Studies on Hemorrhagic and Endotoxin Shock in Relation to Vasomotor Changes and Endogenous Circulating Epinephrine, Norepinephrine and Serotonin *

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STUDIES on traumatic, hemorrhagic and bacteremic shock have resulted in a vast literature characterized by a large body of facts, numerous hypotheses, and many unanswered questions. Although the following investigation may have resulted in the addition of a little bulk to each of these three characteristics, our purpose in carrying out these experiments was to define the role played by the vaso-active hormones, epinephrine, norepinephrine and serotonin in the production of the vascular and hemodynamic changes seen during shock. We also hoped to clarify the relationship of irreversible hemorrhagic shock to lethal shock caused by gram-negative bacterial endotoxin.

The earliest studies on shock were conducted by surgeons who were called upon to treat this enigmatic syndrome. Thus, in 1879, Mapother, in an address to the Surgical Society of Ireland, pointed out that the most marked physical change caused by shock was the contraction of arterioles.[54] Malcolm (1905) later formulated the "vasoconstrictor theory" of shock, in opposition to the then popular theory of "vasomotor paralysis." [51] It was not until the turn of the century that physiologists also became interested in the problem and demonstrated the occurrence of vasoconstriction and increased peripheral resistance during hemorrhage.[4, 14, 53, 58, 65-67] In 1912, Trendelenburg studied the effect of blood loss on the secretion of epinephrine into the adrenal vein of cats.[74] He concluded that this mechanism was *not* effective in combatting hypotension. Subsequent studies, however, indicated that increased amounts of adrenergic amines *were* secreted and did assist in maintaining the blood pressure. These studies are summarized in Table 1.

During World War I, greater attention was focused on the pathogenesis of shock. During this period, Erlanger and his colleagues [23-27] extended Bainbridge and Trevan's [3] observations on the shock-producing properties of epinephrine. They, as well as others, recognized that a depleted blood volume was the basic cause of shock.[59, 61] This fundamental fact became obscured by the theories concerning a "toxic factor" and "increased capillary permeability." The toxic factor was thought to be a histamine-like substance.[13]

In the interval between the wars, several outstanding investigators, notably Blalock and his group,[6] properly oriented surgeons and physiologists to the concept that a decreased blood volume was responsible for the appearance of traumatic shock. How-

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TABLE 1. *Studies on Catecholamine Output During Hemorrhagic and Traumatic Shock*

Peripheral Levels		Adrenal Vein Output	
Investigator	Results	Investigator	Results
Trendelenburg (1912)[74]	No change	Trendelenburg[74] (1912)	Concentration increase. Total output decrease
Bedford (1917)[4]	Increase		
Rapport (1922)[60]	Variable increase	Crile (1913)[15]	No change
Tournade & Chabrol (1925)[73]	Increase	Bedford (1917)[4]	32-fold increase following shock due to manipulation of intestine
Watts *et al.*[77–79] (1956)	22-fold increase after hemorrhage of 40 ml./kg., 100-fold increase when bled to BP of 40 mm. Hg	Stewart & Rogoff (1919)[65]	No change
Manger (1957)[52]	8-fold increase with loss of one-third of blood volume	Saito (1928)[64]	10- to 30-fold increase following loss of one-third of blood volume
Lansing & Stevenson (1958)[47]	Increase	Lund (1951)[50]	Increase
Millar & Benefy[56] (1958)	9-fold increase after graded hemorrhage 36 ml./kg.	Walker *et al.* (1959)[76]	13- to 20-fold increase following loss of one-third of blood volume
Walton *et al.*[80] (1959)	34-fold increase after hemorrhage of 40 ml./kg.		

ever, some investigators, recognizing that other factors may be of importance in the pathogenesis of the shock syndrome, continued to concentrate on the role of vasoconstriction induced by sympatho-adrenal hyperactivity.[30–33] Cannon's earlier work had indicated that the vital centers received preferential treatment with regard to blood flow as a result of vasopressor activity.[12] Freeman attempted to determine whether prolonged or severe vasoconstriction brought about by hypovolemia might not be ultimately harmful.[32] As a result of his studies on the depletion of blood volume following epinephrine infusion and the increased tolerance of sympathectomized dogs to hypotension, he concluded that, ". . . the very mechanism by which the organism strives to survive, brings about its ultimate dissolution."[32] The use of vasoconstricting agents was therefore condemned and the suggestion made that adrenolytic drugs be considered as therapeutic agents.[31]

World War II provided the second great stimulus to master the complexities presented by the shock syndrome. The basic need to restore a manifest and/or hidden loss of blood volume was reaffirmed.[22] The war also stimulated renewed interest in the existence of a "toxic factor" in shock.[2] This, in turn, drew greater attention to the occurrence of circulatory collapse due to bacterial products. Bacteremic shock then came to be recognized as a life-threatening situation which shared many features with traumatic or hemorrhagic shock, but about which little was known.[28, 75] Penner and Bernheim were the first to stress the similarity between the pathologic changes in hemorrhagic and endotoxin shock and the lesions produced by epinephrine infusion.[57] The studies of Delaunay and his colleagues further emphasized the occurrence of vasoconstriction following the administration of endotoxin.[20] They considered endotoxin shock to be a form of traumatic shock.[18, 19] Fine, on the other hand, has presented a

hypothesis which incriminates endotoxin as the lethal factor in irreversible hemorrhagic shock.[34]

It has been suggested that hypovolemia is not necessary for the production of shock by endotoxin (although it may be a contributing factor late in the course of the process.[37]) The concept therefore evolved that the circulatory defects responsible for endotoxin shock consist of vasoconstriction followed by vasodilatation and "vascular pooling."[37] There is also suggestive evidence pointing to a reaction between endotoxin and cells that is similar to an immune response, resulting in the release of histamine, serotonin and catecholamines.[68, 69, 81] Finally, evidence has also been presented showing an altered reactivity of blood vessels in animals receiving endotoxin.[84]

Because of the prominence of vasoconstriction in both hemorrhagic and endotoxin shock, the effects of adrenergic blocking agents were investigated. Wiggers *et al.*[83] and Remington and his co-workers[62] used dibenamine to nullify the effects of vasoconstriction during hemorrhagic shock. Both groups demonstrated its efficacy by impressive increases in the survival rate of pre-treated dogs. Similar results were obtained by others with animals given large doses of endotoxin.[8, 49]

The above considerations led us to the following experiments. The sequential changes of three major endogenous circulating vasoactive substances were measured in both hemorrhagic and endotoxin shock. An attempt was then made to translate the alterations of catecholamines and serotonin into hemodynamic terms and to correlate our findings with those of others who have been concerned with these problems.

