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THE EFFECTS OF CHRONIC HEPATIC VENOUS CONGESTION ON THE METABOLISM OF *d,l*-ALDOSTERONE AND *d*-ALDOSTERONE *

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In 1950 Deming and Luetscher demonstrated increased salt-retaining activity in urine from patients with congestive heart failure (1). In 1953 aldosterone was isolated (2), and subsequently the urinary excretion of this hormone was found to be elevated in nearly all the clinical states associated with edema. Two possible mechanisms could lead to an increase in the plasma level of aldosterone and thus to an increase in urinary aldosterone excretion: 1) hypersecretion of aldosterone, and 2) a decreased rate of metabolism of the hormone. In 1957, it was demonstrated that a sixfold increase in aldosterone secretion occurred in dogs with experimental right heart failure and in dogs with thoracic inferior vena caval constriction (3). The possibility of decreased metabolism of aldosterone was suggested by Yates, Urquhart and Herbst (4) who found a decrease in the 4,5-steroid reductase activity for inactivation of aldosterone by liver tissue from rats subjected to chronic passive venous congestion. They suggested that a decreased rate of reduction of ring A of aldosterone might increase the plasmal level of the hormone in these animals. The primary purpose of the present study was to evaluate the possibility of a decreased rate of metabolism of aldosterone by the congested liver. The disappearance of H^3 -aldosterone from plasma was studied in dogs with chronic hepatic venous congestion secondary to thoracic inferior vena caval constriction and in normal animals. In addition, several aspects of the metabolism of *d,l*-aldosterone were studied including the urinary and biliary excretion of

total tritium radioactivity, the disappearance of total methylene chloride-extractable radioactivity from the plasma, the effect of hepatectomy on the biological half-life ($t_{1/2}$) of aldosterone, and binding of H^3 -aldosterone to plasma protein. At the time these studies were performed tritiated *d*-aldosterone was not available; recently, *d*-aldosterone has been tritiated and additional observations have been made with the natural hormone.

METHODS

Studies with d,l-aldosterone. The disappearance of H^3 -*d,l*-aldosterone from peripheral plasma was determined in 9 normal dogs before and after constriction of the thoracic inferior vena cava, and in one additional dog after caval constriction. Ten or 20 μ c (7 to 21 μ g) of the *d,l*-form of randomly ring-labeled H^3 -aldosterone, with a specific activity ranging from 1.0 to 3.0 μ c per μ g was injected intravenously; the efficiency of the liquid scintillation spectrometer was approximately 25 per cent. The concentration of true H^3 -aldosterone in peripheral plasma was measured at intervals of 5, 10, 15, 20, 30, 45, 60, and 90 minutes. In the dogs with thoracic inferior vena caval constriction, large volumes of ascitic fluid formed and sodium retention was almost complete. In a preliminary experiment it was found that equilibration of H^3 -aldosterone between ascitic fluid and plasma was very slow in the presence of several liters of ascitic fluid. Therefore, in the definitive study, the ascitic fluid was removed as completely as possible on the day of the experiment to enhance the rate of equilibration of tritiated aldosterone between plasma and ascites. During the course of chronic ascites formation, frequent abdominal inferior vena caval pressures were measured to obtain an index of the severity of the hepatic venous congestion. In 2 dogs with caval constriction the influence of hepatectomy on the disappearance of aldosterone from plasma was studied. The hepatectomy consisted of shunting the portal blood to the femoral vein, ligating the hepatic artery, and placing a large polyethylene tube within the inferior vena cava in the region of the hepatic veins; this tube occluded venous outflow from the liver only. All

* Reported in preliminary form to the Endocrine Society, June, 1960, and at the Laurentian Hormone Conference, Sept., 1960.

dogs (15 to 25 kg) were kept in metabolic cages and fed a diet containing 60 mEq of Na and 18 mEq of K per day.

The biliary and urinary excretion of *d,l*-aldosterone was studied. The bile duct was cannulated in 2 normal dogs and 90-minute collections of bile were obtained for the determination of methylene chloride-extractable radioactivity and of true H^3 -*d,l*-aldosterone. The urine was collected by an indwelling catheter for the first 90 minutes after the injection of H^3 -*d,l*-aldosterone, and 24-hour urine collections were obtained for 7 days for measurements of total radioactivity.

The binding of H^3 -*d,l*-aldosterone by proteins of plasma from normal dogs and from dogs with constriction of the thoracic inferior vena cava, and by human plasma proteins was determined by equilibrium dialysis. Ten ml of plasma was placed in cellophane dialysis tubing and dialyzed against 20 ml of phosphate buffer (pH 7.4) at 37° C for 24 hours; the material was mixed during dialysis with a shaker. An aliquot of plasma and of dialysate was extracted with methylene chloride, counted, and the binding calculated.

Randomly ring-labeled H^3 -*d,l*-aldosterone was prepared by the Wilzbach technique (5). The H^3 -aldosterone was separated from all other tritiated products by the method of Peterson (6). The tritium-labeled aldosterone monoacetate was dissolved in 0.5 ml ethanol; 1.5 ml of water was added and the tritiated aldosterone monoacetate was extracted with 10 ml of methylene chloride. The solvent was evaporated with a stream of nitrogen, the aldosterone monoacetate was transferred to Whatman no. 1 chromatography paper (7.5 × 22 inches) with ethanol, followed by two rinses of methylene chloride, and chromatographed in a cyclohexane:benzene:water (100:100:50) system for 13 hours. The tritiated aldosterone monoacetate was eluted with ethanol and evaporated to dryness with a stream of nitrogen. The residue was dissolved in 0.5 ml of ethanol, and 5 ml of glycylglycine buffer (0.1 M, pH 7.4) was added. To this mixture 500 U of acetyl cholinesterase in 0.1 to 0.2 ml water was added and the sample was incubated at 37° C for 16 hours. The aqueous mixture was extracted with 6 vol of methylene chloride and evaporated to dryness with a stream of nitrogen. The free aldosterone was then transferred to Whatman no. 1 paper with ethanol, followed with two rinses of methylene chloride, and chromatographed for 16 hours in a cyclohexane:dioxane:water (100:100:25) system for 17.5 hours. The specific activity of H^3 -aldosterone was determined by counting aliquots in a liquid scintillation spectrometer and measuring its absorption in a Beckman DU spectrophotometer at 242 m μ (molar extinction coefficient, 15,800).

