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Urinary Excretion of Aldosterone in Dogs with Elevated Plasma Titers of Antirenin.* (30526)

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A substantial body of evidence supports the selective role of the renin-angiotensin system in the control of aldosterone secretion(1-5) and numerous investigations are concerned with the physiological mechanisms involved(4,6-9). The ability of antirenin, a neutralizing antibody to renin, to block *in vivo* the renin-angiotensin pressor system(2, 10), has suggested another avenue of approach to study the interactions of this mineralocorticoid control system. It would be anticipated that an inverse relationship would be demonstrable between these two parameters, *i.e.*, as antirenin titers rose, aldosterone secretion and excretion would diminish. Electrolyte clearance studies on dogs with elevated antirenin titers lent credence to this hypothesis, since these animals were found to excrete significantly larger amounts of sodium in their urine without alteration in renal hemodynamics, plasma electrolytes and potassium excretion(11,12). The present investigations were undertaken to substantiate possible correlations between the development of antirenin titers and aldosterone elaboration and to extend the observations contained in a preliminary report(13).

Methods. Six-hour urine collections were made in 7 normotensive female dogs which were trained to lie supine on padded animal boards for 8-hour periods. Food (Purina Dog Chow containing 0.5% NaCl) was removed from the animal cages on the eve of the experimental day. The following morning 20 ml/kg body weight of water (*via* stomach intubation) was given 5 minutes before the start of urine collection (time zero between 9:55-10:15 A.M.). To insure adequate hydration of the animal, additional water (10 ml/kg body weight) was given by intubation 2 hours and 4 hours after beginning urine collection. Plasma electrolytes and hematocrit were determined on venous blood samples taken 1½ hours and 5 hours after the start of the experiment. Hourly specimens of the urine obtained by bladder catheterization, which had been collected in ice-chilled containers, were refrigerated until termination of the collection. The hourly samples for each dog were pooled, the volume of the total 6-hour urine specimens measured and aliquots taken for electrolyte determination and aldosterone analysis.

Urine and blood data were collected for a 6-month period to establish control values. After this period, daily intramuscular injections of 5 Goldblatt dog units†/kg body weight of crude hog renin (1 unit/mg protein) from whole kidney were given to the animals to elicit formation of antibody(14). Plasma antirenin (AR) titers,‡ demonstrable within 3 to 4 weeks by bioassay, were maintained by daily administration of renin for a period of 20 weeks. Injections were discontinued after this 4½-month period and antibody titers were allowed to fall to zero

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† Definition: Goldblatt dog unit of Renin—That amount of renin which will produce a 30 mm Hg elevation of mean arterial blood pressure on intravenous injection into a trained unanesthetized dog.

‡ Definition: Antirenin unit (AR)—That amount of antibody capable of neutralizing completely the pressor response to 1 Goldblatt dog unit of renin.

TABLE I. Antirenin Titers and Urinary Aldosterone and Electrolyte Excretion of 7 Dogs Before and After Immunization with Hog Renin.

No. of samples	Antirenin titer (AR units/ml plasma) Mean	Range	Aldosterone excretion (μg/6 hr/m^2) Mean	± S.E.	Urinary electrolytes (μEq/min/m^2) Sodium Mean	± S.E.	Potassium Mean	± S.E.
8	0		1.366	0.405	32.7	4.8	16.5	2.4
5	6	(4- 8)	0.562	0.094	28.7	4.6	8.1	0.9*
5	0		0.502	0.108	34.5	7.5	25.2	2.3
7	13	(6-20)	0.990	0.141*	64.8	5.5†	18.0	2.7
8	0		1.134	0.401	45.2	8.0	11.7	1.2
7	14	(6-35)	0.911	0.241	47.6	6.8	11.7	1.6
5	0		0.416	0.055	49.1	14.0	8.7	1.0
7	24	(12-30)	0.890	0.223	54.5	7.9	14.1	2.4
6	0		0.704	0.158	34.5	4.4	16.0	3.6
7	30	(20-40)	1.888	0.845	74.8	6.9‡	10.9	1.8
7	0		1.342	0.262	32.2	4.4	9.3	0.6
7	42	(30-80)	0.216	0.094†	49.7	7.1*	27.7	7.6*
5	0		0.642	0.136	24.4	4.2	15.0	1.7
7	54	(40-80)	0.166	0.043†	37.8	8.2	27.2	4.0†

