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Studies on Peripheral Blood Catecholamine Levels During Hemorrhagic Shock in Dogs.* (28981)

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Watts(1) showed that bleeding dogs to a mean arterial pressure of 38 mm Hg increased the peripheral arterial blood epinephrine (as the base) from 0.9 to 20.5 μg/l. Several investigators(2-5) have substantiated this observation and have also shown that there is a significant increase in arterial norepinephrine in dogs during shock. These results from different laboratories obtained by several methods for catecholamine determination show there is a 5-10-fold increase in norepinephrine and a 50-100-fold increase in epinephrine during hemorrhagic shock in dogs. In the present experiments peripheral blood catecholamines in dogs have been determined under a variety of conditions, to establish more precisely the origin and physiological role of these vasopressor agents during hemorrhagic shock. These studies include:

(1) Comparison of values obtained by simultaneous determinations of arterial epinephrine levels by bioassay and fluorimetry. (2) Arterial epinephrine—norepinephrine levels at decreasing increments of arterial pressure. (3) Effect of pentobarbital, chloralose and local anesthesia on arterial blood epinephrine levels during shock. (4) Simultaneous estimation of epinephrine in blood from the inferior vena cava above the adrenals, the femoral artery and the femoral vein, to obtain information on the secretion and disappearance of epinephrine during hemorrhagic shock.

Methods. Dogs of both sexes weighing from 8.2 to 15.9 kg were anesthetized with pentobarbital (30 mg/kg), chloralose (80 mg/kg), by infiltration of 1% procaine in the area to be cannulated. The dogs were bled from the femoral artery into a pressure compensator to a mean blood pressure of 40 mm Hg either by 30 mm Hg increments or by 50 ml increments at 10-minute intervals. Carotid blood pressure was monitored by means of a Sanborn 150 M recorder with a Statham transducer. Control arterial blood samples for bioassay were collected at the beginning of each experiment and after each bleeding. The method of Gaddum and Lembeck(6) as modified by Franko *et al*(7) was used for bioassay of epinephrine in 0.5 ml plasma or whole blood samples. To compare the bioassay method with the fluorimetric method four to eight 50 ml blood samples corresponding to the samples for bioassay were collected in chilled polyethylene centrifuge tubes containing 50 mg ethylenediaminetetraacetic acid disodium salt (Eastman) and immediately centrifuged at 4°C and 2500 rpm for 25 minutes. The plasma was decanted, the volume measured and stored at 4°C until assayed. The fluorimetric method of Vendsalu(8) was used until it was found that catecholamine adsorption on aluminum oxide according to the procedure of Goldfien *et al*(9) followed by the trihydroxyindole procedure of von Euler and Lishajko (10) was just as adequate and less time consuming. In several experiments the epinephrine levels in blood from the superior vena cava, femoral artery and femoral vein were compared. The samples were drawn

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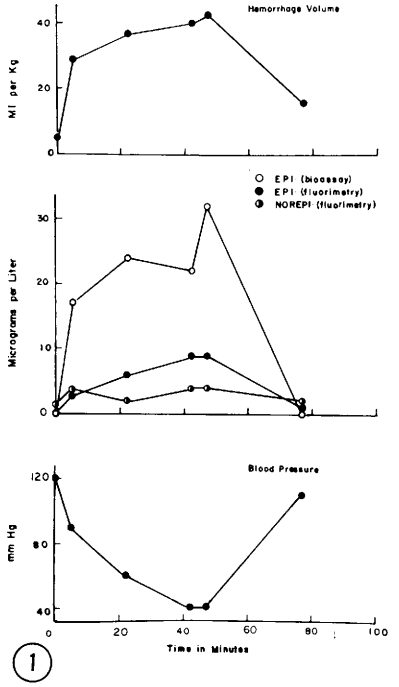

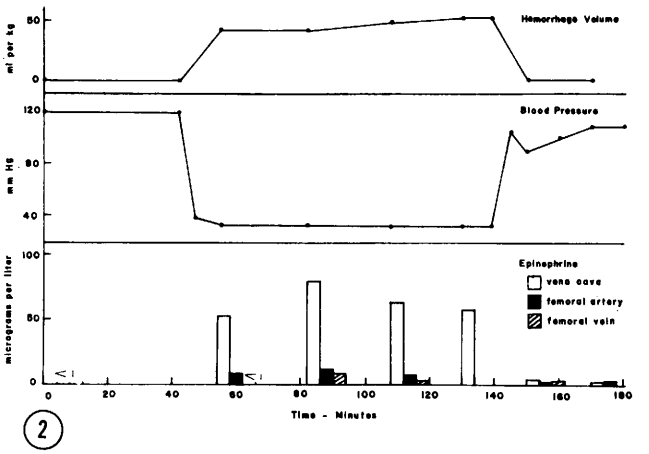

FIG. 1. Typical experiment showing arterial plasma catecholamine levels during hemorrhage and after reinfusion of blood in a 13.6 kg male dog. Pentobarbital anesthesia; catecholamine determinations made on plasma by fluorimetric method.

