Relationship of Altered Vascular Volumes to Plasma 17-Hydroxycorticosteroids in Dogs. (28396)

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Vogt, utilizing the survival of adrenalectomized rats exposed to low temperatures as an assay, reported a lack of correlation between adrenal vein corticosteroids and decreased arterial pressures in anesthetized animals(1). Since this assay could not detect adrenocortical steroids in blood removed from a large artery or the right side of the heart, the method was undoubtedly not sensitive enough to detect minor changes which may have occurred. With the development of more sentitive methods for quantitative determination of 17-hydroxycorticosteroids (17-OHCS), many contradictory results have been published concerning the effects of hemorrhage on adrenocortical steroid secretions. Increases(2,3), no change(3,4) and decreases(5) in minute steroid output have been reported. As suggested by these investigators, the rate and degree of hemorrhage, type of anesthetic, degree of trauma, and individual variability under the same conditions(6) are factors which may influence adrenocortical secretions during hemorrhage. The present investigation was undertaken to determine the relationship of gradations in reduction and expansion of vascular volumes to total free plasma 17-OHCS in anesthetized dogs.

Materials and methods. Twenty-four male dogs, weighing 8.5-21.8 kg, were divided into 4 equal groups and anesthetized intraperitoneally with sodium pentobarbital (30 mg/kg). To obtain a variety of different initial blood volumes, the body weights of dogs in Groups I, II and III were matched and spaced at approximately 2 kg intervals within the range of 8.7 to 21.8 kg. The remaining 6 dogs (Group IV) weighed 10.0 $\pm$ 0.6 kg.

Polyethylene cannulae were inserted into the proximal and distal ends of the right carotid artery and joined together with a plastic t-tube. Heparin (3 mg/kg) was administered and blood flowed essentially unimpeded while lateral pressure was continuously monitored with a Statham transducer and recorded on a Grass Polygraph. Removal of blood for hemorrhage and chemical analyses was accomplished *via* the carotid artery while the right cephalic vein was used for infusion.

Approximately 30 minutes after surgery, blood samples were removed for determination of plasma volume (Evans blue), 17-OHCS(7), micro-hematocrit and plasma proteins (American Optical Total Solids Meter). At the same time additional blood was rapidly removed (50 ml/min) from the animals of Group I to produce a hemorrhage of 10% of the initial calculated blood volume (7% of body weight). Forty minutes after completion of the 10% hemorrhage, the same procedure was repeated with removal of an additional 15% (total 25%) of the original blood volume. Blood samples were removed for analyses 40 minutes later. The vascular volume of Group II was expanded 10%, then an additional 15% (total 25%) by the infusion of 6% w/v dextran in saline (50 ml/min) at times similar to those of the hemorrhagic animals. Group III was maintained in a normovolemic state by immediate replacement of the blood withdrawn for analyses by an equal volume of dextran. After the initial determination of blood volume, Group IV was hemorrhaged 5% of the calculated blood volume every 10 minutes until 35% had been removed.

Results. The means and standard errors of control values for 18 animals comprising Groups I, II and III were 8.5 $\pm$ 1.0 γ 17-OHCS/100 ml plasma, 62 $\pm$ 9 γ 17-OHCS/plasma volume, 133 $\pm$ 5 mm Hg mean arterial pressure, 41 $\pm$ 1% hematocrit, 6.42 $\pm$ .09 g% plasma proteins, 1273 $\pm$ 92 ml blood volume, and 748 $\pm$ 46 ml plasma volume. When using the standard "t-test" these

* Performed during the tenure of a USPHS predoctoral fellowship.

TABLE I. Effect of Altered Vascular Volume on Plasma 17-OHCS.