Plasma, bile, and ascitic fluid were analyzed for aldosterone by the following procedure, which involved slight modifications of the double isotope derivative assay (7, 8).

Each sample (6 to 10 ml of plasma, 3 ml of bile, or 10 ml of ascitic fluid) was extracted with 7.5 vol of methylene chloride. The extract was washed with 0.1 vol of 0.05 N sodium hydroxide, and subsequently with an equal volume of 0.1 N acetic acid, and dried with a stream of nitrogen. The residue was transferred to a 6.5-ml conical glass-stoppered tube with 2 ml ethanol, followed by two rinses of 2 ml of methylene chloride, and dried with a stream of nitrogen. Acetylation was carried out by adding 0.08 ml acetic anhydride and 0.08 ml pyridine under anhydrous conditions and incubating at 37° C for 24 hours. Aldosterone diacetate- C^{14} (800 cpm) was added to correct for losses during chromatography. The steroids were extracted with ethanol (0.16 ml), water (0.8 ml), and methylene chloride (3 ml), and subsequently washed with water and dried with a stream of air. Each sample of steroids was chromatographed in the first two systems employed for routine analysis of plasma for aldosterone by the double isotope derivative assay (7, 8), and radioactivity was counted in a liquid scintillation spectrometer (7, 8).

Tritium to carbon-14 ratios were determined in eight samples after each of three chromatographies. The ratios were constant after the second chromatography, indicating that the aldosterone was completely separated from its tritiated metabolites. No correction was made for losses before chromatography because previous work (7) and weekly analyses of samples of peripheral plasma with added aldosterone have demonstrated that acetylation was complete.

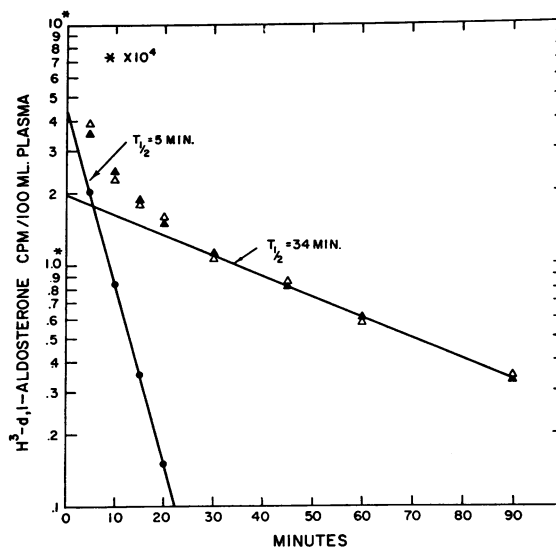


FIG. 1. DISAPPEARANCE OF H^3 -*d,l*-ALDOSTERONE FROM PERIPHERAL PLASMA OF 9 NORMAL DOGS. Either 5,685,000 or 11,370,000 cpm of H^3 was injected into each animal. Δ = experimental values; \blacktriangle = values obtained by a least squares fit. There are 2 components to the disappearance curve. The rapid phase extrapolates to the ordinate at a_{11} , while the slow component extrapolates to a_{12} . (See text for equations describing both components.)

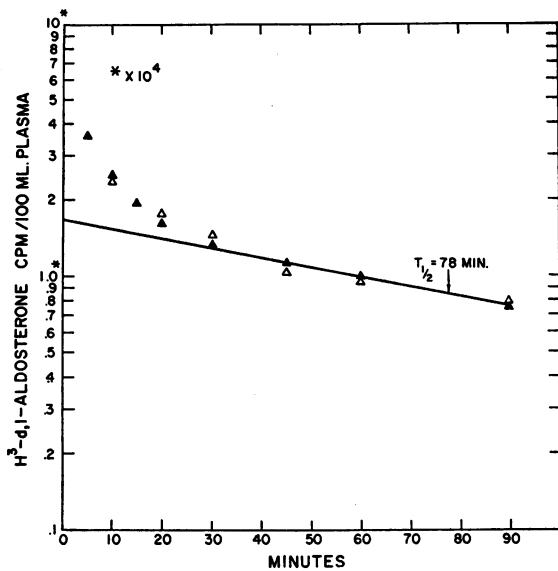


FIG. 2. DISAPPEARANCE OF H^3 -*d,l*-ALDOSTERONE FROM PERIPHERAL PLASMA OF 6 DOGS WITH CHRONIC HEPATIC CONGESTION SECONDARY TO THORACIC CAVAL CONSTRICTION. The amount of H^3 injected was the same as in the normal dogs. The $t_{1/2}$ of aldosterone is prolonged from a normal value of 34 minutes to 78 minutes. See Figure 1 for description of symbols. The experimental and fitted value were the same for the 5-minute sample.

The total methylene chloride-soluble radioactivity of plasma and bile was determined by extracting 1 to 2 ml with methylene chloride, drying the extract with a stream of air, transferring the material to counting vials, adding 5 ml phosphor, and counting in a liquid scintillation spectrometer. Urine total radioactivity was determined by adding 2 ml ethanol to a 0.01-ml aliquot of urine in 5 ml phosphor and counting in the spectrometer. Suppression of counts by the chromogens in urine and bile and by the ethanol was corrected by adding a known amount of H^3 -aldosterone to each sample, recounting, and determining recovery.

Observations with d-aldosterone. The disappearance of H^3 -*d*-aldosterone from peripheral plasma was studied in 7 normal dogs and in 7 animals after thoracic caval constriction. In 3 of the normal dogs, the red blood cells were washed with an equal volume of normal saline and the saline analyzed for aldosterone. The blood was centrifuged within 1 to 2 minutes after withdrawal from the femoral vein. Randomly ring-labeled tritiated *d*-aldosterone prepared by the Wilzbach technique (5) was used; the specific activity was 0.4 to 0.8 μc per μg . The experimental procedure and the analytical techniques used for measuring the disappearance of *d*-aldosterone were identical to those for *d,l*-aldosterone. Also, the binding of *d*-aldosterone to plasma proteins was studied by the same procedure used for *d,l*-aldosterone. For the binding experiments, 7 H^3 -*d*-aldosterone supplied by the

Endocrinology Study Section was used; the specific activity of this material was 20 μc per μg .