Methods

Adult mongrel dogs of both sexes weighing about 20 Kg. were the subjects of these experiments. General anesthesia was not used. All procedures were carried out with the dog under heavy morphine sedation (2 mg./kg.) and using local anesthesia (1% procaine). On the day the experiment was performed, blood was drawn from a donor animal and used to replace the blood removed for analytical purposes. In all instances, venous blood samples were obtained by means of a polyethyelene catheter in the inferior vena cava. Arterial cannulas were also inserted to measure blood pressure and to bleed the animal. Two control samples were drawn 15 minutes apart and then the animal was bled or given endotoxin. The latter consisted of dried *E. coli* organisms prepared by Spink's modification of Braude's method.[9] Hemorrhage was produced by a modified Lamson-Fine technic which has been previously described.[48] The animals were heparinized prior to being bled (2 mg./kg.) and their pre-hemorrhage blood volume was determined using Evans blue dye. They were bled into a reservoir and the blood pressure

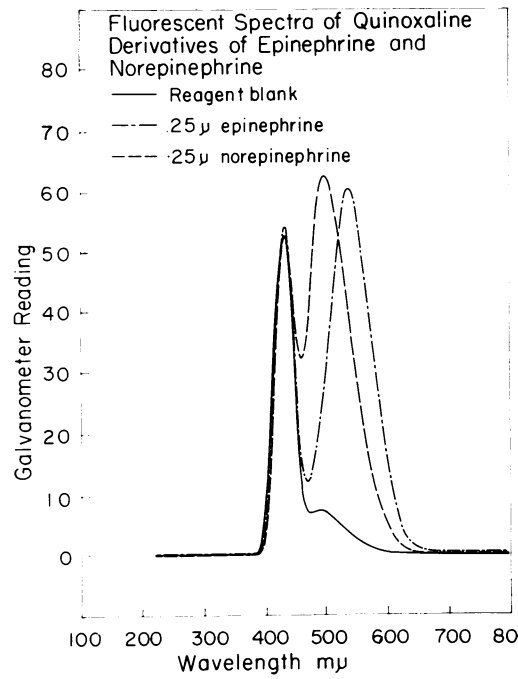


FIG. 1. Fluorescent spectra of quinoxaline derivatives of catecholamines were obtained by activating the compounds at a wavelength of 420 mu. using an Aminco-Bowman spectrophotofluorimeter attached to a direct writing Moseley recorder.

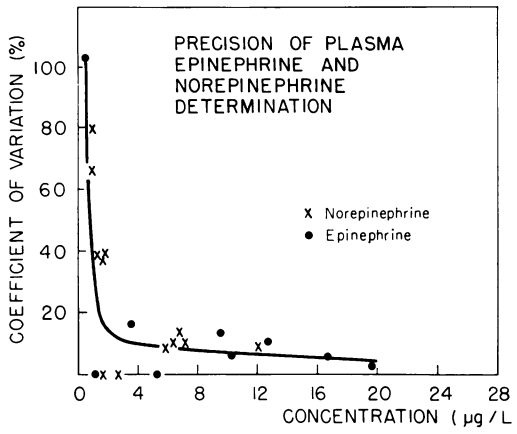


FIG. 2. Coefficient of variation (standard deviation × 100/mean) plotted against mean concentration of four replicate plasma samples results in a hyperbolic curve. Concentrations greater than 2.0 μg./L. have a 10% variation or less.

was maintained at about 45 to 50 mm. Hg. mean arterial pressure for four and a half hours. These animals were then reinfused with their shed blood. Sampling took place periodically after onset of hemorrhage or the administration of endotoxin, i.e., at 5, 30, 60, 90, 180, 270, 280, 310, 340, 370, and 400 minutes. Animals receiving endotoxin were observed for 630 minutes. The blood that was drawn was used to determine epinephrine, norepinephrine and serotonin concentrations, the platelet count, and hematocrit. The rectal temperature was also measured.

The plasma catecholamine concentration was estimated from a 15 ml. sample of blood by a modification of Weil-Malherbe and Bone's fluorimetric analysis.[82] Epinephrine and norepinephrine concentrations were differentiated on the basis of their distinct fluorescent spectral characteristics of their quinoxaline derivatives (Fig. 1). Prior to undertaking these determinations in shock animals, an extensive study of the vagaries of the method was performed. It must be pointed out that concentrations of epinephrine and norepinephrine less than 2.0 μg./L. have a coefficient of variation (standard deviation × 100/mean) which may be as high as 100 per cent. Greater concentrations can be measured with greater reliability (10% variation or less; Fig. 2).

Serum serotonin was measured on a ten ml. sample of blood by Davis' method.[16]

TABLE 2. *"Mock" Shock (Controls)*

	Mean Values*											
	Controls											Pooled Std. Dev. (s_p)**
	A	B	5′	30′	60′	90′	180′	270′	280′	310′	340′	
Blood Pressure (mm. Hg)	115	115	111	114	115	115	114	111	113	115	115	±21
Platelet Count (10^3/min^3.)	250	260	233	230	237	213	213	217	227	223	220	±22
Plasma Epinephrine (μg/L.)	0.73	0.45	0.66	0.73	0.69	0.95	0.11	0.30	0.30	0.12	0.04	± 0.54
Plasma Norepinephrine (μg/L.)	2.97	2.66	2.43	2.94	1.79	5.47	3.79	3.12	3.22	3.93	3.22	± 6.17
Serum Serotonin (μg %)	34	29	37	42	42	42	35	41	42	33	47	±21

* Data obtained from a series of four experiments.