	Vascular volume*	Hemorrhage Group I	Infusion Group II	Control Group III
17-OHCS	a	8.0 ± 1.1	7.1 ± 1.8	10.5 ± 2.0
γ/100ml plasma	b	15.8 ± 1.5†‡	7.1 ± 1.3	11.6 ± 1.9
	c	22.3 ± 1.3†‡	7.6 ± 1.4	11.3 ± 1.9
Total 17-OHCS	a	50 ± 10	66 ± 19	72 ± 14
γ/plasma vol	b	90 ± 15†	74 ± 19	79 ± 13
	c	110 ± 8‡	96 ± 19	86 ± 13
Mean art. press.	a	139 ± 12	134 ± 5	126 ± 5
mm Hg	b	129 ± 8	134 ± 4	125 ± 4
	c	92 ± 7†‡	132 ± 3	125 ± 5
Hematocrit	a	44 ± 2	38 ± 2	39 ± 2
%	b	46 ± 2†	34 ± 2†	37 ± 2†
	c	47 ± 2†	28 ± 1†‡	35 ± 2†
Plasma proteins	a	6.23 ± .26	6.37 ± .21	6.65 ± .29
g%	b	6.03 ± .18	5.82 ± .23†	6.28 ± .26†
	c	5.67 ± .13†‡	5.17 ± .26†‡	5.82 ± .23†‡
Blood vol.	a	1145 ± 115	1450 ± 205	1225 ± 140
ml	b	1070 ± 125†	1505 ± 190	1120 ± 120
	c	960 ± 115†	1800 ± 205†	1125 ± 130†
Plasma vol.	a	635 ± 50	885 ± 105	730 ± 60
ml	b	565 ± 50†	990 ± 115	695 ± 50
	c	505 ± 40†	1290 ± 120†‡	705 ± 70
Body wt kg		14.5 ± 1.6	14.5 ± 1.7	14.8 ± 1.6
Length cm		82.8 ± 3.3	89.5 ± 4.1	85.0 ± 2.9
Surface area m^2		.63 ± .04	.69 ± .05	.65 ± .05
Adrenal wt mg/kg		85 ± 11	97 ± 6	105 ± 8

* a = mean ± standard error of control values; b = 10% hemorrhage in Group I and 10% infusion in Group II; c = 25% hemorrhage in Group I and 25% infusion in Group II. Group III blood volume held constant.

† Significance ($P<.05$) from control (a) based on non-independent and independent

‡ t test.

values were not significantly different from the control data of each individual group. Since the selection of various body sizes resulted in a wide range of initial blood volumes, the non-independent "t-test" was also calculated using the initial period of each animal as its control(8).

The observed effects of altering vascular volumes, 10% and then 25%, are summarized in Table I. Blood and plasma volumes significantly decreased at each level of hemorrhage in Group I, but increased in Group II only after expanding the original blood volume 25% with dextran. Group III exhibited a very slight though significant decrease in blood volume at the end of the experiment concomitantly with a decrease in hematocrit. The infusion of dextran in Group II caused a decrease in hematocrit from 38% to 28% and plasma proteins from 6.37 g% to 5.17 g%. Similar findings were also observed in Group III, but to a lesser degree. On the other hand, hemorrhaging in Group I produced a significant increase in hematocrit along with a decrease in plasma proteins. Mean arterial pressures remained essentially unaltered in all 18 animals with the exception of Group I which showed a significant decrease after 25% hemorrhage.

In Group I, an initial hemorrhage of 10% produced significant increases in 17-OHCS of 7.8 γ/100 ml or 40 γ/plasma volume, whereas an additional 15% hemorrhage caused further increases of 6.5 γ/ml and only 20 γ/plasma volume. The 17-OHCS of hypervolemic animals (Group II) tended to increase when corrected for circulating plasma volume; however, this was not evident when reported as γ 17-OHCS/100 ml plasma. The control animals (Group III) showed no significant alterations in 17-OHCS throughout the entire experiment, although the initial level of these steroids was slightly higher than those of Groups I and II. The differ-

TABLE II. Effect of Hemorrhaging in 5% Decrements (Group IV) on Plasma 17-OHCS.

