studies of kinase activity in vaccinia-infected mouse cell cultures indicated that induction occurred earlier in this system as compared to the chick embryo system. It is possible that differences in the kinetics of expression of the viral genome in the two systems studied could account for the differences in the inhibition of deoxythymidine kinase activity. Another possibility is that the mouse antiviral substance which is thought to be induced in interferon treated cells acts somewhat differently from the antiviral substance of chicken cells. Speculatively, the different action could reflect a different spectrum of interferon activity on the various virus messenger RNAs.

Summary. The effect of interferon on the induction of deoxythymidine kinase has been studied in vaccinia-infected chick embryo and mouse cell culture systems. Antecedent treatment of vaccinia-infected chick embryo cells with interferon inhibited deoxythymidine kinase induction. The inhibitor of deoxythymidine kinase was shown to share many properties with interferon. In the presence of concentrations of cytosine arabinoside or bromodeoxyuridine which suppressed vaccinia replication, interferon effectively inhibited deoxythymidine kinase induction, indicating an interferon effect on virus prior to replication of viral DNA. These results are consistent with other studies which indicate that interferon may act to prevent the function of viral mRNA. In contrast, under similar experimental conditions, mouse interferon did not inhibit deoxythymidine kinase induction in the mouse embryo cell culture system although interferon effectively inhibited viral multiplication.

We wish to thank Dr. Hilton Levy for helpful discussion during the course of this work. The excellent technical assistance of Charles E. Buckler and Henry O. Schulze is gratefully acknowledged.

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Received July 27, 1967. P.S.E.B.M., 1968, Vol. 127.

Deoxycorticosterone Secretion in Chronic Experimental Heart Failure and during Infusion of Angiotensin II* (32647)

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Hypersecretion of aldosterone occurs in chronic experimental heart failure (1, 2) and aldosterone, in association with an extra-adrenal sodium-retaining factor (3), leads to marked sodium retention. The quantitative

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importance of other sodium-retaining hormones in edema formation has not been determined. The present study was undertaken to examine the possibility that deoxycorticosterone (DOC) secretion is increased in experimental heart failure and to determine the contribution of DOC to total sodium-retaining activity in heart failure. If the rate of secretion of DOC is several times greater than that of aldosterone, an increase in DOC secretion in heart failure might indicate that DOC also plays an important role in sodium retention. It should be pointed out, however, that aldosterone is about 30 times as potent in sodium-retaining activity as DOC (4).

To examine this hypothesis, simultaneous measurements of DOC, aldosterone, and corticosterone were made in dogs with chronic experimental heart failure and in normal dogs. Also, the response in DOC to the intravenous infusion of angiotensin in normal dogs was studied because there is evidence for increased activity of the renin–angiotensin system in chronic experimental heart failure (2).

Methods. Chronic experimental heart failure was produced in five female mongrel dogs by a modification (5) of the method of Barger (6). Tricuspid insufficiency was induced surgically and a loose ligature was placed around the trunk of the pulmonary artery. Several days later the pulmonary artery was constricted until signs of right-sided congestive heart failure developed. Evidence for heart failure included a high right atrial pressure marked sodium retention, ascites, and hepatomegaly. To evaluate the state of sodium balance, the animals were placed on metabolic balance studies and fed a synthetic diet containing 60 meq/day of sodium and 18 meq/day of potassium. The normal dogs that served as controls were fed the same diet.

To determine the importance of DOC in heart failure, steroid secretion was measured in conscious dogs. A chronic indwelling catheter was placed in the left adrenolumbar vein for collection of adrenal venous blood. Blood was collected once daily for 2–7 days.

To study the response of DOC to the intravenous infusion of angiotensin II, eight normal dogs with a chronic adrenal venous catheter were anesthetized with sodium pentobarbital (30 mg/kg). The animals were anesthetized to minimize the variation in ACTH release with the subsequent effect of ACTH on steroid secretion. This design was necessary since the acute study was performed over a 4- to 5-hour period during which a conscious animal might become excited. After two collections of adrenal vein blood for the controls, synthetic angiotensin II (hypertensin, Ciba) was infused intravenously at a rate of 0.05 μg per kg per min for 3 hours. Collections of adrenal vein blood were made at 0.5, 1, 2, and 3 hours during angiotensin infusion.

