "T" just above the point of attachment of the 22 gauge cannulating needle. After the injection of vasoactive test substances, the meniscus level of the delivery pipette rose or fell in proportion to the increased or decreased resistance to flow through the ear. A rise in the level accompanied vasoconstriction, while a fall in the level

occurred with vasodilation. Endotoxin preparations were introduced directly into the ear circulation or mixed with the perfusion medium. The addition of small amounts of normal rabbit serum (0.1 per cent) to the perfusion medium considerably increased the over-all reactivity of the ear vessels to both constrictor and dilator agents. Changes in vascular reactivity were arbitrarily expressed by comparing the concentration of epinephrine required to produce a standard rise in the perfusion meniscus, after exposure to endotoxin, with the concentration which elicited a comparable increment during the control period.

Endotoxins.—The majority of the experiments were done with a lyophilized lipopolysaccharide endotoxin of *Escherichia coli* prepared by the Difco Company, Detroit. Fresh solutions of endotoxin, in sterile pyrogen-free distilled water, were prepared for each experiment. Corroborative studies were carried out with four other endotoxin preparations: a highly purified *E. coli* endotoxin (8) prepared by Doctor O. Westphal, Freiburg, Germany (10), a crude meningococcal culture filtrate prepared as described elsewhere (9), a solution of *Brucella melitensis* endotoxin supplied by Doctor Wesley Spink, Minneapolis, and purified *Serratia marcescens* endotoxin prepared by Doctor Murray Shear, Washington.

EXPERIMENTAL

In Vivo Studies of the Rat Mesoappendix

In preliminary experiments, the lethal dose range of Difco *E. coli* endotoxin was determined in a series of 36 rats. These animals were much more resistant to the material than rabbits, tolerating doses as high as 2 mg. per rat without succumbing. Death was produced in 50 per cent by doses between 4 and 5 mg., and all animals were killed by 7 mg. Regardless of the dose, a latent interval of approximately 1 hour was consistently observed between the time of injection and the beginning of symptoms. After this interval the animals showed rapid, labored respiration, marked pallor of the ears and extremities, copious secretion of red-stained fluid from the eyes, and varying degrees of weakness and prostration. Death occurred in most instances between 4 and 18 hours after injection. At autopsy, the usual findings were an enlarged, deeply congested liver, scattered hemorrhages throughout the lungs, hemorrhagic necrosis of the abdominal lymph nodes, frank hemorrhages into the walls and lumen of the small intestine, and patchy areas of extreme pallor on the surface of the kidneys.

In the studies to be reported below, two distinct patterns of altered reactivity to epinephrine were produced by endotoxin, depending on the dose of endotoxin. With relatively small, sublethal doses, ranging from 25 to 200 μg., the most conspicuous change was an augmentation of reactivity, which varied in degree and duration with the dose of endotoxin. After larger doses, ranging from 250 μg. to several mg., a biphasic response occurred, with a brief period of hyperreactivity of the terminal arterioles being replaced by a state of refractoriness to epinephrine. These two types of response will be considered separately.

The Effect of Small, Sublethal Doses of Endotoxin on Reactivity to Epinephrine.—In normal rats,[1] the application of threshold doses of epinephrine (usu-

ally 0.2 to 0.4 μg. per cc.) brought about transient constriction of the terminal arterioles and precapillaries, accompanied by slowing and brief cessation of the capillary circulation. The entire reaction lasted no longer than 10 to 20 seconds, after which the caliber and flow returned to normal.

When endotoxin was injected by vein, in doses ranging from 50 to 200 μg., the same doses of epinephrine elicited an intense, widespread vasoconstriction involving the small arteries, arterioles, venules, and small veins, with complete ischemia of the capillary bed lasting several minutes. Similar changes in reactivity were observed with norepinephrine (levophed), although the degree of potentiation was somewhat less than with epinephrine.

It was noted that with repeated applications of epinephrine, at successive intervals after endotoxin, the constriction persisted in the vascular bed for increasingly long periods of time. In order to determine whether this was a function of the time of testing after endotoxin, or due to the repeated stimulation by epinephrine, a series of animals were subjected to a single test at various intervals of time following the administration of endotoxin. It was found that the progressively increasing and more prolonged reactions to epinephrine were related to the time of testing and the dose of endotoxin, and were not dependent on the number of epinephrine tests.