	% of original blood volume removed							
	0	5	10	15	20	25	30	35
17-OHCS, γ/100 ml plasma	13.9* ± 1.4	14.1 ± 1.7	14.8 ± 1.8	16.5 ± 1.4	17.6 ± 1.4	19.3† ± .9	19.5† ± 1.8	21.7† ± 1.6
Total 17-OHCS, γ/plasma vol.	71.7 ± 6.1	69.2 ± 7.3	68.4 ± 8.2	71.5 ± 4.9	71.7 ± 4.1	75.0 ± 3.7	72.5 ± 7.7	76.1 ± 6.1
Mean art. pres., mm Hg	129 ± 6	130 ± 7	125 ± 6	120 ± 6	112 ± 6	98† ± 5	67† ± 9	52† ± 7
Hematocrit, %	37 ± 2	38 ± 2	38 ± 3	39 ± 3	39 ± 3	39 ± 3	39 ± 3	39 ± 3
Plasma protein, g %	6.30 ± .19	6.32 ± .17	6.20 ± .19	6.13 ± .16	5.98 ± .18	5.87 ± .15	5.75 ± .17	5.60† ± .15
Blood vol., ml	840 ± 39	804 ± 40	763 ± 47	730 ± 36	690† ± 33	653† ± 31	615† ± 33	580† ± 34
Plasma vol., ml	525 ± 17	498 ± 19	467 ± 19	438† ± 16	414† ± 18	393† ± 12	371† ± 14	351† ± 14

* Mean ± standard error.

† $P < .05$ significant from control (0).

ence was undoubtedly due to the larger adrenal glands of Group III.

In experiments in which blood was removed in smaller decrements (Group IV), γ 17-OHCS/100 ml plasma progressively increased while arterial pressure gradually decreased until at 25% hemorrhage these changes became significant (Table II). Total plasma 17-OHCS and hematocrits did not increase throughout the experiment, while a significant decrease in plasma proteins was noted only after the final hemorrhage (35%).

Discussion. The alterations in vascular volumes produced the generally accepted changes in plasma and red cell volumes. The large decreases in blood volume of Group I elevated the hematocrits, since red blood cells were released into the circulatory system at a greater rate than the physiological replenishment of fluid. When the animals were hemorrhaged in smaller decrements (Group IV), the diminution in red cell volume paralleled the plasma volume, hence no observable change in hematocrit occurred. This would indicate that the amount of blood removed at each interval of hemorrhage can affect the erythrocyte releasing response.

Plasma proteins decreased in all animals. In Group I this was undoubtedly due to the removal of proteins by hemorrhage and the known tendency to increase plasma volume by protein deficient fluids(9,10). When blood volume was decreased by smaller amounts (Group IV), the concentration of plasma proteins gradually decreased and attained significance only after 35% hemorrhage. A decrease in plasma proteins and hematocrits in Group II, and to a lesser degree in Group III, would be partially dependent upon the dilution effect of dextran infusion. The extravascular transfer of fluids probably caused the decrease in blood volume noted in Group III, since plasma proteins were diminished by replacing the blood removed for analyses with dextran.

In approaching an understanding of adrenocortical steroid secretions during altered vascular volumes one should consider such factors as changes in adrenal vein blood flow (3,5,11), increased sensitivity of the cortex to ACTH(2), release and action of epinephrine(2,12) and the antidiuretic hormone (13), and perhaps chemoreceptors(14). When 17-OHCS are determined in peripheral blood, other factors must be considered, *e.g.*, metabolism(15) and urinary excretion. In addition, the hemorrhagic state is associated with oliguria or anuria(16) as well as delayed metabolism of cortisol(17). Thus, the level of peripheral 17-OHCS is a composite reflection of adrenocortical activity, metabolism, accumulation and excretion.

In the present investigation peripheral plasma 17-OHCS increased two- to threefold following 10% and 25% hemorrhaging

(Group I). These data are in accord with those of Sayers *et al.*(18), who reported that a non-fatal hemorrhage produced over a period of one hour reduced adrenal cholesterol and ascorbic acid concentrations, and others (2,3,19) who noted that the minute output of 17-OHCS in adrenal venous blood was increased after hemorrhaging in conscious and anesthetized dogs. The data presented herein also demonstrate that, although the removal of an additional 15% blood volume from animals which had been previously hemorrhaged 10% causes approximately similar increases in 17-OHCS per unit of plasma (6.5 *vs* 7.8 γ), total increase in peripheral plasma steroids after the second hemorrhage (20 γ) was only half of that observed after the first hemorrhage (40 γ). This discrepancy is also obvious in the hyper volemic group in which dextran infusions diluted the circulating steroids. An increase of 30 γ/plasma volume occurred after expanding the vascular volume 25%, whereas only a 0.5 γ increase was noted in 17-OHCS/100 ml. Therefore, when measuring peripheral 17-OHCS during altered vascular volumes, the simultaneous measurement of circulating plasma volume would afford a better description of adrenocortical activity and the subsequent fate of these steroids. However, it must be remembered that the product of plasma 17-OHCS level times the total circulating plasma volume underestimates the actual amount of free 17-OHCS circulating in blood since approximately 25% may be associated with the erythrocytes over a wide range of steroid concentration(20). Thus, the total amount associated with erythrocytes in our experiments would vary with the slight alterations in red cell volume.

