Steroids in Adrenal Venous Blood of the Dog: Venous-Arterial Differences Across the Adrenal.* (21091)

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In a previous communication from this laboratory the isolation of 14 fractions from dog adrenal venous blood was reported(1). A number of these substances may enter the gland by way of its arterial blood supply and cannot be considered secretory products. A comparison of the concentrations of the isolated substances in the affluent and effluent blood of the adrenal has yielded information on this important problem.

Methods. Mongrel male dogs were anesthetized with sodium pentobarbital. Adrenal venous blood was collected from the left lumboadrenal vein (the adrenal vein was ligated between the adrenal gland and the inferior vena cava). At the same time and at approximately the same rate of flow, arterial blood was collected from a femoral artery. Arterial blood pressure, recorded by means of a mercury manometer connected to a cannula in a carotid artery, was maintained at or near normal levels by the infusion of pooled dog blood at a rate approximately equal to the rate of bleeding. Heparin was injected intravenously to prevent clotting. The collected blood was diluted 1:1 with water and extracted twice with chloroform. The chloroform extracts were evaporated and the residues partitioned between hexane and 70% ethanol. The 70% ethanol fractions were chromatographed on paper by the method of Burton *et al.*(2); propylene glycol and toluene were the stationary and mobile phases, respectively. The design of chromatography was essentially that described in a previous communication from this laboratory (1). For quantitative analysis each chromatogram was divided at right angles to the length of the strip into 1- or ½-inch cuts, and the steroids were eluted from individual cuts with methanol. The methanol solutions were analyzed by 1) absorption in the ultraviolet, 2) reaction with phenylhydrazine, and 3) reaction with sulfuric acid. In 3 experiments, one liter of adrenal venous blood and one liter of arterial blood were collected over 4 to 6 hours. In 2 of these experiments, an additional liter of arterial blood was collected to which 17-hydroxycorticosterone and corticosterone were added to estimate recovery of added steroids and to test the possibility that degradation of the major steroid components occurs during chromatography. In each experiment, the adrenal venous blood, the arterial blood and the arterial blood containing added steroids were extracted and partitioned at the same time and chromatographed together in order to minimize the effects of minor variations in technic.

Results. 1) *Adrenal venous blood.* In an earlier study(1), 14 fractions were isolated by paper chromatography from the chloroform extract of a 3500-ml sample of dog adrenal venous blood. In Exp. I of this study (Table I), 13 fractions were isolated and shown to be similar to the corresponding fractions isolated in the previous study in regard to 1) relative position on the chromatograms, 2) type of reaction with phenylhydrazine, and 3) absorption characteristics in the ultraviolet. Fraction 14 was not detected. In Exp. II (Table I), 12 fractions were isolated; one of these fractions covered an area on the chromatogram usually occupied by Fractions 3 and 4; another covered an area usually occupied by Fractions 13 and 14. In Exp. III (Table I), 14 fractions, corresponding to those described in a previous report, were isolated.

The results of these 3 experiments indicate that, in general, the steroid patterns of adrenal venous effluent from different dogs are qualitatively similar. However, certain differences are to be noted. For example, in Exp. I, the adrenal did not produce Fraction 11 (11-de-

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TABLE I. Substances Found in Arterial and Adrenal Venous Blood of the Dog.

Fraction No.*	Identification	(1) Conc. in adrenal venous blood, μg/100 ml			(2) Conc. in arterial blood, μg/100 ml			(1)−(2) Adrenal contribution, μg/100 ml		
		Exp. I	Exp. II	Exp. III	Exp. I	Exp. II	Exp. III	Exp. I	Exp. II	Exp. III
1	Not identified	3.6	4.8	2.8	4.2	2.3	2	−.6	2.5	.8
2	*Idem*	†	†	†	†	†	†	†	†	†
3	"	6.8		4.1	5.6		2	1.2		2.1
			9.5			2.4			7.1	
4	"	4.4		1.9	3.6		1.4	.8		.5
5	"	†	†	†	†	†	†	†	†	†
6	17-hydroxycorticosterone	66.4	259	294.4	5.4	3.8	12.2	61	255.2	282.2
7	Not identified	10.9	14.5	18.1	10.3	15.6	15.9	.6	−1.1	2.2
8	*Idem*	12.5	4.9	10.4	.7	0	0	11.2	4.9	10.4
9	"	†	†	†	†	†	†	†	†	†
10	"	†	†	†	†	†	†	†	†	†
11	11-desoxy-17-hydroxycorticosterone	1.6	39	46.1	1.9	3.3	1	−.3	35.7	45.1
12	Corticosterone	40.5	116	120	3.5	6.4	7.2	37	109.6	112.8
13	Not identified	15		23.4	0		11.6	15		11.8
			44.5			28.8			15.7	
14	*Idem*	0		29.2	0		10.3	0		18.9

* Fraction number refers to those fractions described in an earlier publication(1), and are arranged in order of polarity.