The time of occurrence, duration and magnitude of hyperreactivity to epinephrine following various doses of Difco *E. coli* endotoxin are illustrated in Fig. 1. It will be seen that augmented responses appeared within 30 minutes after intravenous injection of endotoxin. With smaller doses (50 and 75 μg.) the reactivity to epinephrine returned to normal after 60 to 90 minutes. Larger doses (100 to 200 μg.) caused more prolonged hyperreactivity, in many instances remaining at maximal levels for periods of 3 to 5 hours without evidence of a return toward normal.

During the period of heightened vascular reactivity, the change in the behavior of the muscular venules and veins was particularly striking. After an injection of endotoxin these vessels, which in normal animals are the least reactive to epinephrine, became so highly reactive that their threshold responses were obtained with lower doses than in the terminal arterioles. Thus, in endotoxin-treated rats, the venous outflow from the capillary bed could be brought to a halt by quantities of epinephrine which had no appreciable effect on arteriolar caliber.

It was noted that curtailment of capillary blood flow persisted for long periods when test applications were made with concentrations of epinephrine greater than threshold doses. For example, in experiments in which the effective threshold concentration of epinephrine had been reduced to 0.02 μg./cc. following endotoxin, the topical application of 0.2 μg. produced an intense degree of vasoconstriction and cessation of capillary blood flow lasting 5 or 6 minutes, after which the capillary flow remained drastically reduced for the next 60 to 90 minutes. In normal rats the application of epinephrine in com-

parable doses, *i.e.* 10 times the threshold dose, results in transient episodes of vasoconstriction which terminate completely within 3 to 4 minutes at the longest.

A quantitative estimation of the degree of epinephrine hyperreactivity produced by different doses of endotoxin is shown in Table I. Here, the degree

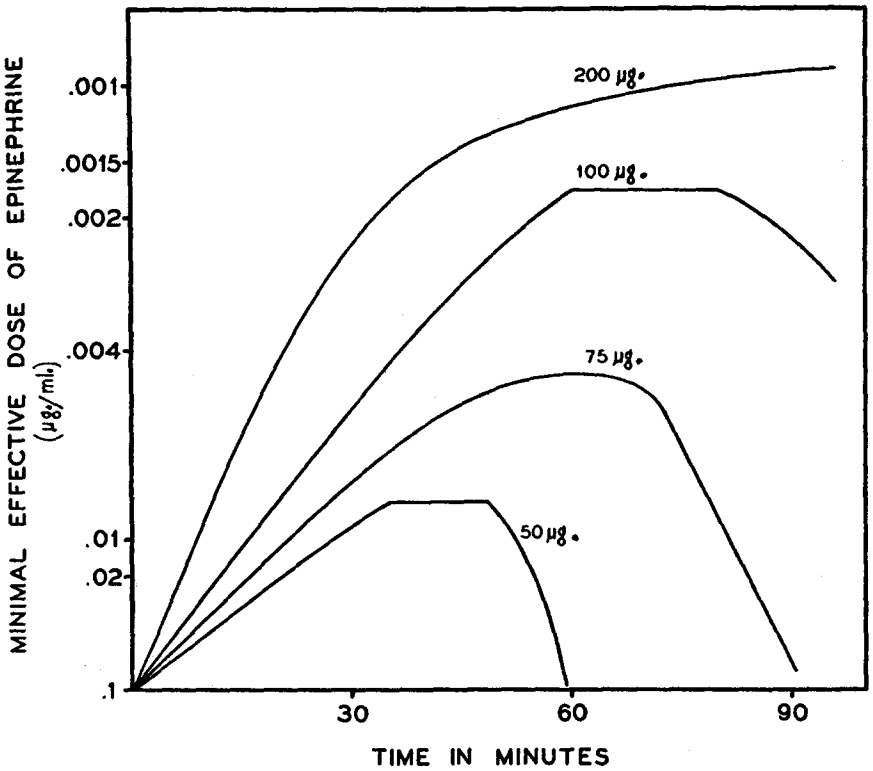

FIG. 1. Hyperreactivity of Mesenteric Vessels to Epinephrine following Intravenous Injection of Endotoxin. Each curve represents the average of results obtained in 4 to 5 rats. The animals received intravenous *E. coli* endotoxin, in the doses indicated above each curve, and the threshold concentrations of epinephrine required to elicit a standard, minimal contraction of the terminal arterioles were determined at approximately 15 minute intervals.

of augmentation is indicated by the ratio between the threshold doses of epinephrine before and after the injection of endotoxin. It will be seen that the optimal dose of Difco *E. coli* endotoxin was 100 μg., which produced 400-to 700-fold magnification of reactivity to epinephrine. With doses of 1000 μg. or more, the reactivity fell to subnormal levels, a phenomenon which is discussed in the section to follow.

