TABLE I. Comparison of Effects of 3 Different Carbonic Anhydrase Inhibitors on Urine Electrolytes of the Dog and the Alligator.

Compound	Dog					Alligator				
	Na	Cl	K	CO_2	H	Na	Cl	K	CO_2	H
Chlorothiazide	++	++	+	+	—	0	+?	0	—?	+?
Dichlorphenamide	++	+	+	+	—	0	+++	++	——	+
Acetazoleamide	++	0?	++	++	——	+?	+++	+	——	+

Direction of the change is indicated as follows: +++, great increase; +, increase; ——, great decrease; —, decrease; 0, little or no change.

amide after 30 days of injections was noted nor was there any sign that the decreased plasma Cl prevented the excretion of Cl. The degree of K loss was not directly correlated with hydrogen ion excretion. Two new carbonic anhydrase inhibitors, chlorothiazide and dichlorphenamide were tested. Dichlorphenamide was more active in promoting K and Cl loss at low dose levels than acetazoleamide. Chlorothiazide showed no significant activity. Since the effects noted after the inhibition of carbonic anhydrase by acetazoleamide and dichlorphenamide are not the same, it is probable that something more than simple inhibition is involved.

The authors wish to thank Lederle Laboratories Division, American Cyanamid Co. for generous supply of Diamox and Merck Sharp and Dohme, for a supply of Diuril and dichlorphenamide.

1. Berliner, R. W., Kennedy, T. J., Jr., and Orloff, J., *Am. J. Med.*, 1951, v11, 274.

2. Hernandez, T., and Coulson, R. A., *Science*, 1954, v119, 291.

3. Maren, T. H., *Trans. N. Y. Acad. Sci.*, 1952, v15, 53(Ser.II).

4. Coulson, R. A., and Hernandez, T., *Am. J. Phy.*, 1957, v188, 121.

5. Beyer, Karl H., Baer, John E., Russo, Horace F., and Haimbach, Audrey S., *Fed. Proc.*, 1957, v16, 282.

6. Ford, Ralph V., Moyer, John H., Handley, Carroll, and Spurr, Charles L., *Med. Records and Annals*, 1957, v51, 376.

7. Coulson, R. A., and Hernandez, T., PROC. SOC. EXP. BIOL. AND MED., 1955, v88, 682.

8. Moyer, J. H., Ford, R. V., and Spurr, C. L., *ibid.*, 1957, v95, 529.

9. Miller, Wilber H., Dessert, Alice M., and Roblin, Richard O., *J. Am. Chem. Soc.*, 1950, v72, 4893.

10. Personal Communications from Merck Sharp and Dohme, West Point, Pa.

Received July 19, 1957. P.S.E.B.M., 1957, v96.

Blood Epinephrine Levels and Automatic Reinfusion of Blood During Hemorrhagic Shock in Dogs.* (23553)

D. T. WATTS AND A. D. BRAGG

Department of Pharmacology, West Virginia University Medical School, Morgantown, W. Va.

Vasoconstriction is a prominent feature of the early stages of hemorrhagic shock in dogs. Most authorities agree that this is initially a protective mechanism mediated through the carotid sinus to maintain a blood supply to vital organs. However, if this vasoconstriction is of adequate severity and duration it is possible for the ischemia to produce irreversible tissue damage(1). This concept is supported by the fact that several investigators have produced shock by the intravenous infusion of epinephrine(2), and that adrenergic blocking agents protect dogs against hemorrhagic shock(3). Various procedures for producing hemorrhagic shock in dogs have been standardized by Wiggers(4) and other investigators. When dogs are bled to a mean arterial pressure in the range of 40-60 mm Hg by means of an arterial pressure compensator (5) spontaneous reinfusion will commence in about one hour and continue until the animal

* This investigation was supported in part by research grants from Life Insurance Medical Research Fund and National Heart Inst., Bethesda, Md.

succumbs. Glasser and Page(6) associated this reinfusion with irreversibility in hemorrhagic shock. Beck and Dontas(7) have shown by direct nerve potential measurements that sympathetic activity was greatly elevated during the initial stages of hemorrhagic hypotension and then decreased as automatic reinfusion progressed. Watts(8) has recently shown that high concentrations of epinephrine appear in the peripheral blood of dogs during acute hemorrhagic hypotension and there was evidence that this epinephrine level decreased in some animals after about one hour. The automatic reinfusion of blood at a constant arterial pressure is probably due to an increased vascular space resulting from decreased vasomotor tone. This could be due to failure of the sympathetic vasoconstrictor mechanism, decrease in the concentration of humoral vasoconstrictor substances, or damage to vascular muscle cells. In the present experiments, peripheral blood epinephrine levels have been followed in dogs during the initial stages of hemorrhagic shock and during the automatic reinfusion at a mean arterial pressure of 40 mm Hg to determine whether or not this vasoconstrictor material was disappearing from the blood during the stage of reinfusion.