** $s_p = \sqrt{\frac{n(s_1^2 + s_2^2 \cdots + s_n^2)}{(n - k)}}$.

TABLE 3. *Early Changes During Endotoxin Shock*

Dog No.	Differences Between Control and 5 Minutes After Administration of Endotoxin: Blood Pressure (mm. Hg)	Plasma Epinephrine (μg./L.)	Plasma Norepinephrine (μg./L.)	Serum Serotonin (μg. %)	Platelet Count (10^3/mm.^{3})	Serotonin: Platelet Ratio*
1735	−17.0	+26.4	+ 8.1	−48	− 80	− 18
1788	+17.5	+ 4.4	+ 7.4	−31	−100	− 9
22	− 2.5	− 0.9	+24.3	−47	− 90	−13
1394	−10.0	+82.1	+16.4	−99	− 80	−44
1645	−47.5	0.0	+30.1	—	− 90	—
1829	−45.0	+ 5.8	− 0.9	—	−135	—
1610	−90.0	+19.5	+20.2	−28	− 85	− 7
Mean	−33	+19.5	+15.1	−51	− 94	−18
p**	<.02	.08	<.01	<.01	<.001	<.001

* $\left(\frac{\mu g.\ \%}{10^5/ml.}\right)$.

** Calculated from a single-tail "t" test.

This is also a fluorimetric method with which we have had extensive prior experience. All the recommended precautions required to preserve the integrity of the platelets were observed in collecting blood for this determination. When blood had to be drawn from heparinized animals, the syringe was coated with a film of Protamine or Polybrene in order to promote coagulation of the blood. Platelet counts were performed on heparinized blood, using Rees-Ecker solution and a Levy-Hauser hemocytometer.

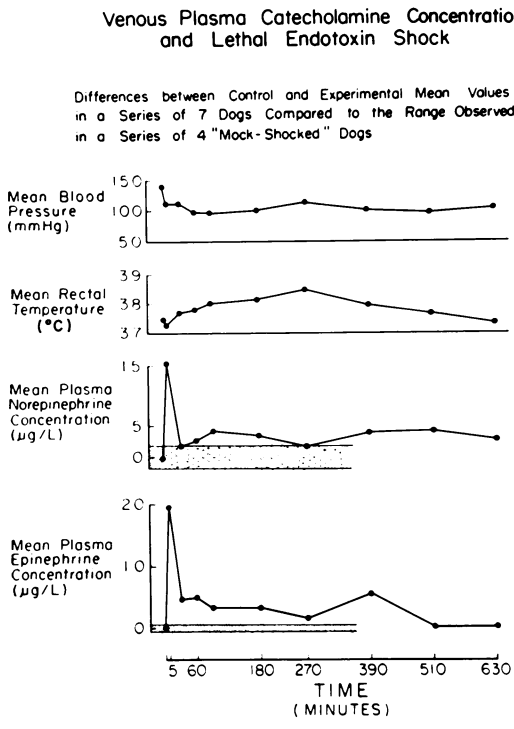
Results

"Mock" Shock (Controls). This euphemistic term was applied to a group of four animals that, except for the actual production of shock, were handled in precisely the same manner as those that were given endotoxin or subjected to hemorrhage. They were observed for a period of 340 minutes. The data obtained from these "sham" controls is summarized in Table 2. It demonstrated the stability of the preparation used in these experiments. No statistically significant differences among any of the means were found by analysis of variance using the omnibus test.

These data served as a baseline for evaluating the changes which occurred in hemorrhagic and endotoxin shock. The values for serum serotonin in this sham group of dogs were lower than those observed as the control levels in the other groups of animals. This was most probably the result of a sampling error; a larger series of sham animals would undoubtedly have obliterated this difference. Davis has also noted a wide variation in canine serum serotonin levels (range of 21 to 110 μg.%)[17] and we have also reported a similar situation with regard to the 5-hydroxyindole content of whole blood in rabbits.[63] In a series of duplicate serum serotonin samples from 31 morphinized dogs, a mean (± standard deviation) of 66 ± 53 μg. per cent was found.

This variation (80%) was much greater than the ± 9 per cent variation found for the differences between serotonin values in the same animal. Because of this variability, changes in serum serotonin were sometimes evaluated by expressing them as a percentage of the mean control value. However, whenever possible, absolute values or differences between values were used.

A similar situation was encountered with respect to catecholamine concentration



FIGS. 3a and 3b. Changes in mean epinephrine and norepinephrine (a) and serotonin (b) during endotoxin shock are plotted as the difference from the mean control value. The closely stippled area at the origin of the graph represents the range of mean values observed in the group of control ("mock shock") animals.

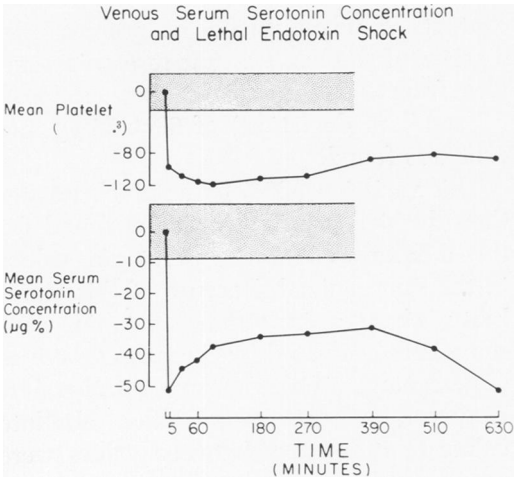


FIGURE 3b

among animals. In the same group of 31 morphinized dogs, the mean plasma epinephrine concentration was 0.7 ± 0.85 μg./L. The mean norepinephrine concentration was 1.84 ± 1.33 μg./L. The variation in epinephrine concentration between duplicate samples in the same animal was $\pm$ 25 per cent; for norepinephrine it was $\pm$ 15 per cent.