Each of the four other endotoxin preparations yielded results similar to

those described above. The doses of each which were required to produce enhancement of epinephrine reactivity are shown in Table II.

The Effect of Large Doses of Endotoxin on Reactivity to Epinephrine.—After doses of endotoxin greater than 250 μg., a biphasic response was observed in which the initial potentiation of epinephrine constriction was interrupted by a stage of rapidly decreasing reactivity, reaching levels much below normal.

TABLE I

The Effect of Various Doses of E. coli Endotoxin on the Vascular Response to Epinephrine in the Rat Mesentery

Dose of endotoxin	No. of rats	Change in reactivity*	Duration of effect
μg.			min.
25	4	Increased 2–3 times	20–30
50	10	" 4–5 "	45–60
75	3	" 20–30 "	60–75
100	12	" 400–700 "	120–180
200	10	" 150–200 "	180–240
500	6	" 15–20 "	180–240
1000	6	Decreased 3–5 "	200–240
2000	6	" 10–100 "	240

* Changes in reactivity are based on differences between the threshold concentrations of epinephrine during control period and after indicated doses of intravenous endotoxin.

TABLE II

The Effects of Five Endotoxin Preparations on Vascular Reactivity to Epinephrine in the Rat Mesentery

Endotoxin	Dose producing change in epinephrine reactivity	
	Increased reactivity	Decreased reactivity
E. coli (Difco)	50–200 μg.	800–2000 μg.
E. coli (Westphal)	25–100 μg.	250–400 μg.
S. marcescens	25–75 μg.	500–2000 μg.
Meningococcus	0.1–0.25 cc.	0.3–0.5 cc.
Br. melitensis	0.05–0.25 cc.	0.3–0.4 cc.

Typical biphasic reactions following different doses of *E. coli* endotoxin are illustrated in Fig. 2. The larger the dose of endotoxin, the more refractory became the terminal arterioles and precapillaries. The hyporeactive stage appeared as early as 60 minutes after injection of endotoxin, and persisted for as long as 4 hours.

The following sequence of events occurred with uniformity after a lethal dose of endotoxin: During the first hour, vascular reactivity to epinephrine was moderately enhanced, but the degree of augmentation was often some-

what less than that observed after smaller doses. No change in blood flow or in arterial blood pressure occurred during this stage. Then early in the 2nd hour, the terminal vascular bed, and also the larger arteries and veins, exhibited a gradual depression of epinephrine reactivity, and during this time the animal usually showed signs of being ill, respiration became labored, and the arterial pressure fell (mean value with Hg manometer in carotid ar-