RESULTS

Data analysis and derived results. For purposes of analysis, the data within each experimental group of animals for both *d,l* and *d*-aldosterone were averaged to yield a simple composite set for each study. These data were plotted on semilogarithmic coordinate paper (Figures 1-4) and fitted to sums of exponentials. Only 2 exponential terms could be resolved and these were expressed as:

$$x_1 = a_{11}e^{-\alpha_1 t} + a_{12}e^{-\alpha_2 t} \quad [1]$$

where a_{11} and a_{12} are the y intercepts of the two components (see Figure 1), α_1 and α_2 are the slopes, and t is the time. Following tracer kinetic theory (9), a two-compartmental model (Figure 5) is proposed to characterize the distribution and metabolism of aldosterone. In this model, compartment 1 represents the pool into which aldosterone was first injected and x_1 is the concentration of aldosterone in counts per minute per 100 ml of peripheral plasma given by the data. The k_{ij} represent turnover rates from the j^{th} to

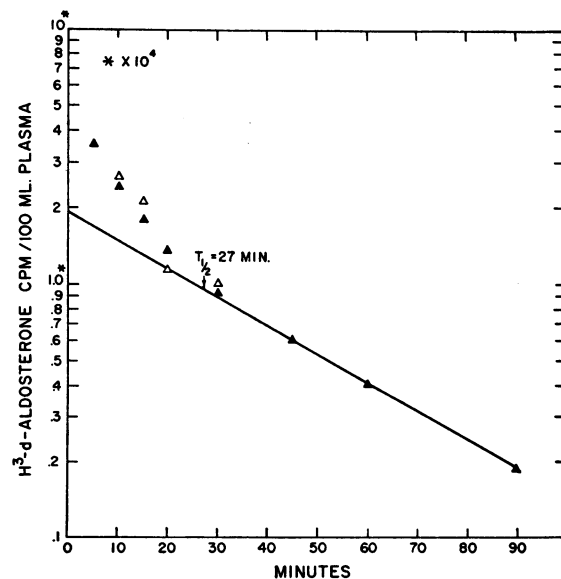


FIG. 3. DISAPPEARANCE OF H^3 -*d*-ALDOSTERONE FROM PERIPHERAL PLASMA OF 7 NORMAL DOGS. 11,370,000 cpm of H^3 was injected into each animal. See Figure 1 for description of symbols. In the samples with only a solid symbol, the experimental and fitted values coincided.

the i^{th} compartment. It is a consequence of the theory that the available data are not sufficient to permit a unique solution for the values of all the k_{ij} . A generalized solution in terms of, at most, one unknown variable, y , however, may be specified as follows:

$$k_{01} = \frac{a_{11}\alpha_1 + a_{12}\alpha_2}{a_{11} + a_{12}} - y(\alpha_1 - \alpha_2) \quad [2a]$$

$$k_{02} = \frac{a_{11}\alpha_2 + a_{12}\alpha_1}{a_{11} + a_{12}} - \frac{a_{11}a_{12}(\alpha_1 - \alpha_2)}{(a_{11} + a_{12})^2} \cdot 1/y \quad [2b]$$

$$k_{21} = y(\alpha_1 - \alpha_2) \quad [2c]$$

$$k_{12} = \frac{a_{11}a_{12}(\alpha_1 - \alpha_2)}{(a_{11} + a_{12})^2} \cdot 1/y \quad [2d]$$

$$k_{11} \equiv k_{01} + k_{21} = \frac{a_{11}\alpha_1 + a_{12}\alpha_2}{a_{11} + a_{12}} \quad [2e]$$

$$k_{22} \equiv k_{02} + k_{12} = \frac{a_{11}\alpha_2 + a_{12}\alpha_1}{a_{11} + a_{12}} \quad [2f]$$

The sizes of compartment 1 (S_1) and compartment 2 (S_2) may be determined from the relations

$$S_1 = \frac{\text{total counts per minute injected}}{a_{11} + a_{12}} \times 100 \text{ ml and} \quad [2g]$$

$$S_2 = \frac{k_{21}S_1}{k_{22}} \quad [2h]$$

Equations 2a to 2d suggest that a single assumption about any of the k_{ij} is sufficient to determine all the others. Two reasonable assumptions were made for which the following two models resulted: 1) $k_{02} = 0$, which requires that losses or degradation of aldosterone take place from compartment 1 only (model I); and 2) $k_{01} = 0$, which requires that losses or degradation of aldosterone take place from compartment 2 only (model II).

Specification of either 1 or 2 permits the solution of y from Equations 2a or 2b, respectively, and consequently the solution for the values of all other k_{ij} .

Two procedures were employed for the solution of the values of the k_{ij} for the studies presented here. In the first procedure, the data were fitted to sums of exponentials by a least squares method

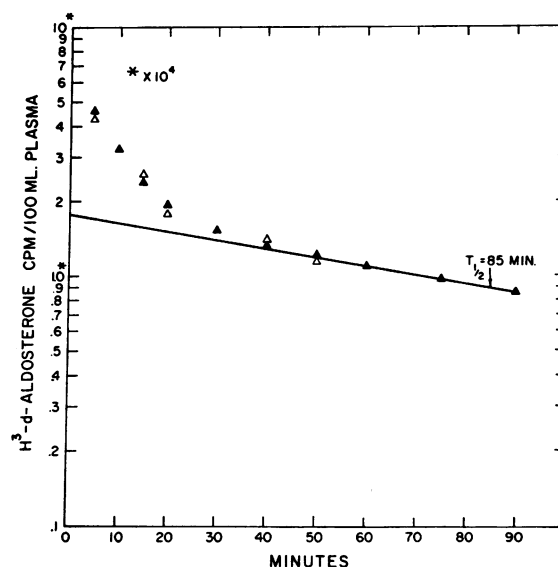
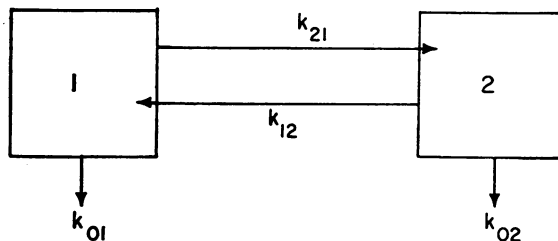


FIG. 4. DISAPPEARANCE OF H^3 - d -ALDOSTERONE FROM PERIPHERAL PLASMA OF 4 DOGS WITH THORACIC CAVAL CONSTRICTION GIVEN THE SAME DOSE OF H^3 AS THE 7 NORMAL ANIMALS. See Figures 1 and 3 for description of symbols.

using a digital computer program¹ (10). The obtained values of a_{11} , a_{12} , α_1 , and α_2 were then substituted into Equations 2a to 2f and solutions for the values of k_{ij} for the two special models

¹ A computer program is a coded set of instructions that enables a digital computer to execute automatically a prescribed sequence of calculations on data presented to it. The program employed here was designed to fit data with specified mathematical functions or equations which may or may not describe a physical model. In fitting the data, the parameters of the functions or equations are adjusted until a least squares fit is obtained. The values for the parameters and their standard errors are the end products of the procedure.