* Probability level P <0.5. † Probability level P <0.1. ‡ Probability level P <0.01.

levels. Following the complete disappearance of antirenin, another 3-month period of control observations was made. Urine collections during the control periods were obtained at approximately 4-week intervals and during the periods of antirenin titer at bimonthly intervals. Determinations of plasma electrolytes, urine volume, urinary sodium and potassium and urinary aldosterone excretion were made on samples obtained before, during and after antirenin formation for each individual animal. Sodium and potassium measurements were made by flame photometry (Baird Atomic) and aldosterone levels were ascertained following extraction(15) by the double isotope derivative technique(16, 17) from aliquots of the 6-hour specimens of urine.

The data were analyzed statistically by multiple classification analyses of variance and the student's T test as described by Batson(18).

Results. Substantial antirenin titers, averaging greater than 12 units, were demonstrable in the plasma of all but one of 7 dogs during the renin injections. The overall range of antirenin titers varied from 4 to 80 units per ml of plasma; ranges of antirenin titers for the individual animals are shown in association with their respective mean values (Table I). Two animals formed

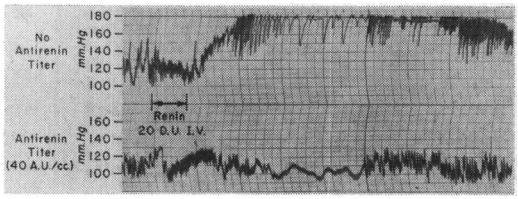

FIG. 1. Pressor response to an intravenous injection of 20 dog units of hog renin in an unanesthetized dog having no antirenin titer (upper tracing) and an unanesthetized dog having a plasma antirenin titer of 40 units/ml (lower tracing).

significantly higher titers of antirenin than the other 5 dogs and low mean antirenin titers were found in 3 dogs. The effectiveness of an antirenin titer in blockade of renin was demonstrated by intravenous administration of 20 units of hog renin (Fig. 1).

Considerable variations were observed in rates of excretion of aldosterone by the same dog on different collection days, which minimized the statistical significance of the alterations observed. Average urinary excretion of aldosterone compiled from the data of all 7 dogs under the conditions of these experiments during the control period was 0.948 ± 0.105 μg per 6 hours per squared meter of body surface area (mean μg/6 hr/m^2 ± standard error); individual values ranged between 0.14 and 3.75 μg/6 hr/m^2 (Table I). During the 20-week period of immunization,

the mean urinary aldosterone was 0.863 ± 0.164 with a range of values from 0 to 6.36 μg/6 hr/m^2. Thus, mean aldosterone excretion was not statistically significantly altered by the induction of high antirenin titers. Aldosterone excretion was elevated in 3 dogs, decreased in 3 and unchanged in one. It should perhaps be noted that an amount and consistency of change of aldosterone excretion adequate to be statistically significant individually occurred in only 2 dogs with a depression of excretion, and that these 2 also exhibited the highest antirenin titers. The one other dog which had also showed diminished aldosterone excretion, interestingly, had produced the lowest levels of antibody titer (4 to 8 units).

Average urinary excretion of sodium during the pre-immunization period varied between 24.4 and 42.8 μEq/min/m^2 (Table I). Sodium loss in the urine was elevated in the presence of antirenin; the mean values were found within a range of 37.8 to 74.7 μEq/min/m^2. Six of the 7 dogs in these experiments displayed an increased urinary sodium excretion although the differences were statistically significant by individual comparisons with the control in only 3 of 6 animals. Urinary potassium excretion was significantly elevated in 2 animals after immunization with renin. The diminished rate of potassium excretion displayed by one dog was also significant ($P<0.05$); however, in the remaining 4 animals rates of potassium loss were neither consistently nor significantly altered. An analysis of variance revealed no significant difference in potassium loss before and after antirenin formation; sodium excretion, however, was significantly elevated ($P<0.02$) in the presence of antirenin.