E. coli Endotoxin Shock. *Early Changes.* Five minutes after the intravenous injection of *E. coli* endotoxin in a series of seven dogs, the blood pressure fell and the catecholamine concentration rose (Fig. 3). The increase in epinephrine concentration was more pronounced but the norepinephrine elevation appeared to be more closely associated with the magnitude of the hypotension. However, statistical analysis did not substantiate the presence of a significant linear correlation. The early changes observed in this group of animals are given in Table 3.

The statistical significance (p) of the epinephrine increase was actually greater than 0.08. This p-value is based on the assumption that the response to endotoxin five minutes after its administration conforms to a normal distribution. Such an assumption does not appear to be true. By using the Chi square distribution and comparing the plasma epinephrine levels above 2 μg./L. following endotoxin administration to those observed in the control series, a p-value of considerably less than 0.001 was obtained.

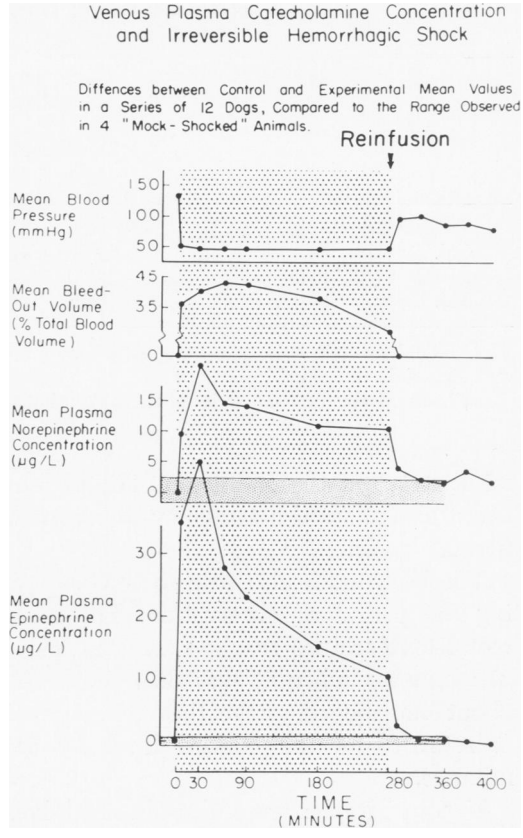
An additional group of eight dogs received 10 mg./kg. of the *E. coli* preparation and measurements of arterial plasma catecholamine concentrations were made. Four of these animals had simultaneous venous plasma determinations performed. Significant arterio-venous differences were not found although there was a tendency for the arterial samples to have higher concentrations than the venous samples. It is noteworthy, however, that we never ob-

served the extremely high elevations in catecholamine concentration in arterial plasma that were occasionally found among venous samples.

The decrease in serum serotonin concentration was accompanied by a fall in platelets (Fig. 4). The platelet decrease was not of the same order of magnitude as the decrease in serotonin. Thus, the hyposerotoninemia cannot be completely ascribed to platelet destruction or sequestration. The serotonin concentration at five minutes was 22 per cent of the control value whereas the platelet count fell to only 60 per cent ($p < .02$). These results are comparable to those previously reported by us for the rabbit.[63] A better index of the absolute decrease in serotonin concentration was obtained by comparing the serotonin-platelet ratios before and five minutes after the injection of endotoxin (Table 3). Heparinization did not affect the early changes in serotonin or platelets (Table 4).

Late Changes. The subsequent course of the animals receiving a 7.5 mg./kg. dose of the *E. coli* cell suspension is shown in Figure 3. The epinephrine concentration was significantly elevated at 90 minutes when is was 4.46 ± 2.0 μg./L. ($p < .05$). It was also elevated at 30 minutes and 60 minutes (5.8 ± 3.7 and 6.0 ± 3.8 μg./L. respectively, $p < 0.1$) but the statistical significance was less because of the variation in the response to endotoxin. In contrast to epinephrine, norepinephrine remanied elevated throughout the period of observation. The elevation was statistically significant at a 95 per cent confidence level, or greater.

Serotonin concentrations remained depressed until the animal expired. There was a tendency for the concentration to increase slightly as time elapsed. However, shortly before the animal died, the serum serotonin concentration fell back to the same level it had attained immediately after the ad-



FIGS. 4a and 4b. Changes in mean epinephrine and norepinephrine (a) and serotonin (b) are plotted in the same manner as in Figure 3. The widely stippled area represents a four and a half hour period of hemorrhagic hypotension.

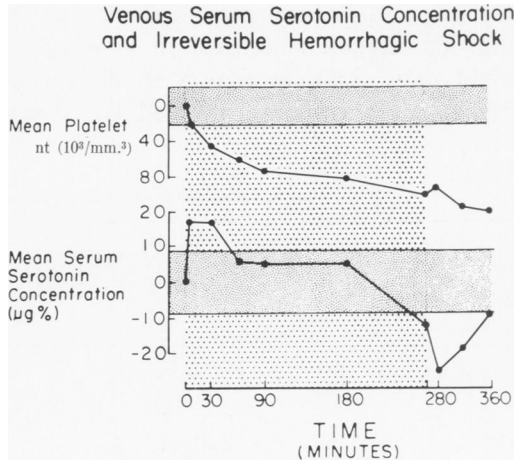


FIGURE 4b

TABLE 4. *Serum Serotonin and Platelet Concentration During Lethal (7.5 mg./kg.) E. coli Endotoxin Shock in Heparinized Dogs*

	Mean Concentration							
	Controls*							
	A	B	C	5′	30′	60′	90′	180′
Venous Serum 5-HT (μg. %)	127	119	109	18	34	50	82	100
Std. Error†	±66	±69	±58	±6	±12	±19	±28	±41
Platelets (10^3/mm.^{3})	230	217	210	107	113	100	120	137
Std. Error	±15	± 7	±17	±9	±13	±15	±15	±26

* Samples obtained 15 minutes apart prior to injection of endotoxin. Between samples B and C, the animals were heparinized, 3 mg./kg.