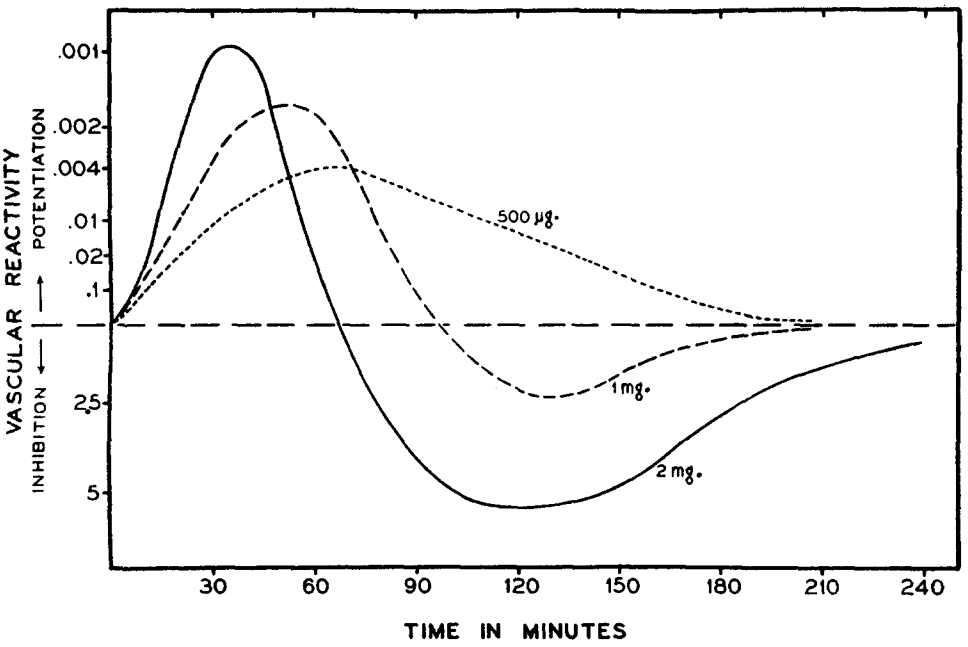

FIG. 2. Biphasic Change in Epinephrine Reactivity Following Large Doses of Endotoxin. The curves indicate the biphasic nature of the vascular response to topical epinephrine following 3 different doses of endotoxin. Each curve is based on the average of results obtained in 6 rats, in which the mesentery was tested at 30 minute intervals after endotoxin. The central dotted line represents the average threshold dose of epinephrine prior to the injection of endotoxin, expressed arbitrarily as unity, and hyperreactivity and hyporeactivity are indicated respectively by the rise or fall in relation to this line.

tery: 65 to 75 mm.). The small arteries and veins became dilated and filled with blood, especially the muscular venules. The entire capillary bed became filled with blood which moved slowly through all of its ramifications, with sluggish outflow by way of the collecting venules. Petechial hemorrhages developed around capillaries soon after the development of stasis in these vessels. Although some petechiae appeared spontaneously, it was observed that they became much more numerous and extensive soon after testing an area with topical epinephrine.

At this stage of events, during the 2nd and 3rd hours, the larger arteries and veins began to narrow down spontaneously and again became highly reactive to epinephrine. In contrast, topical epinephrine elicited no visible reactions in the arterioles, precapillaries, or venules. Narrowing of the larger arteries progressed until they became thread-like structures, while the terminal vessels remained unresponsive and dilated, with the result that large volumes of blood remained stagnant in the capillaries and venules; the latter became engorged and frequently had the appearance of varicosities. Increasing numbers of petechiae developed along the capillaries and venules, and an active circulation was only demonstrable through direct shunts, which completely bypassed the capillary bed.

Similar reactions were produced by the other four endotoxins studied in doses in or near the lethal range. Table I illustrates the degree and duration of hyporeactivity observed with various doses of Difco *E. coli* endotoxin, and Table II shows the dosage range of each of the five endotoxin preparations which brought about hyporeactivity to epinephrine.

Epinephrine Reactivity in Rats Rendered Tolerant to Endotoxin.—When animals are subjected to repeated daily injections of small doses of endotoxins, they become refractory to all the recognized biological effects of these substances. The state of induced tolerance has been widely studied, and is believed by some to reflect an augmented detoxifying capacity of the reticuloendothelial system (10). The acquired resistance disappears within a few hours after an injection of colloidal substances presumed to "blockade" the reticuloendothelial cells, such as thorium dioxide (thorotrast) (11) and colloidal saccharated iron oxide (proferrin) (12).

To learn whether tolerance to endotoxin also applies to the observed changes in vascular reactivity, the following experiment was performed:—

Rats were given daily injections of Difco *E. coli* endotoxin for 8 days, beginning with a dose of 50 μg., and increasing in stepwise increments to a final dose of 500 μg. For a period of 4 to 6 days after the last injection such animals were capable of resisting a challenge dose of 4 mg. without apparent ill effects. Microscopic observations were made of the circulation of 10 endotoxin-tolerant rats, employing doses of endotoxin known to produce both hyperreactivity and hyporeactivity to epinephrine in normal animals.

In all instances, intravenous injections of endotoxin in doses ranging from 50 to 2000 μg. had no discernible effect on the mesenteric vascular reactivity to epinephrine, nor did spontaneous abnormalities of blood flow or petechiae occur.