There were no statistically significant alterations in blood electrolytes. Plasma values for sodium and potassium were 146 and 4.1 mEq/l, respectively, without antirenin and 145 and 3.9 mEq/l after antirenin formation.

Discussion. The data presented here, similar to those of abstract reports(13,19), failed to substantiate the hypothesis that an inverse relationship obtains between development of antirenin titers and excretion of aldosterone. The data indicate that urinary aldosterone excretion was not diminished in the presence of antirenin as might be anticipated if the renin-angiotensin system, as the sole regulator of mineralocorticoid secretion, were completely blocked.

A display of the data for aldosterone excretion expressed as a change from the control rate (Fig. 2) would suggest that mineralocorticoid excretion had diminished. However, the data for the animals, as individuals, do not support this and in 2 instances (Fig. 2), a reversal of this downward tendency is noted.

Measurements of urinary aldosterone are limited in their ability to reflect subtle secretory changes. Isotope dilution procedures(20, 21) for indirect measurement of aldosterone secretion might have proven more valuable. However, in clinical situations where secretion rate methodology was not available, measurement of acid-released aldosterone has been shown to be adequate(16). Moreover, Kliman(20) has pointed out that when an investigation involves a determination of changes in aldosterone production by the same subject, or when daily values are of interest, measurement of the urinary metabolite levels may be employed. Secretion rate technics are considered more appropriate when changes in metabolism or excretion, due to altered liver or kidney function, might be anticipated(20). The degree to which altered metabolism of aldosterone might have influenced the results obtained in the present investigation cannot now be assessed.

The relative increase in sodium outflow for the entire group, which occurred during renin administration, is delineated in Fig. 2. In view of the fact that aldosterone excretion was not significantly lowered, the increased sodium excretion observed in both the present study and in a previous study(11), apparently cannot be explained solely on the basis of diminished aldosterone secretion. The cause of the sodium diuresis in the presence of antirenin is thus uncertain. Previous studies (11) revealed no significant alterations in either glomerular filtration or renal plasma flow after formation of antirenin and thus suggest that a tubular mechanism may be involved.