† Data obtained from a series of three experiments.

ministration of the endotoxin. The platelet count also showed a tendency to return to normal levels.

The hematocrit also rose somewhat during the period of observation. The mean rectal temperature reached a maximum at 270 minutes when it was 38.4 ± 0.5° C.; about one degree above the control level. It returned to the control level by 510 minutes.

Venous samples were also obtained from animals when they were in an agonal condition. A massive increase in catecholamines occurred at this time (Table 5), epinephrine concentration being greater than norepinephrine concentration.

Arterial samples were obtained at regular intervals following the injection of 10 mg./kg. *E. coli* endotoxin in a group of eight dogs. Samples drawn at 30, 60, 90, 180 and 270 minutes revealed concentrations of epinephrine and norepinephrine that were appreciably higher than the values obtained from venous samples in the group of dogs which are presented in Figure 3a. Furthermore, the arterial epinephrine levels were elevated for a 90-minute period following the administration of endotoxin in contrast to the venous norepinephrine elevation found in the dogs depicted in Figure 3a. As with the early changes following the administration of endotoxin, terminal arterial samples did not reveal the same high concentrations found in venous samples. Except for these differences, the pattern of response was not appreciably different from that found by analyzing venous samples.

Hemorrhagic Shock.* *Catecholamines and Blood Volume.* A striking sympatho-

* All means given with their standard errors.

TABLE 5. *Catecholamine Concentration Following Lethal Doses of E. coli Endotoxin*

		Venous Plasma Epinephrine (μg./L.)		Venous Plasma Norepinephrine (μg./L.)	
		Control	Terminal	Control	Terminal
3 Dogs receiving 10 mg./kg.	Mean ±S.E.*	0.76 ±0.4	167 ±79	2.42 ±1.84	48.4 ±20.5
5 Dogs receiving 7.5 mg./kg.	Mean ±S.E.	2.09 ±0.9	165.5 ±70	0.80 ±0.5	39.1 ±17.5

* Standard Error.

adrenal response to acute hemorrhage was observed (Fig. 4a). Five minutes after the onset of arterial bleeding, the mean volume of blood lost in a group of 12 dogs was 693 ± 42 cc. This constituted a loss of 36 ± 2.8 per cent of the total blood volume. The blood pressure fell to 51 ± 4 mm. Hg. The venous plasma epinephrine concentration rose to 35.4 ± 15.9 μg./L., a 70-fold increase over the mean control value within this short period of time. By 30 minutes, when the maximum bleed-out volume was approached, the epinephrine concentration was 45.7 ± 17.9 μg./L. The blood pressure at this time was 46.7 ± 1.0 mm. Hg and the bleed-out volume was 813 ± 67 cc., or 40 ± 3.4 per cent of the total blood volume.

Norepinephrine concentration also increased during this period, but not to the same extent as epinephrine. The magnitude of the increase in norepinephrine levels at five minutes was about five times the control value. At 30 minutes, plasma norepinephrine concentration rose to 22.3 ± 9 μg./L.

The mean blood pressure was maintained at 47 mm. Hg during the remainder of the four and a half hours of hemorrhagic hypotension. After one hour, the mean bleed-out volume had increased to 850 ± 64 cc. (43 ± 3% of total blood volume) but the epinephrine concentration had fallen considerably to 28.6 ± 10.8 μg./L. The change in norepinephrine was less pronounced. It had fallen to 16.25 ± 7.1 μg./L. During the next three and a half hours, the catecholamine concentration, especially epinephrine, continued to fall, followed by a fall in the bleed-out volume. i.e., the animal began to "take-up" some of its shed blood from the reservoir. At the end of the period of hemorrhagic hypotension, the bleed-out volume had fallen to 563 ± 72 ml., or 27 per cent of the total blood volume. Most of this spontaneous reinfusion took place after the three-hour mark, i.e., within the last one and one-half hours. Prior to complete reinfusion of the shed blood, the epinephrine concentration was 11.1 ± 4.1 μg./L. Norepinephrine concentration was 12.2 ± 2.8 μg./L. All of the changes in catecholamine content of the plasma were statistically significant at a confidence level of 95 per cent, except the increase in norepinephrine concentration at five minutes which was significant at a 90 per cent confidence level. Many of the differences were significant at confidence levels of 99 per cent or greater.**

The correlation between changes in blood volume (expressed as percentage of total blood volume) and the changes in catecholamine concentration were evaluated using Spearman's rank-correlation test. The correlation was not significant between epinephrine and blood volume ($r' = 0.37$) but it was significant at a 90 per cent confidence level between norepinephrine and blood volume ($r' = 0.77$).

Immediately after the dogs had been reinfused with the remaining blood, there was another striking change in catecholamine concentration in the direction of a decrease toward control levels. The mean plasma epinephrine concentration immediately after reinfusion was 2.4 ± 0.6 μg./L.; norepinephrine fell to 5.9 ± 1.1 μg./L. (p of difference from pre-infusion levels, $< .02$). However, these values were still significantly larger than the control levels ($p < .005$).