TWO - COMPARTMENTAL MODEL

FIG. 5. DIAGRAM OF TWO-COMPARTMENTAL MODEL DERIVED FROM AN ANALYSIS OF DATA IN TERMS OF TRACER KINETICS.

TABLE I
Turnover rates and volumes of distribution of H^3 -*d,l*- and *d*-aldosterone *

| <i>d,l</i> -Aldosterone | | <i>d</i> -Aldosterone | | | |
|-------------------------|---------------------|------------------------------------|---------------------|-----------------------|------------------------------------|
| Normal [9] | | After caval constriction [6] | Normal [7] | | After caval constriction [4] |
| Model I † | | | | | |
| k_{12} | | $0.058 \pm 0.006\ddagger$ | | $0.0516 \pm 0.0055^*$ | |
| k_{21} | | 0.070 ± 0.006 | | 0.049 ± 0.0046 | |
| k_{01} | 0.0517 ± 0.0017 | | 0.0261 ± 0.002 | 0.0636 ± 0.0016 | 0.0265 ± 0.0015 |
| SS | | 485 | | 113,600 | |
| S_1 | 17.2 | | 17.0 | | 19.7 |
| S_2 | 32.6 | | 38.0 | | 29.8 |
| Model II † | | | | | |
| k_{12} | | $0.035 \pm 0.004\ddagger$ | | $0.0266 \pm 0.0024^*$ | |
| k_{21} | | 0.104 ± 0.007 | | 0.098 ± 0.007 | |
| k_{02} | 0.0275 ± 0.0018 | | 0.0119 ± 0.0016 | 0.0350 ± 0.0014 | 0.0110 ± 0.0012 |
| | | 416 | | 93,144 | |
| S_1 | 19.8 | | 20.3 | | 15.6 |
| S_2 | 32.9 | | 32.3 | | 40.7 |

* Numbers in brackets are the number of dogs in each group. For the dogs with caval constriction, only the dogs which showed a prolonged t_1 for aldosterone are considered. SS = sum of squares, comparable within each study only; S_1 = volume of distribution for compartment 1 in liters, and S_2 the same for compartment 2.

† Model I is a 2-compartment system with $k_{02} = 0$, whereas in model II, $k_{01} = 0$.

‡ All constants for turnover rates are expressed in fraction of pool/min; standard errors are given.

were obtained. Standard errors of the fitted constants were transformed into standard errors of the k_{ij} by routine statistical methods. In the second procedure, using the same computer program, the values of the k_{ij} in the two chosen models as well as their standard errors were calculated directly. The two procedures are equivalent and yield the same answers. The second procedure, however, is more versatile for special cases and is used in some of the analyses employed later.

The results of calculations for the *d,l*-aldosterone indicated that definite changes in turnover rate due to thoracic caval constriction occurred in the values of k_{01} or k_{02} in the two respective models and that the other turnover rates did not change significantly. In model I, for the normal dogs, k_{01} was 0.0492 ± 0.009 fraction of pool per minute, whereas in the dogs with caval constriction k_{01} was 0.0217 ± 0.005 fraction of pool per minute. Similarly, in model II the turnover rate, k_{02} , was less in dogs with caval constriction (0.016 ± 0.003 fraction of pool per minute) compared with 0.028 ± 0.002 fraction of pool per minute for normals. Since k_{12} and k_{21} did not show statistically significant changes, all the data for the two models for both *d,l*- and *d*-aldosterone were refitted with the constraint that the same

values for k_{21} and k_{12} apply before and after caval constriction. To accomplish this, the fitting had to be done in terms of the model parameters directly. The results are presented in Table I. In addition to the values of the k_{ij} , the sums of squares for a least squares fit and the calculated size of compartments 1 and 2 are presented. The results of these fits indicate that for both *d*- and *d,l*-aldosterone, a somewhat better fit was obtained with $k_{01} = 0$ than with $k_{02} = 0$. This suggests that the model II with $k_{01} = 0$ is preferable to describe the metabolism of aldosterone and the effects of caval constriction. The data were also fitted with the additional constraint that the values for the size of compartment 1 for *d*-aldosterone be the same before and after caval constriction. This, however, resulted in a much poorer fit, and the hypothesis that the size of compartment 1 remains the same before and after caval constriction was discarded.

*Studies with H^3 -*d,l*-aldosterone.* The rate constants of Table I show considerably slower (50 to 57 per cent) turnover rates for *d,l*-aldosterone in dogs with caval constriction than in normal animals for both models I and II. All values for the volumes of distribution of *d,l*-aldosterone are large. With model I the volume of distribution of compartment 1 is 28 per cent larger after caval

defect and a greater fall in plasma fibrinogen and plasminogen than did the lowest concentration (20 SK U per ml). However, interesting differences were apparent between the effects of the 40 and 80 SK U per ml concentrations. Both concentrations effectively reduced plasma plasminogen to zero or near zero levels, but as indicated in the left-hand panel of Figure 2, the speed with which this reduction occurred was different. The slope of initial plasminogen reduction was approximately twice as fast with 80 as with 40 SK U per ml and this increased rate of plasminogen activation was associated with a significantly greater and faster fall of plasma fibrinogen. A corresponding increase in the severity of the coagulation anomaly also occurred, and this suggested that fibrinogen proteolysis could be related to the coagulation anomaly.

Enzymatic proteolysis of fibrinogen: Properties of the digestion products

Plasmin and trypsin degrade fibrinogen so that it becomes totally incoagulable by thrombin. The resulting enzymatic digests, after the addition of a suitable plasmin or trypsin inhibitor, possess novel properties not exhibited by the native fibrinogen. Whereas a solution of native fibrinogen, when added to a plasma or fibrinogen test system, will normally shorten the thrombin clotting time of the mixture, an equivalent quantity of fibrinogen digest will greatly prolong the

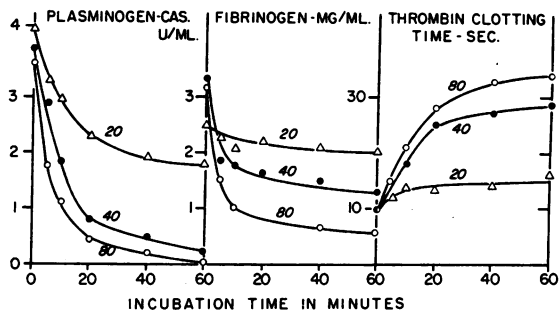


FIG. 2. EFFECT OF ADDING THREE DIFFERENT CONCENTRATIONS OF SK (20, 40, AND 80 U PER ML) TO A SINGLE PLASMA. SK concentrations are designated by a superscript number and serial assays for plasminogen (left-hand panel), fibrinogen (middle panel), and thrombin clotting time (right-hand panel) are shown for each concentration.