FIG. 2. Epinephrine concentration in blood from the inferior vena cava, femoral artery and femoral vein during hemorrhagic shock in a 9.9 kg male dog. Pentobarbital anesthesia; epinephrine determinations made on whole blood by bioassay using the rat uterus.

simultaneously and epinephrine levels measured by bioassay using 0.5 ml samples.

Recovery of epinephrine and norepinephrine by the fluorimetric method was determined by adding known amounts of the amines to whole blood samples or plasma and carrying them through the same procedure as the unknowns. No recovery experiments were run on the bioassay method as it was known from previous work(7) to give essentially complete recovery of epinephrine from both plasma and whole blood.

Results and discussion. Fig. 1 shows the results of a typical experiment in which a dog was bled to a mean arterial pressure of 40 mm Hg by 30 mm Hg increments. With each drop in blood pressure there was a corresponding increase in peripheral plasma epinephrine level. The concentration of norepinephrine was found to be higher than epinephrine in the control plasma sample but in the hemorrhage samples this was reversed. When the blood was reinfused the catecholamines returned to the pre-hemorrhage level.

Table I is a summary of data from experiments on 6 dogs in which plasma catecholamine levels were determined simultaneously by bioassay and fluorimetric methods. The epinephrine and norepinephrine values by fluorimetry are also shown corrected for recovery. It can be seen that the corrected value of 27.6 μg/l for epinephrine determined by the fluorimetric procedure closely approximates the 23.3 μg/l found by bioassay. Likewise the values for epinephrine-norepinephrine obtained in this series of dogs are in close agreement with those obtained by other investigators(2-5). These data show that whether estimated by bioassay or by fluorimetric methods the catecholamines in the peripheral arterial plasma of dogs increase from control levels of less than one microgram per liter to about 25 to 30 μg/l for epinephrine and 5 to 10 μg/l for norepinephrine. Thus the increase in circulating catecholamines is largely due to epinephrine, which is to be expected since the norepinephrine liberated at post-ganglionic sympathetic nerve endings would be largely inactivated in the cells stimulated and only a small part would be expected to escape into the circulating blood. Since there is good correlation between epinephrine determinations made by bioassay employing the rat uterus

TABLE I. Plasma Epinephrine Levels for 6 Dogs Determined Simultaneously by Fluorimetry and Bioassay. Values are means ± standard error of mean.

	Hemorrhage vol, ml/kg	Blood pressure, mm Hg	Epinephrine bioassay, μg/l	Epinephrine fluorimetry, μg/l	Norepinephrine fluorimetry, μg/l
Control (n = 6)	3.9 ± 1.4	143 ± 10.6	<1.0	.2 ± .1	1.5 ± .7
Hemorrhage (n = 15)	39.1 ± 2.0	56 ± 7.5	23.3 ± 4.4	14.4 ± 4.5	5.1 ± 1.5
			Corrected for % recovered:		
Control (n = 6)	—	—	—	.4 ± .2	3.4 ± 1.6
Hemorrhage (n = 15)	—	—	—	27.6 ± 8.7	11.6 ± 3.4

% recovered by fluorimetry: Epinephrine, 52 ± 7.1; Norepinephrine, 44 ± 7.9.

and 0.5 ml blood samples and the fluorimetric method using 50 ml samples these different techniques can be used to advantage in different types of experiments. When rapid, serial estimations are needed with minimum blood loss the bioassay procedure is adequate and the rat uterus is reasonably specific for epinephrine. Unfortunately isolated tissues such as the rat colon(6), which might be used to estimate rapidly norepinephrine in small blood samples, are not sufficiently sensitive to detect catecholamines in peripheral blood even under severe stress such as hemorrhagic shock. Thus when a differential estimation of epinephrine-norepinephrine is needed and 30-50 ml blood samples can be obtained the fluorimetric method is more useful.