Reinfusion did not bring the blood pressure back to its pre-hemorrhage level. It rose to 96.3 ± 9 mm. Hg; the blood pressure prior to hemorrhage was 134 ± 6 mm. Hg. Thirty minutes after reinfusion, the epinephrine concentration was within normal limits and remained there for the duration of the period of observation (two hours after reinfusion). Norepinephrine levels, however, were still significantly greater than the control level at one and two hours after reinfusion was effected. The values at these

** Confidence levels obtained from a single-tail "t" test for matched pairs.

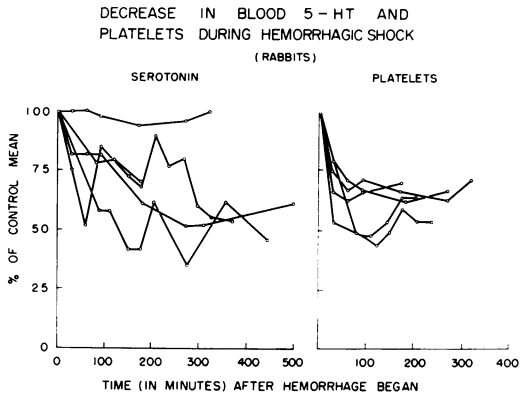


FIG. 5. Alterations in serotonin content of whole blood and platelets in five rabbits bled to a blood pressure of 40 mm. Hg for 2–3 hours and then re-infused. Sampling continued until the animals expired.

times were 4.2 ± 0.5 and 3.5 ± 0.8 μg./L., respectively (p of difference from control, $< .01$ and $< .05$)

Serotonin and Platelets. At the time of removal and reinfusion of blood from these animals, significant changes took place in both the platelets and serotonin (Fig. 4b). The profound loss of serotonin from the blood that was found in endotoxin shock was not observed. The decrease in platelets was greater than the fall in serotonin with an increase in serotonin concentration and the serotonin-platelet ratio. This could indicate that platelet destruction occurred with subsequent reabsorption of the released serotonin into the remaining platelets (Fig. 5). Figure 6 depicts the difference between hemorrhagic and endotoxin shock with respect to blood serotonin content in rabbits that were observed until death occurred.[63]

Discussion

Shock Due to Bacterial Endotoxins. Although the general problem of bacteremic shock includes both gram-positive and gram-negative organisms, we must confine this discussion to the form of shock produced by Gram-negative bacteria. Recent work has indicated that shock produced by staphylococci, for example, may be due to an exotoxin which causes circulatory collapse in a fashion somewhat different from that produced by endotoxins.[70] Endotoxin designates a ". . . relatively homogeneous group of toxic substances which exist as phosphorus-containing, polysaccharide - protein - lipid complexes in the intact-cells of a wide variety of gram-negative micro-organisms, or are liberated . . . during autolysis of the bacteria."[71] The importance of the shock syndrome caused by endotoxin has increased markedly in the past two to three decades. Finland has analyzed the changes which have taken place at the Boston City Hospital in the relative frequency of various bacteremic infections since the introduction of antibiotics.[29] He found that enterobacteria accounted for less than one in eight cases and one in eleven deaths before the chemotherapeutic era (1935). However, in 1957 one-third of all the cases of bacteremia and two-fifths of the deaths were produced by Gram-negative organisms.

The manner in which Gram-negative bacteremia produces the demise of an individual is undoubtedly complex, but shock is usually a prominent clinical feature. Factors such as direct tissue injury and plasma loss are probably contributing mechanisms. However, quite often shock persists despite an apparent adequate blood volume, al-

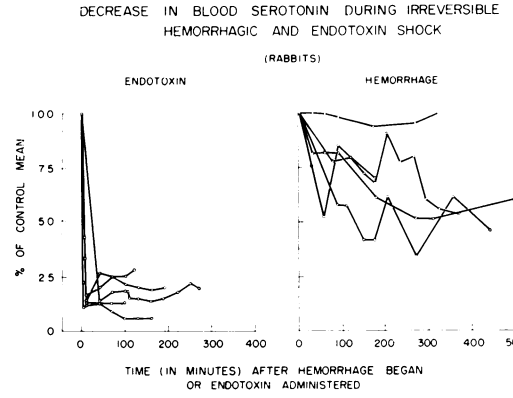


FIG. 6. Comparison of effect of endotoxin and hemorrhage on serotonin content of whole blood in rabbits (five animals in each group).

though actual circulating blood volume may be decreased. The reasons for this are obscure but are thought to be related to the hemodynamic changes produced by endotoxin.

The reticulo-endothelial system acts as the first line of defense in removing endotoxin from the circulation and thereby prevents these bacterial products from exerting their deleterious action.[5, 35, 72] The type of interaction which takes place between tissues and endotoxin, when the lipopolysaccharide is not effectively removed by the reticulo-endothelial system, bears a great similarity to an immunological reaction. Stetson's studies in this area suggest that normal animals may be hypersensitive to endotoxins.[69] Weil and Spink considered endotoxin shock to be another example of "anaphylactoid shock," and classified it with the toxic reactions, ". . . produced by a diversity of unrelated agents including peptones, carbohydrate polymers, parasitic extracts and snake venoms. . . ." [81] One might account for the beneficial effects of adrenal steroids in bacteremic shock on the basis of the salutary action of corticoids in most forms of allergic reactions.[36]

It was the similarity between endotoxin shock and anaphylactic shock which prompted us to investigate the changes in serotonin concentration following the administration of endotoxin. In 1953, Humphrey and Jacques demonstrated the release of histamine and 5-hydroxytryptamine (serotonin) during antigen-antibody reactions.[46] Histamine release had also been postulated as a possible mechanism for the hemodynamic changes which occurred following administration of endotoxin. Only recently has this been accurately documented.[45] However, the release of histamine cannot account for all of the vascular and hemodynamic effects of endotoxin. It is obvious that other mechanisms are involved.

In describing the changes occurring during endotoxin shock, it is convenient to consider the early (within the first 15 minutes) and later phases. In man, the early changes are not prominent—in contrast to the immediate and dramatic changes exhibited by the dog.[37] In interpreting these experiments, one should be cognizant of the many significant species differences which are known to exist. A prominent component of the early alterations brought about by endotoxin is vasoconstriction, the "villain" of hemorrhagic or traumatic shock. The basis for the vasoconstriction has eluded clarification.[1] The most lucid studies in this area are those of Zweifach *et al.;* [84] but even their results have been contradicted.[55] They demonstrated an increased reactivity of blood vessels to epinephrine following administration of endotoxin.