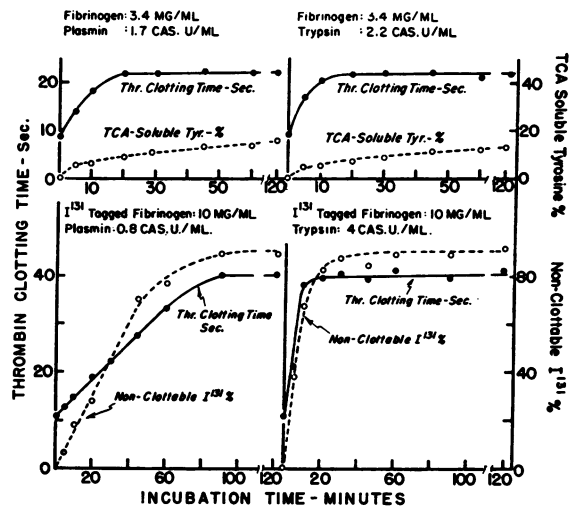


FIG. 3. FIBRINOGEN PROTEOLYSIS BY TWO CONCENTRATIONS OF PLASMIN (2 LEFT-HAND PANELS) AND BY TWO CONCENTRATIONS OF TRYPSIN (2 RIGHT-HAND PANELS). Thrombin clotting time (all panels) refers to the ability of the digest to prolong the thrombin clotting time of the standard assay system. This parameter is correlated with the development of TCA-soluble tyrosine (above), and using I¹³¹-labeled fibrinogen with the formation of non-clottable I¹³¹-labeled fragments (below).

thrombin clotting time of the mixture.¹² Indeed, under suitable experimental conditions, the thrombin clotting time of the mixture may be prolonged to infinity.

Figure 3 illustrates serial findings, especially with relation to the development of "antithrombin" activity, occurring during the enzymatic proteolysis of I¹³¹-labeled fibrinogen by plasmin or trypsin. The original concentration of fibrinogen was 3.4 mg per ml in the two top sections of the diagram and 10 mg per ml in the two bottom sections; the left-hand sections illustrate experiments with two concentrations of plasmin and the

¹² Conventional usage would suggest that the term "antithrombic activity" should be applied to designate the property of fibrinogen digestion products in prolonging the thrombin clotting time of normal plasma. Similarly it would be customary to state that during the digestion process an "antithrombin" was formed. This nomenclature is clearly unsatisfactory, for its use implies that a specific mechanism of inhibition has been demonstrated where such is not the case. Nevertheless, because other suitable terms are lacking, the words "antithrombin" and "antithrombic activity" will be used sparingly in this communication, solely for descriptive convenience and without any implications as to the mechanisms of action.

TABLE II

Comparison of the abdominal inferior vena caval pressure of the dogs with an increased $t_{1/2}$ of H^3 -*d,l*-aldosterone after caval constriction with the group of dogs that had no change in $t_{1/2}$ of H^3 -*d,l*-aldosterone after thoracic caval constriction

| Dogs | Group I N=3 | | Group II N=6 | |
|---------------------------------|-----------------------|------------------|----------------------|------------------|
| | $t_{1/2}$ | IVC pressure | $t_{1/2}$ | IVC pressure |
| Normal | min 31 (30-33)* | mm water | min 24 (20-30) | mm water |
| After thoracic IVC constriction | 32 (30-35) | 217 (202-240) | 60 (43-101) | 260 (190-295) |

* Values in parentheses show ranges.

Five of the six dogs that showed a prolonged $t_{1/2}$ after constriction of the thoracic inferior vena cava had abdominal vena caval pressures higher than the three dogs with no change in $t_{1/2}$ after thoracic caval constriction (Table II). The six dogs with a prolonged $t_{1/2}$ had a mean abdominal inferior vena caval pressure of 260 mm water compared with a pressure of 217 mm water in the three dogs with no change in $t_{1/2}$.

To evaluate the role of the liver in the metabolism of aldosterone, hepatectomy was performed in two of the dogs with caval constriction immediately before the intravenous injection of H^3 -*d,l*-aldosterone. The $t_{1/2}$ for aldosterone was prolonged in both animals and data from the dog with the greatest prolongation of the biological half-life of H^3 -*d,l*-aldosterone after hepatectomy

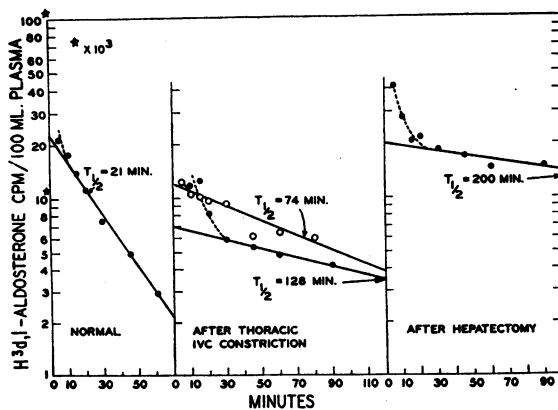


FIG. 8. DISAPPEARANCE OF H^3 -*d,l*-ALDOSTERONE FROM THE PLASMA OF A DOG BEFORE AND AFTER THORACIC INFERIOR VENA CAVAL CONSTRICTION AND SUBSEQUENT HEPATECTOMY. The $t_{1/2}$ of 74 minutes was obtained after 8 days of caval constriction while the longer $t_{1/2}$ of 128 minutes was observed after 21 days of hepatic congestion.

are presented in Figure 8. The $t_{1/2}$ was greatly prolonged ($t_{1/2} = 200$ minutes) indicating that the liver is the major organ responsible for the metabolism of aldosterone.