Having established that endotoxin-tolerant rats were resistant to alteration of epinephrine reactivity by endotoxin, it was next of interest to learn whether the resistance could be abrogated by colloidal "blockading" agents. Saccharated iron oxide (0.4 ml./150 gm.) was injected intravenously in 10 rats with induced tolerance, and the effect of endotoxin on the mesenteric vascular bed

was studied during the ensuing 4 hours. Epinephrine hyperreactivity, followed by hyporeactivity, were produced by doses of endotoxin even smaller than those which are effective in normal rats, indicating that the state of resistance had been eliminated by proferrin.

In Vitro Perfusion Studies of the Isolated Rabbit Ear

Although the response of different ear preparations to vasoconstrictor doses of epinephrine was highly variable, the vasoconstrictor response in each individual preparation was found to be consistent and reproducible. As many as 15 test reactions with epinephrine could be elicited at 4 or 5 minute intervals with a reproducibility of approximately 15 per cent, as measured by the change in the meniscus level in the delivery pipette. It was therefore possible to use the endpoints obtained with successive epinephrine tests as an indication of the vasoactive effects of endotoxin.

The injection of a single dose of Difco *E. coli* endotoxin, in doses ranging from 0.5 to 2.0 μg., markedly enhanced the constrictor reaction to epinephrine. By themselves, these doses had no observable effect either on the state of contraction of the ear vessels, as indicated by the meniscus level, or on the rate of perfusion flow. A total of 25 ear preparations were studied with 0.5 μg. of this endotoxin, and 3 with the Westphal *E. coli* preparation. In each case the effective threshold concentrations of epinephrine were reduced to levels indicating potentiation of the epinephrine response, usually from 200 to 850 times the reactivity before endotoxin (Table III). The change in reactivity was of such magnitude that the use of epinephrine doses just above the control threshold level resulted in a complete shutdown of flow through the ear for periods of 15 minutes or longer.

To illustrate the type of reaction which was observed, a representative protocol is shown in Fig. 3.

The response to a priming dose of 0.5 μg. of epinephrine served to indicate the reactive stage of the preparation before endotoxin. Extremely small doses of epinephrine were then tested, until the threshold level was determined, in this instance 0.015 μg. An injection of 0.5 μg. of *E. coli* endotoxin was then made, in a volume of 0.1 cc. The actual concentration perfusing the ear was estimated to be approximately 0.03 μg. per cc. No spontaneous change in the flow pattern was detectable as the result of endotoxin alone. Now, the injection of the original threshold dose of epinephrine (0.015 μg.) brought about vasoconstriction which was as intense as that elicited before endotoxin by the initial test dose (0.5 μg.). This augmented response reached a maximum 5 minutes after endotoxin, and was no longer demonstrable after 8 minutes.

The phenomenon could be reproduced several times in the same ear, although the magnitude of augmentation was not always identical. Doses of endotoxin below 0.3 μg. were ineffective. However, two divided doses of 0.25 μg. each, spaced 3 minutes apart, produced the same response as a single in-

jection of 0.5 μg. When the interval between such divided doses was 5 minutes or longer, no additive effect was demonstrable.

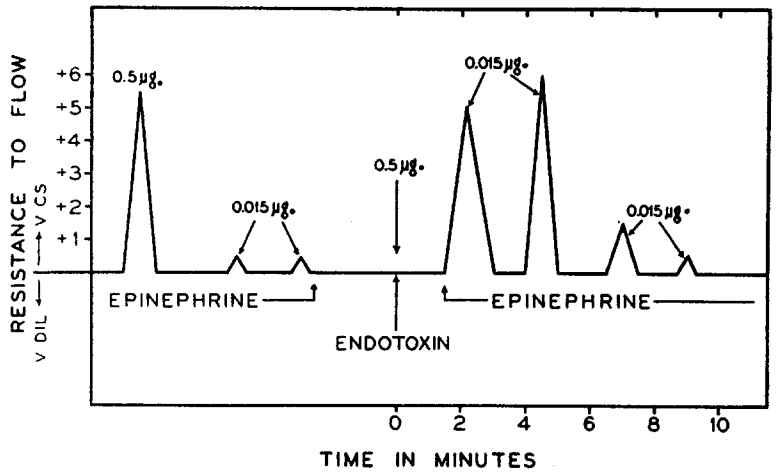

FIG. 3. Potentiation by Endotoxin of Response to Epinephrine in Isolated Rabbit Ear. An experiment illustrating the type of augmented response to epinephrine following perfusion with 0.5 μg. of *E. coli* endotoxin. The height of the excursion, indicating vasoconstriction, is shown in arbitrary units; one unit is equivalent to a 0.1 cc. shift in the meniscus level of the delivery pipette.