In the series of 8 dogs in which arterial pressure was lowered by 30 mm steps readily detectable levels of catecholamines did not appear until the pressure had dropped below 80 mm Hg. When the blood pressure was lowered to 30 ± 3 mm Hg the arterial epinephrine and norepinephrine concentrations were 46.1 ± 11.7 and 8.4 ± 3.4 μg/l respectively (fluorimetric determinations corrected for recovery). Freeman *et al*(11) have shown that infusion of epinephrine at rates of 3.4 to 16.4 μg/kg/min will produce shock and death in dogs. Their lowest infusion rate of 3.4 μg/kg/min known to produce shock in dogs has been shown to give an arterial blood level of 39 μg/l(12) which is apparently adequate to produce shock and death in normovolemic animals. Thus the endogenously released epinephrine level of 46.1 μg/l observed in these experiments must be considered an important factor in producing shock in hypovolemic dogs.

Fig. 2 shows the epinephrine levels, as determined by bioassay in whole blood obtained from 3 areas of the dog; the inferior vena cava at the level of the sternal notch, femoral artery and femoral vein. The epinephrine increased markedly at all 3 sites reaching a maximum at about 80 minutes after the beginning of hemorrhage and returning to near control levels after reinfusion of the shed blood. As expected the greatest increase was observed in the superior vena cava followed by the femoral artery and femoral vein. The high concentration of epinephrine observed in the inferior vena cava was reduced by dilution through venous return from the superior vena cava and inactivation in the lungs so that a much lower level was found in the femoral artery. This was reduced to an even lower concentration in the femoral vein by inactivation in the tissues of the leg. These results focus attention on the importance of epinephrine in the sympathoadrenal response to hemorrhagic hypotension. Epinephrine is the catecholamine most markedly increased in the peripheral circulation during shock and would be largely responsible for the hyperglycemia, acidosis and visceral ischemia characteristic of shock.

It was expected that arterial blood epinephrine would be higher under chloralose anesthesia which is known to sensitize baroreceptor reflexes in the dog(13) than under pentobarbital which inhibits these reflexes. No significant difference in peripheral arterial epinephrine levels was found with these anesthetics nor was there any noticeable difference when a local anesthetic only was used for obtaining femoral arterial blood in a single experiment. Thus one must conclude that severe hemorrhage with arterial blood

pressures of 40-60 mm Hg is a supramaximal stimulus for the adrenal medulla and the secretion of epinephrine is not markedly affected by the type of anesthetic.

Summary. Peripheral blood catecholamines were studied in dogs during hemorrhagic shock. When determined by the fluorimetric procedure, with values corrected for recovery, epinephrine increased from control levels of 0.4 ± 0.2 μg/l to 27.6 ±8.7 μg/l; norepinephrine increased from 3.4 ± 1.6 μg/l to 11.6 ± 3.4 μg/l. Thus during hemorrhagic shock in dogs the catecholamines found in the peripheral arterial blood are largely epinephrine (70%); and to a lesser extent norepinephrine (30%). Significant quantities of epinephrine-norepinephrine usually appear in the blood when the mean arterial pressure has dropped to about 80 mm Hg. As the pressure is lowered further the circulating catecholamines increase very rapidly. This increase is largely due to epinephrine and to a lesser extent to norepinephrine. Simultaneous estimation showed epinephrine concentration to be greatest in the inferior vena cava followed by the femoral artery and lowest in the femoral vein. These experiments indicate most of the catecholamines in the circulation during hemorrhagic shock in dogs are released from the adrenal medulla and are rapidly destroyed by the tissues.

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Inhibition of Mammary Secretion in Rats by Iproniazid.*† (28982)

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Monoamine oxidase (MAO) inhibitors are reported to produce alterations in the metabolism of serotonin and catecholamines in the central nervous system (CNS), and hence may influence CNS function(1). Since the CNS, particularly the hypothalamus, is involved in regulation of pituitary secretions (2), these MAO inhibitors may bring about changes in pituitary function. Thus it has been reported that iproniazid and certain other MAO inhibitors retarded sexual maturity in immature female mice(3) and rats (4), prevented development of decidual tissue in mice(5), interrupted pregnancy in mice and rabbits when administered before midpregnancy(6,7), and reduced fertility in mice and rats when given chronically throughout the periods of mating(8). Only one laboratory reported that iproniazid had no effect on implantation and litter size in mice(9). The foregoing suggests that iproniazid may inhibit gonadotropin secretion through the CNS(3-9).

Many agents, including electrical stimula-

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