† These substances could not be quantitated in terms of μg steroid. They appeared to be present in the same concentrations in arterial and adrenal venous blood, as estimated by U.V. absorption in the instances of fractions 2, 5, and 10 and by sulfuric acid chromogen in the instance of fraction 9.

soxycorticosterone), whereas in Exp. II and III this substance was secreted in significant amounts. Furthermore, it is not yet possible to state the exact number of steroids present in adrenal venous blood. A number of the fractions isolated may be a mixture of steroids. Indeed, recent work on Fraction 8(3) indicates that this fraction consists of at least 2 steroids. Final statements as to similarities and differences in the steroid patterns of various dogs should be deferred until such time as it is possible to more completely characterize the individual components of the mixture.

2) *Comparison of adrenal venous blood and arterial blood.* (*Table I*). Comparison of the concentrations of the steroids found in femoral arterial blood with those found in adrenal venous blood provides information on the secretory activity of the adrenal cortex. *Fraction 6* (17-hydroxycorticosterone) is clearly of adrenal origin, since the concentration of this steroid in adrenal venous blood was many times that in arterial blood in each experiment. *Fraction 7* (an unidentified steroid which absorbs at 230 mμ, exhibits a 340 mμ maximum with phenylhydrazine and shows maxima at 240, 285, and 370 mμ in sulfuric acid) was the largest single steroid component in arterial blood in each experiment. There was no evidence that the blood concentration of this substance was increased by passage through the adrenal. In view of the relatively high concentration of this steroid in peripheral blood further study is warranted. It is noteworthy that neither the method of Nelson *et al.*(4) nor that of Sweat (5) for the analysis of steroids in peripheral blood would detect the presence of this substance. *Fraction 8*, although present in low concentrations, was clearly of adrenal origin in each experiment. The major constituent of Fraction 8 is a steroid which absorbs at 240 mμ and gives a 400 mμ maximum with phenylhydrazine reagent. This fraction has been found to be 25 times as potent as desoxycorticosterone in producing sodium retention in the adrenalectomized rat(6). The sodium-retaining activity of this fraction does not appear to be associated with the major com-

TABLE II. Comparison of Steroid Content of Arterial Blood to Which 17-Hydroxycorticosterone (Exp. II, 100 μg/100 ml; Exp. III, 150 μg/100 ml) and Corticosterone (50 μg/100 ml) Had Been Added with That of Arterial Blood.

Fraction No.*	Identification	(1) Conc. in arterial blood, μg/100 ml		(2) Conc. in arterial blood with added steroids, μg/100 ml		(2)-(1) Recovery, μg/100 ml	
		Exp. II	Exp. III	Exp. II	Exp. III	Exp. II	Exp. III
1	Not identified	2.3	2	4.9	2.4	2.6	.4
2	*Idem*	†	†	†	†	†	†
3	"		2		1.7		−.3
		2.4		4.7		2.3	
4	"		1.4		1.6		.2
5	"	†	†	†	†	†	†
6	17-hydroxycorticosterone	3.8	12.2	100.4	140.3	96.6	127.9
7	Not identified	15.6	15.9	23	10.4	7.4	−5.4
8	*Idem*	0	0	0	0	0	0
9	"	†	†	†	†	†	†
10	"	†	†	†	†	†	†
11	11-desoxy-17-hydroxycorticosterone	3.3	1	2.4	2.6	−.9	1.6
12	Corticosterone	6.4	7.2	33.6	48.1	27.2	40.9
13	Not identified		11.6		8.1		−3.5
		28.8		21.8		−7	
14	*Idem*		10.3		11.9		1.6

* Fraction number refers to those fractions described in an earlier publication(1), and are arranged in order of polarity.

† These substances could not be quantitated in terms of μg steroid. They appeared to be present in the same concentrations in arterial and adrenal venous blood, as estimated by U.V. absorption in the instances of fractions 2, 5, and 10 and by sulfuric acid chromogen in the instance of fraction 9.

ponent, but rather with a substance which exhibits an absorption maximum at 240 mμ but which does not react with phenylhydrazine to yield a derivative which absorbs at 400 mμ (3). This finding is an example of a steroid of great metabolic importance present in very low concentration in adrenal venous blood.