Methods. Twelve healthy mongrel dogs with an average weight of 11.6 kg (8.4-15.0) were anesthetized with 30 mg/kg sodium pentobarbital injected intraperitoneally 30 minutes before hemorrhage. This amount of pentobarbital was usually sufficient to maintain anesthesia, but small supplementary doses were used as needed to keep the animal quiet. A polyethylene catheter (0.070 in. internal diameter) was passed through a femoral artery as far as the aorta. Heparin (3 mg/kg) was given intravenously to prevent clotting of blood during hemorrhage and collection of samples. Control blood samples were drawn and the dog was allowed to hemorrhage into a polyethylene reservoir. Level of blood in the reservoir was maintained at 40 mm Hg until death of the dog. The blood was agitated with a magnetic stirrer driving a small plug covered with polyethylene. The use of non-wettable polyethylene catheters

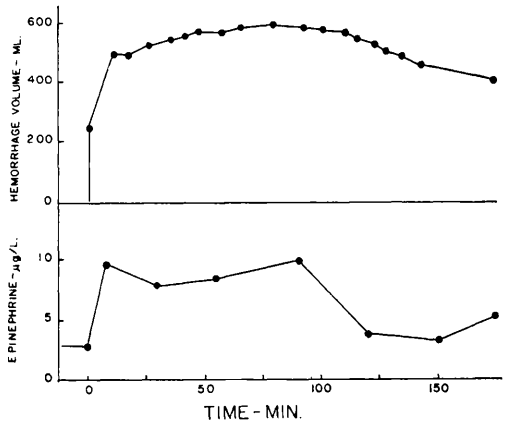

FIG. 1. Hemorrhage vol and blood epinephrine levels in 10.6 kg male dog while mean arterial blood pressure was held at 40 mm Hg. Blood samples were drawn from the femoral artery and epinephrine values are given as the base.

and containers minimized hemolysis. Temperature of the blood was maintained at 38° C. *Epinephrine levels* in arterial blood were determined at 6 to 30 minute intervals beginning with control samples taken before hemorrhage. With minor modifications the method of Gaddum and Lembeck(9), employing the rat uterus was used for the bioassay for epinephrine. Modified Ringer solution was used as the perfusion fluid. Carbachol (1 mg/l) was used to stimulate the uterus. Lysergic acid diethylamide (5 µg/l) was added to the wash solution to prevent stimulation of the uterus by serotonin in the blood samples(10). Two to 3 ml blood samples were drawn from the femoral artery into iced polyethylene tubes and assayed within 30 seconds since extensive experience with the method has shown that epinephrine disappears very rapidly from shed blood. Under these conditions from 93 to 99% of epinephrine added to blood samples can be recovered. Hemorrhage volume, respiration, and heart rate were recorded at the time each blood sample was obtained. Tests for significance were made by Fisher's(11) method for small samples using the "t" test to determine probability.

Results. Fig. 1 is a typical experiment showing hemorrhage volume and blood epinephrine levels during hemorrhagic hypotension at 40 mm Hg with automatic reinfusion

TABLE I. Epinephrine Levels in Peripheral Arterial Blood of Dogs during Hemorrhagic Hypotension at 40 mm Hg with Automatic Reinfusion.

	Epinephrine—μg/l, as the base				Hemorrhage vol—ml/kg	
	Control	Max hemorrhage	Early reinfusion (first 10%)	Late reinfusion (10% to death)	Max	At death
	.9	17.3	9.5	5.8	51.2	31.0
	0 *	4.0	6.7	8.8	45.0	0
	3.4	7.7	6.0	4.1	54.8	43.3
	0	5.2	8.7	5.1	32.3	10.1
	0	9.3	5.9	4.9	46.7	26.0
	0	14.2	6.4	1.2	40.5	24.8
	2.9	9.7	5.3	3.4	40.6	24.5
	0	10.8	4.9	4.5	70.6	44.1
	0	7.5	6.8	6.1	34.7	26.0
	0	4.9	3.8	2.5	50.4	
	0	21.0	6.5	4.3	45.5	
	0	38.0	22.2	11.6	48.1	
Mean ± S.E.	.6 ± .33	12.5 ± 2.75	7.7 ± 1.39	5.2 ± .80	46.7 ± 2.72	25.5 ± 4.7

* 0 indicates epinephrine content of blood was less than sensitivity of the method which is about 1 μg/liter.

in a 10.6 kg male dog. Automatic reinfusion began 90 minutes after the start of hemorrhage and continued until the animal died at 178 minutes. The blood epinephrine levels paralleled the hemorrhage volume. The maximum epinephrine concentration was found soon after hemorrhage was started and remained at this elevated level until about the time automatic reinfusion began. As the reinfusion continued the epinephrine level decreased and was near the minimum level observed at the time of death.

Table I summarizes the data from similar experiments on 12 dogs. Since the time to reach maximum hemorrhage, for automatic reinfusion, and for death, was quite variable, the blood epinephrine levels are given for the control period, the time to reach maximum hemorrhage and for periods of early and late reinfusion. Early reinfusion was arbitrarily taken as the time required for reinfusion of 10% of the hemorrhaged blood; late reinfusion as the time from the point of 10% reinfusion until death. The average maximum hemorrhage of 46.7 ml/kg was reached in 89 minutes (range, 60-135). Automatic reinfusion commenced at this point and 10% of the maximum hemorrhaged volume had returned to the circulation at the end of 127 minutes (range, 90-210). The reinfusion continued and the animals died after an average elapsed time of 232 minutes (range, 140-395) when average hemorrhage volume was 25.5 ml/kg.