TABLE III

Potentiation by Endotoxin (0.5 μg.) of Vasoconstrictor Effect of Epinephrine in Isolated Rabbit Ear

Exp. No.	Threshold dose of epinephrine		Degree of potentiation*
	Control	After endotoxin	
	μg.	μg.	
1	0.5	0.0015	300 times
2	0.025	0.00003	850 "
3	0.025	0.00006	420 "
4	0.025	0.00012	200 "

* Expressed as multiples of control threshold value, to indicate the relative magnitude of the effect on vascular reactivity.

As was observed in the *in vivo* experiments in rats, larger doses of endotoxin produced reversal of the epinephrine constrictor reaction, and actually brought about vasodilation in response to epinephrine. This observation, which was made in a series of 10 consecutive experiments with different ear preparations, is indicated in Table IV. In the experiment illustrated by the protocol in Fig. 4, a large dose of epinephrine (0.5 μg.), which elicited vigor-

ous vasoconstriction during the control period, was used as the test challenge throughout. It will be noted that administration of 40 μg. of endotoxin converted the response to this dose of epinephrine from constriction to pronounced vasodilation—a phenomenon never encountered in any of the

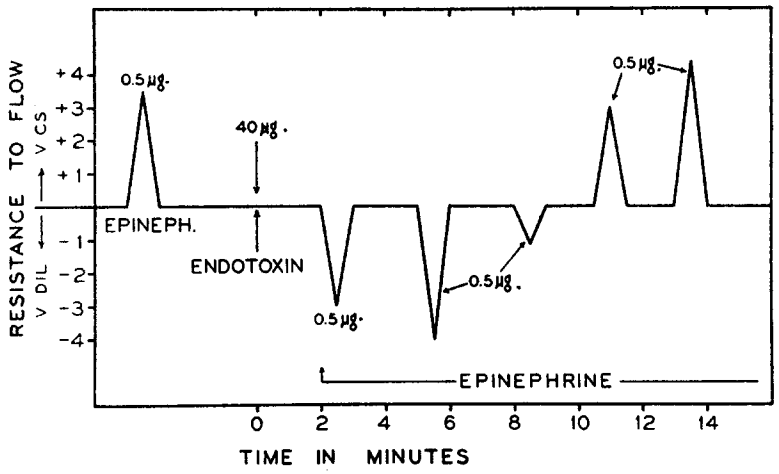

FIG. 4. Reversal of Epinephrine Response in Isolated Rabbit Ear by a Large Dose of Endotoxin. The effect of perfusing 40 μg. of *E. coli* endotoxin is illustrated. Vasoconstriction (above base line) and dilation (below base line) are expressed in arbitrary units as in Fig. 3.

TABLE IV

Vasodilation by Epinephrine after Endotoxin (50 μg.) in the Isolated Rabbit Ear

Exp. No.	Epinephrine dose	Vascular response*	
		Control	After endotoxin
	μg.		
1	0.05	Constriction (0.6)	Dilation 0.5
2	0.015	" (0.8)	" 0.5
3	0.001	" (0.7)	" 0.6
4	0.0001	" (0.3)	" 4.0
5	0.00001	" (0.1)	" 1.3

* Degree of vasoconstriction or dilation arbitrarily expressed by the rise or fall of the meniscus of delivery pipette, in cubic centimeters.

control preparations. The dilator effect persisted for 8 to 10 minutes, after which the normal constrictor reaction reappeared.

In several experiments, larger doses of endotoxin (75 μg. or more) produced a state of complete unresponsiveness to epinephrine, during which the ear vessels failed to react either by constriction or by dilation to doses of epinephrine as large as 2 μg. Similar unreactivity appeared after repeated injec-

tions of endotoxin in doses sufficient to cause dilator responses to epinephrine when given singly.

