

# THE DISAPPEARANCE OF 7-H<sup>3</sup>-*d*-ALDOSTERONE IN THE PLASMA OF NORMAL SUBJECTS \*

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The study of the disappearance of the radioactivity in plasma after the injection of labeled aldosterone should elucidate the transport and metabolism of this steroid in man. In particular this would allow the calculation of the turnover rate in various clinical conditions. This value, together with a concomitant determination of the secretion rate estimated from the specific activity of a urinary metabolite, would enable the mean blood concentration of aldosterone to be calculated. This is particularly important at the present time, as no practical direct method has yet been reported for the analysis of aldosterone in peripheral blood.

After the administration of 16-H<sup>3</sup>-aldosterone to one normal subject, the labeled hormone had a half-life in plasma of 20 minutes, as calculated from the change in concentration of radioactivity measured specifically as aldosterone between 2 and 3 hours after the injection (1). This indicated that the rate of metabolism of aldosterone was greater than that of cortisol and corticosterone. The data obtained were not sufficient to give a reliable estimate of volumes of distribution. Also, the low specific activity of aldosterone then available made it necessary to administer 2  $\mu$ g of hormone. This amount represents about 25 per cent of total body pool of hormone. Peterson reported a corresponding half-life of 0.5 to 0.8 hour in normal subjects after injection of tritiated *d,l*-aldosterone (2). Again in this study large quantities of the hormone were injected. Also it is not known whether the optical isomers of aldosterone are metabolized in an identical manner. The natural hormone is in the *d* form exclusively.

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The availability of 7-H<sup>3</sup>-*d*-aldosterone of specific activity 20  $\mu$ c per  $\mu$ g has made it possible to inject 0.1  $\mu$ g of the hormone (about 1 per cent of the body pool). A more detailed analysis and interpretation of the disappearance curve in plasma has been carried out.

## METHODS

4-C<sup>14</sup>-aldosterone<sup>1</sup> was fractionated by partition column chromatography before use. 7-H<sup>3</sup>-aldosterone was prepared as previously described (3). It was stored at a concentration of 11  $\mu$ c (0.55  $\mu$ g) per ml in ethanol and checked at regular intervals for radiochemical purity over a period of 2 months. *d,l*-Aldosterone diacetate was prepared by acetylating *d,l*-aldosterone-21-monoacetate.<sup>2</sup> Methylene dichloride was prepared by washing with an equal volume of distilled water and dried over anhydrous sodium sulfate. After filtration, charcoal was added and the solvent decanted and again filtered. It was allowed to stand over potassium hydroxide, refluxed for 1 hour and distilled. It was stored at -10°C. Pyridine and acetic anhydride were prepared as previously described (3).

Two-tenths ml of the solution of radioactive aldosterone in ethanol was added to 11 ml physiological saline; 10 ml of this saline solution, containing 2  $\mu$ c and 0.1  $\mu$ g 7-H<sup>3</sup>-aldosterone was injected into the antecubital vein of female subjects at 8 a.m. Blood was withdrawn from the other arm with a heparinized syringe at various times after injection. The blood was immediately centrifuged and the plasma added to the tube which contained a dry sample of 4-C<sup>14</sup>-aldosterone [usually 80 dpm (1  $\mu$ g) per 10 ml plasma]. The plasma was then extracted with 3  $\times$  1.3 vol of methylene dichloride. The combined solvent extracts were washed once with water (0.3 of the volume of the plasma) and taken to dryness. The mixture was transferred to a smaller flask and acetylated with 1 ml pyridine and 0.5 ml acetic anhydride overnight at room temperature, and taken to dryness. After addition of 8  $\mu$ g *d,l*-aldosterone diacetate in 1 ml ethanol, the dried residue was transferred to a partition column in 1 ml + 2  $\times$  0.5 ml mobile phase. The column, 60 cm

<sup>1</sup> 4-C<sup>14</sup>-aldosterone was generously donated by Dr. R. Travis and Dr. G. Farrell.