Adrenal vein plasma was analyzed for DOC, aldosterone, and corticosterone by the double isotope derivative assay of Kliman and Peterson (7). All three steroids were determined on each sample of plasma so that data on simultaneous secretion rates are available. The assay procedure used routinely (8) in our laboratory for analysis of aldosterone and corticosterone was altered so that DOC could be determined on the same plasma samples. The DOC-4-^{14}C was added to correct for losses of DOC during chromatography. After addition of the ^{14}C steroids, extraction, and acetylation with tritium labeled acetic anhydride, the samples were chromatographed in a benzene (30 parts), cyclohexane (100 parts), methanol (100 parts) and water (15 parts) system for 7–8 hours. This system separated DOC from aldosterone and corticosterone which ran together. The three routine chromatographs and the oxidation step (8) were used to separate aldosterone from corticosterone. The DOC was run through a second chromatography (dioxane, 100 parts; cyclohexane, 100 parts; methanol, 50 parts; and water, 15 parts) for 7 hours. To provide better separation of DOC from the other adrenal steroids, the samples were subjected to sodium borohydride reduction before the third chromatography. After elution from the paper of the second chromatography and drying, each sample was dissolved in 0.5 ml of spectral grade methanol. Two-tenths of a ml of a freshly made sodium borohydride solution (20 mg in 1 ml of distilled water) were added to each sample; the sodium borohydride was allowed to react with the steroids for exactly 20 sec (timed with a stopwatch). The reaction was stopped by addition of 3 drops of glacial acetic acid. Water and methylene chloride

TABLE I. Specificity Data for Deoxycorticosterone.

Samples	$^3H/^{14}C$ ratios after successive chromatographies			
	1st	2nd	3rd	4th
1	53.5	21.8	1.04	1.13
2	41.6	21.1	1.09	1.03
3	45.9	24.7	4.32	4.26
4	42.8	22.5	4.14	4.28
5	40.4	22.7	1.04	1.09
6	36.2	16.5	.87	.78
7	42.6	23.9	3.59	3.81
8	40.2	21.3	3.35	3.18
Mean values	42.9	21.8	2.43	2.44

were added and the sample was washed twice with water. Each sample containing DOC was subjected to a third chromatography (14 hours); the same chromatographic system was used as for the first chromatography. It was necessary, however, to use a different chromatography tank than that used for the first system since the tank for the first chromatographic system contained a substantial amount of radioactivity after a few samples were run.

To examine the specificity of the method for measurement of DOC, $^3H/^{14}C$ ratios were determined after each of four chromatographies on a series of eight samples. This experiment required a fourth chromatographic system in which the dioxane, 100 parts; cyclohexane, 100 parts; methanol, 50 parts; and water, 15 parts system was used. The results are presented in Table I. The $^3H/^{14}C$ ratios were essentially the same after the third and fourth chromatographies; the average ratios for the eight samples were 2.43 and 2.44.

Recoveries were made of DOC added to adrenal vein plasma. The concentration of DOC was determined in two pools of adrenal vein plasma. The DOC was added to these plasmas and the concentrations of DOC determined again. The average value for the recoveries was 88% with a range from 65 to 118%.

Results. Relative rates of secretion of DOC and aldosterone. The simultaneous rates of secretion of aldosterone and DOC were determined in eight normal conscious dogs. The results are presented in Fig. 1 which shows that the rate of secretion of DOC was nine times greater than that of aldosterone.

DOC secretion in chronic experimental heart failure. The rate of secretion of DOC was measured in five dogs with chronic congestive heart failure and compared with the values from the eight normal dogs (Fig. 2). At least two determinations were made in all but one dog. Although there was considerable variation among animals, the mean values for DOC secretion were almost identical for the two groups of dogs. The variation in DOC production among animals in both the normal and the dogs with heart failure is probably a reflection of different plasma levels of ACTH.