In the foregoing experiments, endotoxin was perfused through the vessels of the ear for a relatively brief period, at most 20 or 30 seconds. It was therefore of interest to learn the effect of continuous perfusion of endotoxin. To this end, various concentrations of endotoxin were added to the perfusing fluid, and the response to epinephrine measured at selected intervals during perfusion. In two such experiments, in which 0.5 μg. per cc. of endotoxin were perfused at the rate of 3 cc. per minute, a subthreshold dose of epinephrine (0.0025 μg.) now produced prolonged, intense vasoconstriction.

Another modification was used to demonstrate the vasodilator effect of endotoxin in the presence of constant perfusion by epinephrine. Epinephrine was infused by a motor-driven syringe at a constant rate of 0.0001 μg. per minute. This dose was just below the amount required to produce a measurable, sustained vasoconstriction. Endotoxin was then added to the perfusate, in doses ranging from 10 to 35 μg. In each instance, this was immediately followed by vasodilation which persisted throughout the period of observation (30 minutes). The addition of more epinephrine during this period, in concentrations as low as 0.000001 μg. per cc. evoked further vasodilation.

No effect of endotoxin on the vasomotor response to serotonin (10-hydroxytryptamine) could be demonstrated in the isolated ear, when various doses of the latter substance were tested by methods similar to those described above.

DISCUSSION

In the preceding paper (1), it was shown that endotoxin produces a drastic alteration of the action of epinephrine in the rabbit, converting this hormone from its normal role as a transient vasoconstrictor in the skin to a powerful necrotizing agent capable of causing extensive hemorrhagic destruction. It seemed apparent that the action of endotoxin was exerted directly on the blood vessels, since hemorrhagic necrosis occurred both when endotoxin was given by vein and epinephrine injected separately into the skin, and when the two were mixed and injected together.

The results of the present study provide substantial evidence for a direct effect of endotoxin on the reactivity of small blood vessels to epinephrine, and suggest several ways in which tissue damage may be caused by the combined action of the two substances. In the experiments with the rat mesoappendix, small doses of endotoxin were found to cause extreme degrees of prolonged vasoconstriction, involving both arterioles and venules, following topical application of epinephrine in concentrations which had little or no effect on normal tissues. The small veins, which are normally the least reactive of the terminal vessels to epinephrine, became more sensitive than the precapillary arterioles, thus providing a mechanism for impeding capillary circulation and pooling blood within the collecting venules.

With larger doses of endotoxin, the stage of epinephrine hyperreactivity was shortened in duration and was replaced by a stage of increasing hyporeactivity. During this time, petechiae began to appear along the capillaries and venules, especially in the sites being tested with topical epinephrine. After lethal doses, the terminal arterioles and venules became totally unresponsive, while the reactivity of the larger arteries and veins continued to increase, with the final result that stagnant blood became pooled in all capillaries and venules while the larger vessels contracted to narrow, thread-like structures.

It seems reasonable to suggest that comparable events, occurring in rabbit skin in response to the combined effects of endotoxin and epinephrine, may have been the basis for the lesions of hemorrhagic necrosis. It was considered unlikely that prolonged vasoconstriction by itself could account for lesions of such magnitude, particularly since repeated intradermal injections of large doses of epinephrine caused no necrosis in the skin of normal rabbits. However, the demonstration in the rat mesoappendix that the alteration of epinephrine reactivity may be biphasic, with a period of hyperreactivity followed by pronounced hyporeactivity, offers a more plausible explanation. This finding, together with the direct observation of blood stasis and petechial hemorrhage in dilated capillaries and venules, would fit well with the changes visible in the gross as the rabbit skin lesion develops.

A crucial question, in attempting to explain the endotoxin-epinephrine skin lesion, is whether epinephrine actually causes dilation of blood vessels under the influence of endotoxin. If this were so, the meshwork of dilated small vessels, resembling telangiectases, which appear early in the development of the lesion, could be nicely accounted for. The rat mesoappendix experiments do not provide an unequivocal answer. In the terminal stages of epinephrine hyporeactivity, after lethal doses of endotoxin, the small vessels lost all responsiveness to epinephrine and became progressively more dilated and engorged with blood, and it was noted that topical applications of epinephrine were followed by increasing numbers of petechiae. But it was not observed that epinephrine itself caused an active, definite vasodilator reaction. On the other hand, such reactions were consistently demonstrable in the isolated rabbit ear preparations after exposure to large doses of endotoxin.