Epinephrine concentration in the peripheral blood increased from a control level of less than one μg/l, the minimal concentration of epinephrine that could be detected with the rat uteri used, to a level of 12.5 ± 2.7 μg/l during the period of maximum hemorrhage. This is a significant increase. This epinephrine concentration reached a peak at approximately the same time as the hemorrhage volume. The blood epinephrine level then decreased during the period of early reinfusion to an average of 7.7 μg/l, and further decreased to an average of 5.2 μg/l, during late reinfusion. These epinephrine concentrations during both the early and late stages of automatic reinfusion are significantly less than the level during the period of maximum hemorrhage. The heart and respiratory rates were elevated above control values throughout the period of hemorrhage except for a short interval when death was imminent and both rates dropped precipitously. Respiratory exchange was good in all animals except for the last few minutes before death.

Discussion. These experiments have shown that epinephrine concentration in the circulating blood is significantly higher during hemorrhagic hypotension than under normal conditions. The method of bioassay used was not sufficiently sensitive to detect routinely epinephrine in the control blood samples. Thus

epinephrine levels in normal blood could only be determined to be less than the minimum sensitivity of the method, *i.e.*, less than about one μg/l. This observation accords with the results of Holzbauer and Vogt(12) showing that in a highly purified extract that the plasma epinephrine concentration in a dog under normal conditions was between 0.04 and 0.25 μg/l. The rise in the epinephrine content of the blood during hemorrhage and the maintenance of this high concentration indicate that epinephrine release may not only be one of the compensatory mechanisms effective in shock, but may even be the principal one. The average peak of 12.5 μg/l. of epinephrine represents at least a 10-fold increase over normal physiological levels and could account for the intense vasoconstriction, decreased peripheral blood flow in many tissues, and cardiac effects which are typical of hemorrhagic shock in dogs(4).

The impending failure of the cardiovascular system as indicated by the automatic reinfusion of blood was accompanied by a lowering of the epinephrine content of the peripheral blood. The decrease in blood epinephrine between peak hemorrhage and death is statistically significant, but it should be indicated that throughout the period of hemorrhage and at the time of death, blood epinephrine levels are well above the control level. Although the physiological significance of the maximum blood epinephrine levels observed in these experiments has not yet been established, it is very likely that this circulating epinephrine does contribute to the intense vasoconstriction and tissue ischemia characteristic of the earlier stages of hemorrhagic shock. Since the concentrations observed throughout the period of hypotension are well in excess of normal physiological levels, it appears that the administration of additional epinephrine would not be beneficial, whereas adrenergic blocking agents could be expected to alleviate some of the suspected deleterious effects of this excess circulating epinephrine.

Summary. The epinephrine content of peripheral blood of 12 dogs was determined during hemorrhagic hypotension with automatic reinfusion of blood at 40 mm/Hg. The epinephrine content increased from a control value of less than one μg/l to a maximum of 12.5 μg/l which occurred during the period of maximum hemorrhage. This epinephrine level decreased during automatic reinfusion of the blood at 40 mm Hg and reached a level of 5.2 μg/l during the later period of the reinfusion before the dogs died. It is possible that this increased concentration of epinephrine in the circulation is at least partially responsible for the intense vasoconstriction, decreased peripheral blood flow in certain tissues, and detrimental tissue ischemia, typical of hemorrhagic shock in dogs.

1. Freeman, N. E., *Ann. Surg.*, 1935, v101, 484.
2. Erlanger, J., and Gasser, H. S., *Am. J. Physiol.*, 1919, v49, 345.
3. Wiggers, H. C., Ingraham, R. C., Roenchild, F., and Goldberg, H., *ibid.*, 1948, v153, 511.
4. Wiggers, C. J., *Physiology of Shock,* Commonwealth Fund, New York, 1950.
5. Lamson, P. D., and DeTurk, W. E., *J. Pharmacol. Exp. Therap.*, 1945, v83, 250.
6. Glasser, O., and Page, I. H., *Am. J. Physiol.*, 1948, v154, 297.
7. Beck, L., and Dontas, A. S., *Fed. Proc.*, 1955, v14, 318.
8. Watts, D. T., *Am. J. Physiol.*, 1956, v184, 271.
9. Gaddum, J. H., and Lembeck, F., *Brit. J. Pharmacol.*, 1949, v4, 401.
10. Gaddum, J. H., and Hameed, K. A., *ibid.*, 1954, v9, 240.
11. Fisher, R. A., *Statistical Methods for Research Workers* (12th ed.). New York Hafner, 1954.
12. Holzbauer, M., and Vogt, M., *Brit. J. Pharmacol.*, 1954, v9, 249.

Received July 22, 1957. P.S.E.B.M., 1957, v96.