The data on the binding of H^3 -*d,l*-aldosterone to the plasma proteins of normal dogs and of dogs after thoracic caval constriction and by plasma proteins from one normal human subject are presented in Table III. The plasma proteins from dogs with thoracic caval constriction and from normal dogs showed no difference in binding of aldosterone. The plasma from dogs bound approximately 27 to 33 per cent (average values) of the added H^3 -aldosterone compared with 72 to 74 per cent binding by human plasma. There appeared to be no difference in binding at the two concentrations of H^3 -*d,l*-aldosterone used (0.34 and 3.4 μ g per 100 ml).

TABLE III
Plasma binding of aldosterone at 37° C

| No. | Normal dogs | | Dogs with thoracic caval constriction | | Normal human | |
|---|----------------|---------------|---------------------------------------|--------------|---------------|--------------|
| | 0.34 μ g%* | 3.4 μ g%* | 0.34 μ g% | 3.4 μ g% | 0.34 μ g% | 3.4 μ g% |
| | % | % | % | % | % | % |
| Randomly ring-labeled H^3 - <i>d,l</i> -aldosterone | | | | | | |
| 1 | 17 | 26 | 29 | 24 | 74 | 72 |
| 2 | 35 | 33 | 26 | 27 | | |
| 3 | 34 | 38 | 25 | 38 | | |
| 4 | 33 | 36 | | | | |
| Mean | 30 | 33 | 27 | 30 | 74 | 72 |
| $7H^3$ - <i>d</i> -aldosterone | | | | | | |
| 5 | 45 | 41 | 37 | 30 | | |
| 6 | 16 | 35 | 28 | 56 | | |
| Mean | 30 | 38 | 32 | 43 | | |

* Concentrations of H^3 -*d,l*-aldosterone.

The urinary excretion of H^3 -*d,l*-aldosterone and its tritiated metabolites, which were measurable as total radioactivity, was virtually complete in 3 days (Figure 9). Five to 11 per cent of the total tritium injected was excreted in the urine within the first 90 minutes. Fifty-eight to 63 per cent of the total tritium injected was recovered in the urine. More than half of the total tritium recovered was excreted in the first 24-hour urine collection. Less than 0.1 per cent of H^3 -*d,l*-aldosterone injected was recovered in the bile from two normal anesthetized dogs during the first 90 minutes after injection of H^3 -*d,l*-aldosterone; also, less than 0.1 per cent of tritium injected was recovered as total radioactivity.

*Observations with H^3 -*d*-aldosterone.* The rate constants for *d*-aldosterone (Table I) demonstrate a 58 to 68 per cent slower rate of turnover in dogs with caval constriction than in normal animals. The calculated sizes of compartments 1 and 2 (Table I) indicate large volumes of distribution.

If the slow component is used as an index of the biological half-life, the $t_{1/2}$ for *d*-aldosterone in normal dogs was 27 minutes, which is not appreciably different from the $t_{1/2}$ of *d,l*-aldosterone. After caval constriction, the $t_{1/2}$ for aldosterone was prolonged in four of seven dogs from 27 to 85 minutes (Figures 3 and 4). In the other three

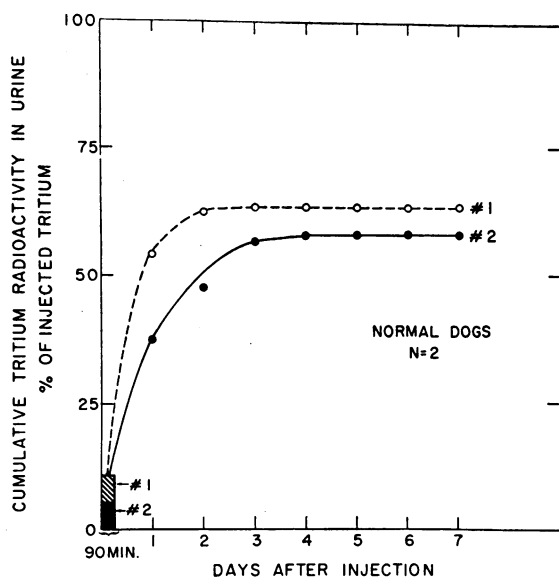


FIG. 9. CUMULATIVE RECOVERY OF TRITIUM EXCRETED IN URINE AFTER THE INTRAVENOUS INJECTION OF H^3 -*d,l*-ALDOSTERONE.

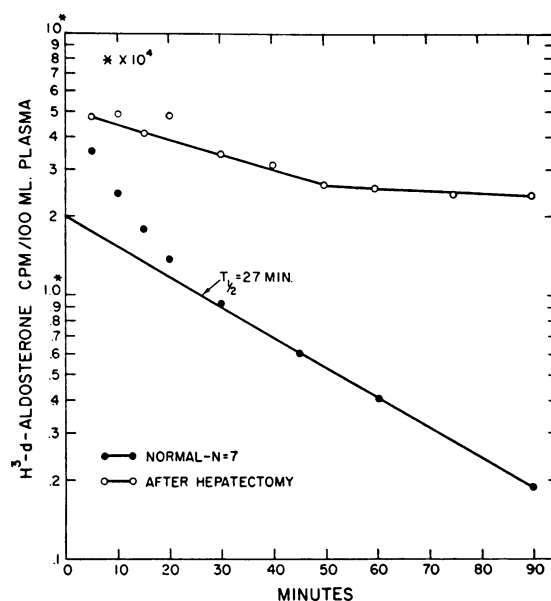


FIG. 10. COMPARISON OF THE DISAPPEARANCE CURVES OF H^3 -*d*-ALDOSTERONE IN A HEPATECTOMIZED DOG WITH THAT IN 7 NORMAL DOGS. Note the higher initial level and slow rate of disappearance of aldosterone in the hepatectomized dog.

dogs with caval constriction the $t_{1/2}$ was normal (24 minutes).

The concentrations of *d*-aldosterone in the saline washes of the red blood cells were measured in 4 to 6 samples from each of three normal dogs. The average ratios of the concentrations of *d*-aldosterone in the saline washes to those in plasma for the 4 to 6 periods for the three dogs were 34.9, 9.6, and 36.4 per cent. The results from study of the degree of binding of *d*-aldosterone to plasma proteins are presented in Table III. No difference was detected between the degree of binding of *d*- and *d,l*-aldosterone, and caval constriction failed to alter the binding of *d*-aldosterone to plasma proteins.