In certain respects, the effects of endotoxin on vascular reactivity were essentially the same in the rat mesoappendix and the isolated rabbit ear. In both, hyperreactivity occurred after small doses, and hyporeactivity after large ones. It should be noted, however, that these changes reached their maximum levels within a few minutes in the ear vessels while an hour or so was required for their full development in the rat mesoappendix. Whether this difference, and also the dilator effect in the rabbit ear, are due to the different animal species involved, or to other factors such as the artificial

environment created by the ear perfusion technique, remains to be determined. It is worthy of note that repeated attempts to produce skin lesions in the rat with combinations of endotoxin and epinephrine have been uniformly unsuccessful, and conceivably the rapidity of action of endotoxin in the rabbit, or its capacity to bring about active vasodilation by epinephrine in this species, may be important factors in the pathogenesis of the skin lesion. The claim by Lundholm (13) that the physiological dilator effect of epinephrine is due to its capacity to stimulate the release of lactic acid in certain tissues is of interest in view of the demonstration, by Thomas and Stetson (14), of greatly increased levels of lactic acid in skin tissue injected with endotoxin. Further studies of the possible relationship between these two phenomena are now in progress.

Apart from this problem, the observations made in this study may have direct bearing on the mechanism of certain other pathophysiological effects of endotoxin, notably the syndrome of shock. The resemblances between traumatic shock and the lethal reaction to endotoxin have been pointed out by Delaunay and his associates (15), Fine (16) and others (10). The events observed in the rat mesoappendix after endotoxin are strikingly similar to those previously described during the development of shock (17). In the decompensatory stage of shock, as after large doses of endotoxin, the small blood vessels became refractory to epinephrine and show no spontaneous changes in the caliber with fluctuations in blood flow; the larger arteries and veins, on the other hand, maintain their tone and become excessively narrowed to less than one-third of their original diameter.

It is suggested that the observed changes in epinephrine reactivity brought about by endotoxin, and the resulting degrees of impairment of peripheral blood flow, may represent aspects of a basic mechanism for tissue damage. The amounts of epinephrine and norepinephrine normally contained in the tissues vary widely from one organ to another, and it is possible that some tissues may be sufficiently supplied as to be placed in jeopardy by the action of endotoxin. The several points of similarity between the systemic intoxicating effects of endotoxin and epinephrine, summarized in the preceding paper, may become explicable in these terms.

SUMMARY AND CONCLUSIONS

The effects of endotoxin on the epinephrine reactivity of blood vessels in the rat mesoappendix have been studied. Following intravenous injection of a relatively small, sublethal dose of endotoxin, the terminal arterioles and venules exhibited greatly augmented and prolonged vasoconstrictor responses to epinephrine and norepinephrine. Hyperreactivity became evident within 30 minutes after injection of endotoxin, and persisted for as long as 6 hours.

After larger doses of endotoxin, sufficient to cause illness or death, the

vascular hyperreactivity to epinephrine was of briefer duration, and was followed by a stage of increasing hyporeactivity reaching levels much below normal. With lethal doses, the terminal arterioles and venules became completely refractory to epinephrine, while heightened reactivity persisted in the larger arteries and veins. The end result was pooling of stagnant blood in distended capillaries and venules, accompanied by the appearance of petechiae. Topical applications of epinephrine during this stage were followed promptly by an increase in petechial hemorrhage at the site of testing.

Rats which were rendered tolerant to the lethal effect of endotoxin, by repeated daily injections of small doses, developed resistance to the effects of endotoxin on epinephrine reactivity. Neither hyperreactivity nor hyporeactivity to epinephrine were demonstrable in these animals, nor were spontaneous abnormalities of blood flow or petechial hemorrhages observed in the mesoappendix.

Analogous results were obtained in perfusion studies of the vessels of the isolated rabbit ear. Perfusion of small amounts of endotoxin was followed within a few minutes by potentiation of epinephrine reactivity. Larger doses caused complete reversal of this effect, to such an extent that epinephrine now produced marked degrees of vasodilation.

The possible meaning of these observations in the interpretation of the endotoxin-epinephrine skin lesions described in the preceding paper is discussed. It is suggested that abnormal reactions to epinephrine or norepinephrine in the tissues of intact animals may represent a basic mechanism in the intoxicating and tissue-damaging properties of endotoxin.

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