Simultaneous measurement of aldosterone with DOC revealed a fivefold increase in aldosterone secretion, a change significant at the 5% level (Fig. 2). Corticosterone production, similar to DOC secretion, was essentially the same in the two groups of dogs.

Effect of angiotensin II on DOC secretion in normal dogs. Since there is evidence for increased activity of the renin–angiotensin system in chronic experimental heart failure, the effect of angiotensin II on DOC secretion was studied. A striking increase in DOC secretion occurred (Fig. 3). This was evident after 30 min of intravenous infusion of angiotensin II.

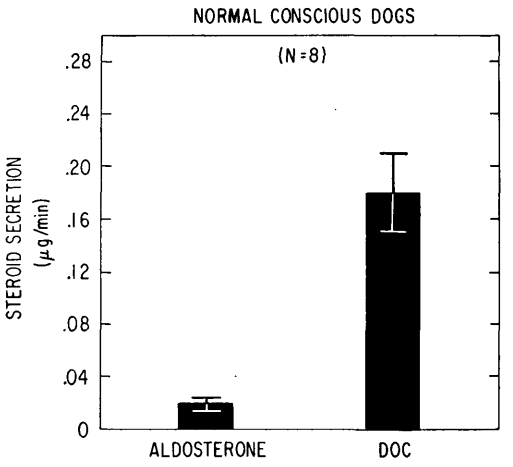

FIG. 1. Simultaneous measurements of aldosterone and DOC in eight normal conscious dogs. Mean values and standard errors of the means (SEM) are presented.

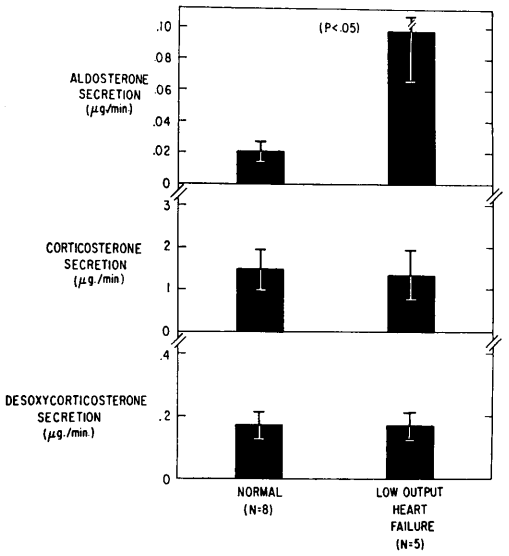

FIG. 2. Simultaneous measurements of adrenal steroid secretion in normal animals and in dogs with low output heart failure. A fivefold increase in aldosterone secretion was present in heart failure but the mean values for corticosterone and DOC secretion for the two groups of animals were the same. The deviations are SEM.

The elevation in DOC secretion was sustained for the 3-hour period of angiotensin infusion.

Simultaneous measurements of aldosterone and corticosterone during the angiotensin infusion demonstrated concurrent rises in secretion of these two steroids in association with the hypersecretion of DOC (Fig. 3).

Discussion. The present observations demonstrated a normal rate of DOC secretion in chronic experimental heart failure. Simultaneous measurements of aldosterone and corticosterone in the same animals revealed a fivefold elevation in aldosterone production during heart failure but corticosterone output was normal. Thus, the greatest part of the sodium-retaining activity in experimental heart failure results from a high plasma level of aldosterone.

It should be pointed out, however, that the ninefold higher rate of DOC than aldosterone secretion in normal dogs may indicate that DOC contributes substantially to total sodium-retaining activity in homeostasis. Since the sodium-retaining potency of aldosterone is 30 times that of DOC (4) while the rate of DOC secretion is 9 times that of aldosterone, on the basis of secreted hormone DOC provides about one third of the sodium-retaining activity under normal conditions. Of course, the plasma levels of DOC and aldosterone depend not only upon secretion but also upon the rates of inactivation of these steroids. Data are not available on the biological half-life or rate of metabolism of DOC in the dog, so a definitive statement cannot be made about the relative contributions of DOC and aldosterone to sodium-retaining activity in peripheral plasma.