Recent studies on the adrenal output of 17-hydroxy-corticosteroids and catecholamines showed that large doses of endotoxin do significantly stimulate catecholamine output whilst smaller doses do not.[21] Measurements of adrenal catecholamine content following endotoxin administration by Heiffer *et al.* support the contention that these substances are secreted in greater than normal amounts after the injection of endotoxin.[42] In an earlier communication they were unable to document increased peripheral levels of catecholamines.[41] However, the data we have presented here definitely establish the presence of increased, albeit variable, catecholamine concentrations early in endotoxin shock. The concentrations which were attained could by themselves account for the early vasoconstriction seen in the dog. The predominant increase in epinephrine concentration indicates that the adrenal medulla is prominently involved in this response. The fact that epinephrine levels soon return to normal while norepinephrine levels remain slightly elevated, indicates that the norepinephrine release, from the adrenal medulla or sympathetic nerve endings, may be of some importance during the later stages of endotoxin shock.

The late changes of endotoxin shock are

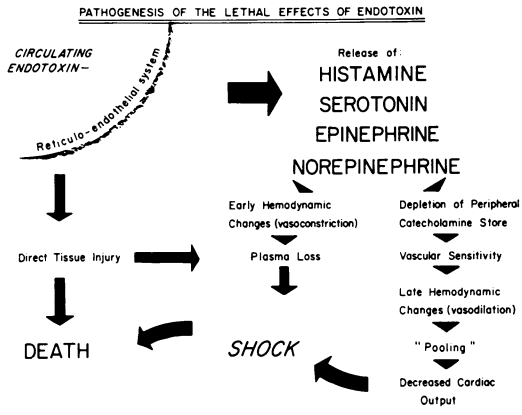


FIG. 7. Schematic representation of pathogenesis of shock caused by endotoxin.

characterized by vasodilatation. The action of serotonin is such that it constricts larger arteries and veins while it dilates smaller vessels.[39] Haddy's work in this area is noteworthy. He has demonstrated that serotonin and histamine produce directionally opposite changes in segmental resistance.[40] Histamine actively dilates arterioles at the same time that it actively constricts veins, a situation most conducive to the pooling of blood in a capillary bed.[40] Since both serotonin and histamine are liberated by endotoxin, *in vitro* as well as *in vivo*,[17] they could be responsible for the late dilatation and pooling of blood which have thus far not been adequately explained. The vasodilatation produced by the release of serotonin during endotoxin shock can be compared to the vasodilatation seen in patients with the malignant carcinoid syndrome during an episode of flushing.

There may be another reason for vascular instability in endotoxin shock. Endotoxin may have the ability to temporarily deplete the peripheral norepinephrine store in much the same fashion that Burns and Rand have shown reserpine to exert its effects.[10, 11] This would be tantamount to pharmacologically "denervating" the vessels and rendering them more sensitive to catecholamine stimulation; a point that may be important in utilizing vasopressor amines therapeutically. The lack of vasomotor tone would also contribute to the occurrence of vasodilatation. This concept does not imply vasomotor "paralysis" or "exhaustion" due to a deficiency in catecholamines. Quite the contrary, as the blood pressure falls in the terminal phase of endotoxin shock, an abundance of epinephrine and norepinephrine is found in the circulation.

Eventually there is sufficient "pooling" within the splanchnic bed so that the venous return to the heart falls. The subsequent fall in cardiac output leads to progressive hypotension and the clinical picture of shock supervenes. This hypothetical sequence of events is schematically shown in Figure 7.

Hemorrhagic Shock. Early deaths during hemorrhagic hypotension are undoubtedly due to cerebral anoxia—but those that occur after the oligemia has been corrected and the blood pressure brought back to normal are more difficult to explain. A sudden fall in pressure stimulates the baroreceptors in the sino-aortic area and by reflex action produces an outpouring of catecholamines from the sympatho-adrenal system.[44] As in endotoxin shock, the adrenal plays the more prominent role in the immediate period but norepinephrine release constitutes the mainstay of the response. The resulting vasoconstriction is, in turn, responsible for a decrease in catecholamine secretion. Heymans has shown that norepinephrine applied to the carotid sinus decreases its reactivity.[43] This produces a decrease in the secretion of sympathetic amines, the vasoconstriction relents somewhat, and thus the phenomenon of "take-up" of blood from a reservoir occurs.

In the patient who has bled, there is no reservoir and the result is a further fall in blood pressure. This "feedback" mechanism decreases the degree of vasoconstriction, which, if persistent, has deleterious effects of its own. However, sympatho-adrenal hyperactivity, as reflected by elevated levels of epinephrine and norepinephrine cannot

be equated with vasoconstriction, increased vascular resistance and decreased flow. It has been shown that adrenergic responses consist of vasodilatation, as well as vasoconstriction, depending largely on the nature of the adrenergic agent (e.g., epinephrine, norepinephrine or a sympathomimetic substance), the amount released or injected and the vascular bed to which the adrenergic amine is applied. Green's recent review of the effects of sympatho-adrenal activity on the control of blood flow through various vascular beds best summarizes this complex situation.[38] He has pointed out that the heart and brain, ". . . show no clearcut evidence of participating in autonomically induced constrictor responses." The vascular bed that responds maximally with vasoconstriction when stimulated by epinephrine and norepinephrine is the kidney, followed, in decreasing order of responsiveness, by the vascular beds of the skin, intestine, skeletal muscle, spleen and liver. Thus, the concept enunciated by Cannon as early as 1914, appears to be basically sound, i.e., the sympatho-adrenal response to stress, in this case hemorrhagic hypovolemia, protects the two organs which are most susceptible to anoxia.[12] The increased resistance in the vascular beds of the kidney, skin, intestine, skeletal muscle, spleen and liver shunts the reduced blood volume to the heart and lungs and the brain. However successful this protective mechanism may be in the immediate period following hemorrhage, its ultimate benefit to the organism can be questioned. Because of the prolonged reduction in nutrient flow, the organs with a greater ability to withstand anoxia become the vulnerable organs in shock. The kidneys, intestine and liver can tolerate temporary ischemia of varying degrees but eventually reach a point of no return, i.e., despite the re-establishment of an adequate blood flow, if ischemia has been prolonged, irreversible changes occur as a result of stagnant anoxia. These irreversible changes produce the demise of the animal. The early deaths are most probably the result of intestinal necrosis.[48] If the shocked animal avoids this catastrophe, a later death may be caused by hepatic failure, renal insufficiency or a combination of the two.