Fraction 11 (11-desoxy-17-hydroxycorticosterone) was not present in higher concentration in the adrenal venous blood in Exp. I, although it was clearly so in Exp. II and III. The role of this biologically inactive steroid in the adrenal secretion is not clear. The variability in its production by the glands remains unexplained. *Fraction 12* (corticosterone) was present in the adrenal venous blood in a concentration many times that in arterial blood in each experiment. This steroid is clearly of adrenal origin. *Fraction 13* (an unidentified steroid which absorbs at 230 to 240 mμ, exhibits a 340 mμ maximum with phenylhydrazine reagent and exhibits maxima at 280 and 370 mμ in sulfuric acid) was present in higher concentrations in adrenal venous blood than in arterial blood in Exp. I and III. In Exp. II, it was not separated from Fraction 14, although the concentration of Fraction 13 plus 14 was higher in adrenal venous blood than in arterial blood in this experiment. *Fraction 14* was not present in the adrenal venous blood in Exp. I, and was not well resolved in Exp. II; in Exp. III it appeared to be present in higher concentrations in adrenal venous blood. This fraction is a nonpolar substance or mixture of substances which chromatographs close to the front in the solvent system, absorbs at 230 to 235 mμ and gives a 290 mμ peak in sulfuric acid. Further study is required to elucidate the nature of this material and the role of the adrenal in its production.

Fractions 1, 3, and 4 are substances which are more polar than 17-hydroxycorticosterone, which react with phenylhydrazine to give maxima in the region of 400 mμ and which fluoresce in sulfuric acid; they were present in both adrenal venous and arterial blood in low concentrations. Their status as adrenal secretory products cannot be definitely established at this time. The small positive arterial-

venous differences in concentration of Fractions 1 and 3 plus 4 in Exp. II suggests that they were secreted at a low rate. However, when arterial blood to which 17-hydroxycorticosterone (Exp. II, 1000 μg; Exp. III, 1500 μg) and corticosterone (500 μg in each experiment) had been added was analyzed and the concentrations of the various fractions compared to those of the control samples of arterial blood (Table II) a small positive difference in concentrations of Fractions 1 and 3 plus 4 of Exp. II was found. The possibility that these fractions may be formed by the degradation of one or more of the major fractions during processing cannot be ruled out.

3) *Recovery of 17-hydroxycorticosterone and corticosterone added to arterial blood.* In Table II are presented the results of the recovery studies. 17-Hydroxycorticosterone added to blood (100 μg/100 ml in Exp. I and 150 μg/100 ml in Exp. II) was recovered in 96% and 86% yields, respectively. Fifty μg/100 ml of corticosterone was added to blood in both experiments and was recovered in yields of 60% and 84%, respectively. Other than the very slight absolute increase in the concentrations of Fraction 1 and Fraction 3 plus 4 in Exp. II (see above) there is no evidence of change in the pattern of steroids. These experiments indicate that none of the major fractions which have been described in adrenal venous blood is an artifact derived by degradation of the major constituents during extraction and chromatography.

Summary. Concentrations of steroids in arterial and venous blood of the adrenal have been determined. Those steroids whose concentrations in the blood are increased by passage through the gland are considered to be adrenal secretory products. Three steroids are consistently of adrenal origin: 17-hydroxycorticosterone, Fraction 8, and corticosterone. In 2 of 3 experiments 11-desoxy-17-hydroxycorticosterone was contributed to the blood by the adrenal gland. A steroid less polar than corticosterone (Fraction 13) also appeared to be an adrenal secretory product in 2 of 3 experiments. Five substances (Fractions 2, 5, 7, 9, and 10) are clearly not secreted by the adrenal gland, since they were found in the same concentrations in arterial blood as in adrenal venous blood. The status of 4 substances (Fractions 1, 3, 4, and 14) as adrenal secretory products has not been established. 17-Hydroxycorticosterone and corticosterone, when added to arterial blood, are recovered without substantial alteration in the pattern of steroids present in such blood.

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Effects of Neonatal Anoxia on Maze Learning in Rats. (21092)

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It is generally agreed that severe oxygen deprivation at the time of birth can result in a variety of behavior disorders involving intellectual deficit. These effects may be due solely and directly to neonatal anoxia(1,4, 6,8). An alternate possibility is that failure to breathe normally at birth is a secondary effect resulting from congenital neurological defects which are also responsible for the psychological abnormality often associated

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