The observation of a normal rate of DOC secretion in heart failure together with the evidence for increased activity of the renin–angiotensin system in this condition deserves comment. These findings could indicate that angiotensin II acts late in the biosynthetic process to promote the conversion of corticosterone to aldosterone but the present studies demonstrate that at least acutely angiotensin II increased DOC secretion. Another mechanism which controls DOC secretion is the plasma level of ACTH (9). Thus, angiotensin II could have stimulated release of ACTH which increased DOC secretion. It should be emphasized that the present data on DOC secretion with angiotensin II were

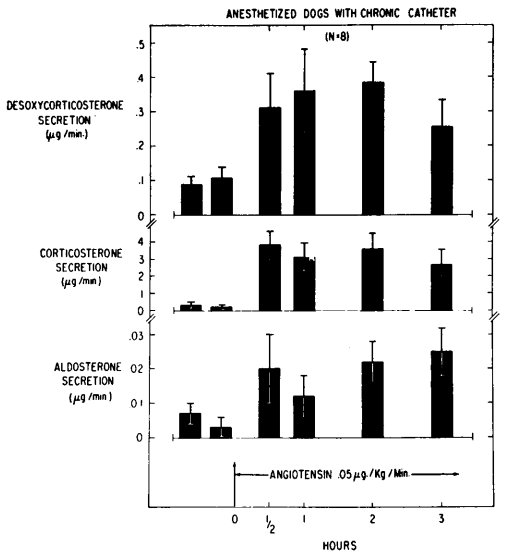

FIG. 3. Simultaneous measurements of DOC, corticosterone, and aldosterone secretion in response to infusion of synthetic angiotensin II in anesthetized dogs with a chronic indwelling venous catheter. The deviations are SEM.

obtained in an acute experiment and the same mechanisms may not be operative in the conscious animal with heart failure. In this connection, the possibility must be considered that DOC secretion is maintained at a normal level in chronic heart failure by the negative corticosteroid feedback mechanism; secretion of both corticosterone (present study) and cortisol (10) was increased by angiotensin II. Evidence for such a feedback mechanism has been obtained previously (11) during sodium depletion. In this experimental situation, an increase in corticosterone as well as aldosterone secretion occurred within 1–2 hours after the intravenous injection of meralluride (Mercuhydrin); subsequently corticosterone production returned to the normal control level while aldosterone secretion remained elevated. The finding (12) that hypophysectomized dogs responded with an increase in corticosterone secretion during sodium depletion is also consistent with operation of a negative corticosteroid feedback mechanism during sodium depletion. The present data do not provide a clear-cut explanation for the apparent discrepancy between the findings of an acute increase in DOC secretion with angiotensin II and a normal DOC secretion in chronic experimental heart failure.

Summary. Experiments were undertaken in dogs to determine the contribution of deoxycorticosterone (DOC) to the increased mineralocorticoid activity in heart failure. Chronic experimental heart failure occurred secondary to induced tricuspid insufficiency and pulmonic stenosis. Simultaneous measurements of DOC, corticosterone and aldosterone revealed average secretion rates of 0.18, 1.50, and 0.020 μg/min respectively for eight normal dogs and 0.18, 1.33, and 0.097 μg/min for five dogs with heart failure. The fivefold increase in aldosterone secretion was significant at the 5% level. Because of earlier evidence for increased activity of the renin–angiotensin system in experimental heart failure, the effect of angiotensin II on steroid secretion was studied. In acute experiments, intravenous infusion of angiotensin II in normal dogs increased DOC secretion. The explanation for the finding of a normal rate of DOC secretion in heart failure whereas acutely angiotensin II increased DOC secretion is not clear.

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Received July 28, 1967. P.S.E.B.M., 1968, Vol. 127.