The effects of hepatectomy on the disappearance of *d*-aldosterone from peripheral plasma were determined in two dogs. The level of *d*-aldosterone in peripheral plasma was initially (after 5 minutes) considerably higher than that in normal dogs, and the $t_{1/2}$ for aldosterone was markedly prolonged (Figure 10).

DISCUSSION

The intravenous injection of H^3 -aldosterone was followed by the very rapid disappearance of

the hormone from plasma. For both *d,l*-aldosterone and *d*-aldosterone two components were present in the disappearance curves. There was a very rapid initial phase of disappearance of aldosterone; for *d,l*-aldosterone a $t_{\frac{1}{2}}$ of less than 5 minutes was observed in the normal dogs. For the slow component the $t_{\frac{1}{2}}$ was 34 minutes for normal dogs with *d,l*-aldosterone and 27 minutes for *d*-aldosterone. The apparent "volumes of distribution" for the two compartments have been calculated. These data show a "volume of distribution" for both compartments 1 and 2 which exceeds that of total body water and, therefore, the calculations suggest that both *d,l*- and *d*-aldosterone are selectively taken up by some region of the body. The present studies show that injected H^3 -*d*-aldosterone equilibrates with or becomes bound to the red blood cells, which therefore constitute a part of the body pool.

The data for H^3 -aldosterone have been analyzed in terms of a two-compartmental model which is suggested by the two components of the disappearance curve. Model II with $k_{01} = 0$ appears to be preferable to model I to describe the metabolism of aldosterone. This finding and the present data which indicate that the liver is the principal site for metabolism of aldosterone are consistent with the presence of the liver in compartment 2 or the outer pool; however, the liver could be part of both compartments and the present data do not strongly favor the inclusion of the liver in the outer pool. The suggestion that the liver is part of the inner pool was made earlier by Tait, Tait, Little and Laumas (11).

There is some question about the metabolism of the *d*- and *l*-forms of aldosterone. Ulick (12) reported that after the injection of racemic aldosterone, the unnatural antipode, *l*-aldosterone was not converted to either the conjugate hydrolyzed at pH 1 or to urinary tetrahydroaldosterone. Peterson (6) has found the $t_{\frac{1}{2}}$ for *d*-aldosterone in normal man to be approximately one-half the value reported earlier (13) of 30 to 48 minutes for *d,l*-aldosterone. In contrast, Tait and co-workers (11) obtained a $t_{\frac{1}{2}}$ of 35 minutes for *d*-aldosterone in normal human subjects and Cope, Island, Cooner and Liddle (14) reported a mean value of 36 minutes for normal humans. The present data in dogs failed to show a difference between the $t_{\frac{1}{2}}$ for *d,l* and *d*-aldosterone;

the $t_{\frac{1}{2}}$ for the *d,l*-form was 34 minutes whereas that for *d*-aldosterone was 27 minutes. These values are similar in magnitude to the $t_{\frac{1}{2}}$ of 30 to 40 minutes for endogenous aldosterone obtained after bilateral adrenalectomy in the dog [Bojesen and Degn (15) and personal communication]. In all of these studies, the slow component of the disappearance curve has been used to determine the $t_{\frac{1}{2}}$ of aldosterone.

From the present study, direct evidence for the metabolism of H^3 -*d,l*-aldosterone was obtained and the major portion of the metabolites of aldosterone was excreted in the urine. The slower rate of disappearance of methylene chloride-extractable tritium than of authentic H^3 -*d,l*-aldosterone reflects the formation of metabolic products of aldosterone. Radioactivity appeared in the urine very rapidly; 5 to 11 per cent of the injected H^3 -*d,l*-aldosterone was recovered in the urine as total tritium radioactivity within 90 minutes, and 58 to 63 per cent of the administered radioactivity was present in the urine after 2 days. In contrast, only a very small amount of tritium was recovered in bile. Peterson and associates (16) have demonstrated that only a small fraction of administered cortisol appears in bile and that it is the sole source of fecal cortisol; no fecal radioactivity was observed after the injection of cortisol-4- C^{14} into patients with biliary fistulae.

To compare the rates of metabolism of *d,l*- and *d*-aldosterone in normal dogs and dogs with caval constriction, two indices have been used: 1) the fractional turnover rates of aldosterone of both inner (compartment 1) and outer (compartment 2) pools on the basis of a two-compartmental model, and 2) the biological $t_{\frac{1}{2}}$. As Tait and colleagues (11) have pointed out, the $t_{\frac{1}{2}}$ of aldosterone does not reflect metabolism alone. H^3 -*d*-aldosterone moves into, is adsorbed on, or is bound to, the red blood cell. Also, the large volumes of distribution for both inner and outer pools indicate selective uptake of *d,l*- and *d*-aldosterone by body tissues. However, the marked prolongation of the $t_{\frac{1}{2}}$ for both *d,l*-aldosterone and *d*-aldosterone in the dog with caval constriction is a very strong indication of a slower rate of metabolism in these animals. Furthermore, statistical analysis of the turnover rates of both *d,l*-aldosterone and *d*-aldosterone indicates a considerably slower rate of turnover from both inner and outer pools

in the dogs with caval constriction than in normal animals.

It seems unlikely that the amount of aldosterone injected was sufficient to overload the mechanisms responsible for metabolism, since only small microgram quantities of H³-aldosterone were injected. For cortisol, Peterson and associates (16) have found it necessary to inject from 50 to 200 mg as a pharmacological dose to overload the mechanism of steroid inactivation. The finding that the $t_{\frac{1}{2}}$ for *d,l*-aldosterone was independent of the amount injected in quantities ranging from 7 to 21 μ g and the observation that three dogs with caval constriction and presumably a considerably larger miscible pool of aldosterone than normal showed a normal $t_{\frac{1}{2}}$ for *d,l*-aldosterone suggest that the amount of H³-aldosterone injected did not influence the rate of disappearance from plasma. The similarity in the values for the biological half-life of endogenous aldosterone reported by Bojesen and Degn (15) and the present data indicates that the present observations reflect the physiologic rate of metabolism of aldosterone.

The degree of binding of both *d,l*- and *d*-aldosterone to plasma protein was essentially the same for normal dogs and for dogs with caval constriction. Only 27 to 43 per cent of aldosterone was bound to plasma protein in the dog. This finding is in contrast to the much higher degree of binding in man. For human plasma the findings of Mills (17), and of Daughaday, Holloszy and Martz (18), and the present data show a value of approximately 70 per cent for binding of aldosterone. By electrophoretic studies of human plasma, Daughaday and co-workers (18) have demonstrated that the major part of the bound aldosterone is bound to albumin.