Although one can regularly produce "irreversible shock" in experimental preparations, there is no way to predict its occurrence clinically. To prevent it, one must vigorously correct any depletion in blood volume. The use of adrenolytic drugs is a poor substitute for transfusions. As these studies show, the reinfusion of blood into hypovolemic animals immediately reduces the extremely high levels of endogenous circulating catecholamines. The use of vasodilating drugs during the hypovolemic phase, as recently employed by Boba, may remove the mechanism which is responsible for maintaining a patient's survival unless even greater amounts of blood or plasma than that originally lost are added to the circulatory system simultaneously with the vasodilating drug.[7] Until the exact relationship among blood flows to various organs in hemorrhagic shock is delineated, blood volume replacement must be stressed as the mainstay of therapy for the patient in hemorrhagic shock. The "protective" role of adrenergic amines in early hemorrhagic shock—and its ultimate "harmful" action—must be substantiated by direct blood flow determinations. Such studies are currently being performed in this laboratory.

These studies also demonstrate that one can characterize either hemorrhagic shock or endotoxin shock on the basis of differences in circulating vasoactive substances: Endotoxin shock in the dog always is accompanied by hyposerotoninemia. This is not true of hemorrhagic shock. Although oligemia may contribute to endotoxin shock, and endotoxin to oligemic shock, the underlying mechanisms of death in these two conditions appear to be quite dissimilar. Experiments on germ-free animals have corroborated the fact that the characteristic

picture of irreversible shock occurs in the absence of bacteria.[85] Nonetheless, the final common path by which death occurs in hemorrhagic and endotoxin shock undoubtedly is a limitation of blood flow to vital organs.

Summary

1. During lethal endotoxin shock, serum serotonin levels fall to 22 per cent of their control value and remain at this general level throughout the remainder of the experiment. Platelets do not decrease to the same degree.

2. Immediately after endotoxin administration, plasma catecholamine concentrations rise. Epinephrine soon returns to control levels but norepinephrine remains slightly elevated.

3. The hemodynamic changes which lead to endotoxin shock result from the release of histamine, serotonin, epinephrine and norepinephrine. It is postulated that a temporary depletion of the peripheral catecholamine store occurs, similar to the situation encountered in denervated vessels. Vascular instability progressing to vasodilatation, pooling and decreased cardiac output are ultimately responsible for the appearance of shock. Direct tissue injury and plasma loss also contribute to the appearance of the shock syndrome.

4. Following onset of hemorrhagic hypotension, peripheral venous levels of endogenous epinephrine rise in a striking fashion to reach a 90-fold increase over the control value. Thereafter they decrease but never approach normal concentrations. Following re-infusion of shed blood, catecholamines decrease in the same striking fashion that they increase.

5. Pronounced changes in serum serotonin concentration do not occur during hemorrhagic shock.

6. Irreversible hemorrhagic shock results from ischemic changes of the bowel, liver or kidney, secondary to vasopressor amine activity superimposed on oligemia. Vasoconstriction serves to preserve blood flow to the heart and brain which do not share in the increased resistance in their vascular beds. Deaths occurring early in the irreversible stage are due to intestinal necrosis—later deaths are due to hepato-renal insufficiency.

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Discussion

Dr. Bernard Zimmermann: Only a couple of rather general thoughts occur to me. This investigation emphasizes the major difference in mechanisms which obtain in the case of hemorrhagic and endotoxin shock, and there is surely a great deal more to be learned about the endocrine and vascular responses to such things as large hemorrhage, small hemorrhage, extensive tissue injury, bacteremia, and so on. The term "shock," however, seems to emphasize the similarities rather than the differences in reactions to these particular situations which makes one wonder whether the whole concept has outlived its usefulness. Central, of course, to the consideration of experimental shock is the phenomenon of irreversibility, a situation which though clearly definable and reproducible in a very special kind of laboratory situation, is much more difficult to identify in relationship to injury in the human.

Finally, the question of the damaging role of sympatho-adrenal hormones comes into the question. Intuitively it is very difficult for me to accept the idea that an animal which is otherwise capable of survival would succumb to his own protective internal secretions. I think we will have to have a good deal more evidence before many of us can agree to this.

Dr. Jacob Fine: A high titer of circulating catechol amines in hemorrhagic shock reflects the response of the adrenal medulla to stress. It does not necessarily reflect the rate of production of these compounds by the sympathetic nerves in the tissues, or their rate of turnover, or their activity at the target site.

Therefore, the lower and less consistently elevated level of these compounds in the blood of endotoxic shock does not permit the inference that their effectiveness at the target site is any less than it is in hemorrhagic shock. Thomas has demonstrated that endotoxins potentiate the activity of the catechol amines injected intradermally so as to result in hemorrhagic necrosis of the skin. We have confirmed this observation by noting that sympathetic denervation of the rabbit's kidney or ear protects the denervated tissues from the inflammation and necrosis that is otherwise produced by endotoxin. The protective effect of dibenamine in blocking the Schwartzman reaction in thorotrast pretreated rabbits is also evidence that endotoxin inflicts damage via the catechol amines. Whatever the level of the catechol amines in the blood, excessive vasoconstrictor activity in endotoxic shock has been repeatedly observed in man and animals. Paralysis of flow owing to a late or terminal loss of vascular tone may be a more dominant features of endotoxic shock than of hemorrhagic shock. If so, it may be because of the role of other hormones, but whether they do so and how they might do so remains to be demonstrated.