The data further suggest that decreases in vascular volumes are more effective than hypotension in causing an increase in peripheral 17-OHCS. Whether reported as γ/100 ml or γ/plasma volume, these steroids increased after the 10% hemorrhage (Group I) while blood pressure remained essentially unaltered. However, with slower hemorrhage (Group IV) the concentration of 17-OHCS did not materially increase until arterial pressure had decreased significantly; and no significant correlation existed between blood pressure and steroid concentration. Comparison of the hemorrhagic and hypervolemic animals demonstrates that reduction in vascular volume is more effective in elevating peripheral 17-OHCS than an equivalent expansion of this volume.

More adrenocortical steroids may have been present in the circulatory system of Group IV than we were able to ascertain by measuring γ 17-OHCS/100 ml. Only initial blood volumes were determined in this group and the remainder of the blood volumes, hence γ 17-OHCS/plasma volume, were calculated on the basis of the amount of blood removed. These data, therefore, do not take into account any physiological replacement of vascular volume that may have occurred. However, comparison of 17-OHCS levels of Groups I and IV reveals some interesting observations. The total experimental times for both Group I and IV were approximately similar, but the time interval between each hemorrhage and removal of blood for chemical analyses was 40 minutes in the former and 10 minutes in the latter group. Tables I and II illustrate that 17-OHCS increased 40 minutes after hemorrhaging 10% in Groups I and IV, although the latter group had been hemorrhaged an additional 15% during this time. Thus, it is possible that the adrenal cortex does not react until approximately 10-40 minutes after hemorrhaging, or that this length of time is necessary for accumulation of these steroids resulting from delayed metabolism(17) and/or excretion. However, the amount of blood removed during each hemorrhage may affect the level of 17-OHCS, since in 40 minutes there was an increase in Group IV of 5.6 γ/100 ml, although 25% of the initial blood volume had been removed, while in Group I 17-OHCS increased 7.8 γ/100 ml after 10% hemorrhage and 14.3 γ after 25% hemorrhage.

Summary. The rapid removal of 10% and 25% blood volume in anesthetized dogs (sodium pentobarbital) produced a marked rise in peripheral 17-OHCS when reported as γ/100 ml or γ/plasma volume, whereas the ex-

pansion of vascular volumes by 10% and 25% with dextran caused only slight increases in steroid levels. In the 10% hemorrhagic group, 17-OHCS increased without a significant decrease in mean arterial pressure. In animals which were hemorrhaged 5% of their blood volume every 10 minutes until 35% had been removed, 17-OHCS progressively increased and became significant only after 25% hemorrhage. The alterations in peripheral plasma 17-OHCS which occur during the expansion or reduction of vascular volumes are better described when these values are corrected for circulating plasma volume.

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Received March 18, 1963. P.S.E.B.M., 1963, v113.

Analgesic and Anti-Inflammatory Effects of Orally Given Streptokinase. (28397)

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According to Lewis(1), the nonapeptide bradykinin is an important mediator of the acute inflammatory reaction. It is, therefore, of interest that orally given proteases were recently shown to antagonize 3 pharmacologic actions of bradykinin: vasodilatation, vascular permeability and smooth muscle contraction(2). The objects of the present investigation were: first, to assess the action of orally given streptokinase against the fourth pharmacologic action of bradykinin, namely, pain production; and second, to appraise the anti-inflammatory action of orally given streptokinase in a controlled animal and clinical experiment.

Methods. 1. *Analgesia study.* The cantharidin blister technic of Armstrong *et al.* (3) was slightly modified. This study comprised 30 apparently healthy students and technicians. A band-aid containing 2 drops of tincture cantharidin was applied to the skin of the flexor surface of the forearm the evening before the day of the experiment. When the band-aid was removed the next morning, the area showed a blister with a diameter of about 1.5 to 2 cm. Blisters remained relatively unchanged for 24-48 hours. Intact blisters were opened and the separated