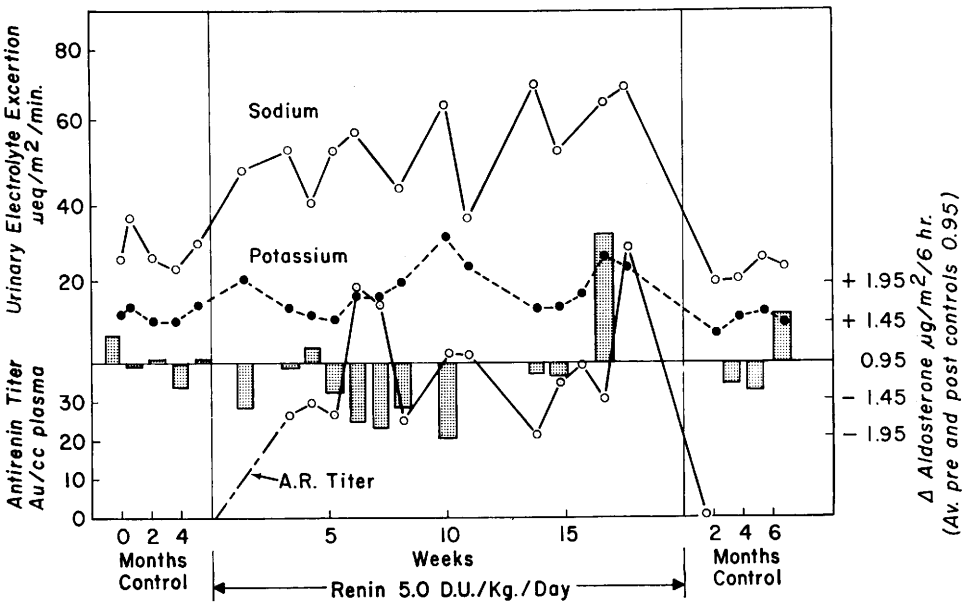

FIG. 2. Rates of sodium and potassium loss in the urine and the change of aldosterone excretion as a function of time in months before and after renin injections and in weeks during antirenin formation in the seven animals employed in this study.

Each point or bar represents the mean of the values obtained from the dogs during that particular week of collection.

Renin was injected daily (5 dog units/kg) during the 20-week period delineated in the middle portion of the graph. Formation and maintenance of antirenin titers are shown to coincide with this four and one-half month period. The various levels of antirenin are expressed in antirenin units/cc plasma.

Aldosterone excretions are expressed as the changes from a control rate (0.948 $\mu g/m^2/6$ hr) of secretion.

Rates of sodium and potassium excretion are expressed as $\mu Eq/m^2/min$.

Urinary potassium excretion in contrast to sodium excretion showed a lack of consistent change (Fig. 2). It is interesting to note that potassium loss was significantly elevated in 2 dogs which displayed the highest antirenin titers and showed markedly depressed levels of urinary aldosterone. These contradictory observations remain unclear, since diminished potassium excretion with depressed aldosterone excretion, as observed in only one animal, would more closely correlate with the anticipated results.

This lack of interrelation between the urinary aldosterone levels and excretion of sodium and potassium suggests that another factor or combination of factors, such as antirenin or the blockade of the renin-angiotensin system itself may be influencing either the disposition or renal transport of these ions. In this regard Vander(22) has shown that angiotensin II can inhibit distal sodium reabsorption by a direct tubular effect.

The continued apparent secretion of aldosterone in the presence of antirenin suggests, among other possibilities, that sufficient amounts of endogenous angiotensin might still be formed to exert an influence on aldosterone formation(19). The sensitivity to angiotensin may differ in regard to the amount required to elicit blood pressure responses, to enhance aldosterone formation and to exert direct effects on the renal tubules. Thus, minute, non-pressor amounts of angiotensin may be adequate to maintain primary control of aldosterone production. The existence of factors acting independently of angiotensin, either directly or permissively to alter formation of mineralocorticoids, has also been suggested by Davis *et al*(23).

Summary. Daily injections of crude hog renin into 7 trained, normotensive dogs elicited formation of antirenin titers ranging from 4-80 AR units/ml plasma. Urinary aldosterone excretion levels determined from 6-hour

specimens of urine varied from 0.14 to 3.75 μg/6 hr/m^2 during zero AD titer periods and between 0 and 6.36 μg/6 hr/m^2 when AR titers were elevated. The data indicate that aldosterone secretion continued in the presence of elevated antibody titers despite neutralization of renin pressor response. The sodium diuresis which occurred in the presence of elevated AR without alteration in K excretion does not appear to correlate with depressed aldosterone formation and the causative factor modifying electrolyte excretion remains uncertain.

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Antiviral Activity of Herbs on Columbia SK in Mice, and LSM, Vaccinia and Adeno Type 12 Viruses *in vitro*.* (30527)

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The difficulty in finding active therapeutic drugs of synthetic origin for experimental virus infections in animals has led us to search in the field of natural products. In addition to propionin, an unidentified antiviral substance extracted from *Propionibacterium freudenreichii*(1,2), we have found 6 higher plants that show antiviral effects against Columbia SK virus infection in mice. Also, 24 plants have been found to show antivaccinia effects in tissue cultures.

Materials and methods. About 130 kinds of herbs, constituting the bulk of the agents commonly used in Chinese medicine in the Pacific-Asian area, were collected or purchased. The plants or portions of plants were extracted by boiling in distilled water for 30

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