Studies of the biological $t_{\frac{1}{2}}$ for H³-*d,l*-aldosterone in the dogs with thoracic caval constriction showed that the highest venous pressures were associated with a prolonged $t_{\frac{1}{2}}$ for aldosterone. It seems likely that these animals had the most marked chronic passive congestion of the liver and the data suggest that the decreased rate of metabolism of aldosterone was secondary to hepatic venous congestion. The results also indicate that hepatic congestion must be severe to impede the metabolism of aldosterone.

The importance of the liver in the metabolism of aldosterone is further indicated by the almost

flat disappearance curve for *d,l*-aldosterone after hepatectomy of dogs with caval constriction. Also, measurements of the $t_{\frac{1}{2}}$ for *d*-aldosterone after hepatectomy of normal dogs showed a marked decrease in the rate of disappearance from plasma. The data after hepatectomy and the decreased rate of disappearance of both *d,l*- and *d*-aldosterone from plasma following caval constriction provide evidence that a decreased rate of metabolism of aldosterone by the liver may contribute to the hyperaldosteronemia in right-sided cardiac failure. However, the more consistent occurrence and the marked degree of hypersecretion of aldosterone in dogs with thoracic caval constriction (3) indicate that hypersecretion is the primary mechanism resulting in hyperaldosteronemia.

From the present data on fractional turnover rates and compartment sizes for *d*-aldosterone (Table I) and from the earlier values for the secretion rates of aldosterone in conscious animals (19), the plasma concentrations of aldosterone have been calculated. For model II, the concentration of aldosterone in peripheral plasma was calculated from the steady state condition that $k_{21}Q_1 - k_{12}Q_2 = \rho$ and $k_{02}Q_2 = \rho$, where ρ is the net secretion rate of aldosterone per unit time and Q_1 and Q_2 are the total amounts of aldosterone in compartments 1 and 2, respectively. Solution of the above equations yields

$$Q_1 = \rho \frac{k_{12} + k_{02}}{k_{21} \times k_{02}}$$

Dividing through by S_1 gives

$$\frac{Q_1}{S_1} = \frac{\rho}{S_1} \times \frac{k_{12} + k_{02}}{k_{21} \times k_{02}}$$

for the concentration in compartment 1. For normal dogs the peripheral plasma concentration is 0.002 μ g per 100 ml, whereas for dogs with caval constriction a value of 0.090 μ g per 100 ml is obtained. This 45-fold elevation in the plasma level of aldosterone in dogs with caval constriction is largely attributable to increased adrenal secretion since the turnover rate per day (k_{02}) in these animals (Table I) is approximately one-half to one-third of that observed in normal dogs. The normal value of 0.002 μ g per 100 ml of plasma is less than the peripheral plasma level of 0.02 μ g per

100 ml for dogs reported by Bojesen and Degn (15), but their animals were sodium depleted.

Several workers (4, 13, 20-22) have demonstrated the importance of the liver in the metabolism of steroids other than aldosterone. In cirrhosis of the liver, Peterson and colleagues (13, 16, 20) found that cortisol is metabolized at a diminished rate; synthesis of cortisol is also reduced in decompensated hepatic cirrhosis and this may be secondary to the homeostatic regulation of cortisol secretion by a feedback mechanism. It was suggested that the defect in cortisol metabolism is attributable to a decreased rate of conversion of cortisol to dihydrocortisol and the possibility of decreased availability of 5α - or 5β -dehydrogenase was considered. In contrast, Peterson (20) found the rate of metabolism of aldosterone to be within normal limits in two patients with cirrhosis of the liver. It appears, therefore, that the degree of liver damage in these two cases of hepatic cirrhosis was considerably less or qualitatively different from that resulting from the severe chronic liver congestion in the present study. Very recently, Coppage and associates (14) reported prolongation of the $t_{\frac{1}{2}}$ for 7 H^3 - d -aldosterone (slow component of disappearance curve) from 36 minutes for normal human subjects to 67 minutes for patients with decompensated hepatic cirrhosis.

The nature of the defect in the metabolism of aldosterone by the severely congested liver is not known. It seems likely that hepatic blood flow is reduced after caval constriction and a resultant state of relative hepatic anoxia might be present. At an enzymatic level, it is possible that a reduced amount of TPNH, or of the specific dehydrogenases for reduction of the Δ^4 bond or the 3-keto group occurs. Tomkins (23-25) has demonstrated that Δ^4 reduction is TPNH dependent. Yates and co-workers (4) found a decrease in Δ^4 reduction of cortisone, cortisol, deoxycorticosterone, and aldosterone by liver homogenates from rats with constriction of the inferior vena cava above the hepatic veins. These data provide strong suggestive evidence for a defect in Δ^4 reduction, but because of the marked differences in the specific enzyme systems among different animal species (24), final solution of the problem awaits study of the enzymatic defect in the dog.

SUMMARY AND CONCLUSIONS

The biological half-lives of tritiated d,l - and d -aldosterone in normal dogs were 34 and 27 minutes, respectively. After thoracic caval constriction and in the presence of marked chronic hepatic venous congestion the $t_{\frac{1}{2}}$ for both d,l -aldosterone and d -aldosterone was markedly prolonged. Subsequent hepatectomy resulted in an almost flat disappearance curve of d,l -aldosterone. Analysis of the data in terms of a two-compartmental model showed a decreased rate of turnover of both d,l - and d -aldosterone by both the inner and outer compartments in dogs with caval constriction. The data provide evidence that a decreased rate of metabolism of aldosterone may contribute to the hyperaldosteronemia in clinical states with severe chronic hepatic congestion, as in right-sided congestive heart failure. However, in dogs with caval constriction the more consistent occurrence and marked degree of hypersecretion of aldosterone indicate that increased secretion is the primary mechanism leading to hyperaldosteronemia.

Urine was found to be the major excretory route for d,l -aldosterone and its metabolites. Only trace amounts of radioactivity from H^3 - d,l -aldosterone were recovered in bile. Saline washes of red blood cells demonstrated the presence of H^3 - d -aldosterone in, adsorbed on, or bound to the red blood cells.

Studies by equilibrium dialysis of the degree of binding of d,l - and d -aldosterone to plasma proteins showed 27 to 43 per cent binding in the dog in contrast to approximately 70 per cent binding to normal human plasma protein. No difference in the degree of binding of aldosterone was observed between plasma from normal animals and dogs with caval constriction.

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