<sup>2</sup> *d,l*-Aldosterone-21-monoacetate kindly supplied by Dr. R. Gaunt, Ciba, Summit, N. J.

long, contained 28 g Celite 545, 14 ml of the stationary phase, water: methanol, 4: 1, vol: vol and was developed with toluene: Skellysolve C, 2: 3, vol: vol as the mobile phase; 5-ml fractions were collected. Two-tenths ml from fractions 11 to 18 (counted from the time of application of the extract) was applied separately to no. 2 Whatman paper, immediately sprayed with reagent and detected by soda fluorescence (4). Usually, the aldosterone appeared in fractions 14, 15 and 16. The 3 fractions containing the steroid were pooled, added to quartz vials (Atomic Associates) and taken to dryness in a vacuum oven at 60°C. Ten ml of scintillation fluid (4 g diphenyloxazole plus 100 mg POPOP<sup>3</sup> per liter toluene) was added plus 0.2 ml ethanol. The vials were then assayed for  $H^3$  and  $C^{14}$  in an automatic liquid scintillator (Tricarb model 314-DC, Packard Instrument Co.). The samples, together with appropriate background and standard vials, were counted for at least eight 30-minute periods at each high voltage position. Quenching corrections were not necessary for fractions obtained after partition chromatography. The counting rates due to  $H^3$  and  $C^{14}$  estimated in this way gave a value for the initial tritium content of the plasma, present specifically as aldosterone (hereafter "radioactive concentrations in plasma after injection of aldosterone" refers to values measured specifically as aldosterone) and corrected for losses during the isolation procedure. Accuracy was within 5 per cent (including counting errors) for all samples. The fractions on either side of the aldosterone diacetate peak were also assayed for tritium and  $C^{14}$  in 6 experiments. In all cases, the radioactivity was slight. The  $H^3/C^{14}$  ratio, when it could be measured, was the same as that in the peak fractions. The recovery of the 4- $C^{14}$ -aldosterone through the isolation procedure in the 3 peak fractions was about 70 per cent. After passing 300 ml of mobile phase through the columns following elution of the steroid and replacing 0.5 g Celite at the top of the column, they could be reused many times. When this procedure was followed there was no deterioration of resolving power, or displacement of the position of the steroid peak or accumulation of radioactivity even after frequent use of the columns.

For some plasma samples (usually taken 22.5 and 30 minutes after injection) 20 per cent of the extract was directly assayed for radioactivity before acetylation. Measurements for  $H^3$  and  $C^{14}$  were carried out as previously described, except that quenching corrections were applied by adding internal  $H^3$  and  $C^{14}$  standards. For all subjects studied, the total tritium radioactivity in the plasma at 22.5 and 30 minutes after injection was equal to the  $H^3$  measured specifically as aldosterone. It was, therefore, considered that the total tritium radioactivity in the plasma taken 7.5 and 15 minutes after injection was entirely due to aldosterone. The total methylene dichloride extracts from the 7.5 and 15-minute specimens were assayed directly for  $H^3$  and  $C^{14}$  without acetylation or chromatography. The quenching correction for  $H^3$  in these crude solutions was about 10 per cent. All sam-

ples taken later than this were chromatographed before counting.

Following the injection of 7- $H^3$ -aldosterone, urine was collected for 24 hours and the secretion rate of the hormone estimated from a measurement of the specific activity of aldosterone released from the urinary "3-oxo conjugate" (3).

Three subjects were clinic patients in the department of gynecology. Two were fully recovered and convalescent after pelvic inflammatory disease. One was studied prior to elective hysterectomy for leiomyomata uteri. All three patients were receiving regular diets. They were in the normal range of weight and height, and showed no signs of disturbance of electrolyte metabolism. Two volunteer normal women were studied by a single injection technique over a 3 hour period. The characteristics of their disappearance curves were similar to those of the clinic patients. One normal woman was studied by a constant infusion technique after a priming dose; 1.05  $\mu$ c 7- $H^3$ -aldosterone was injected instantaneously in 10 ml physiological saline at the start of the experiment. Thirty minutes later, a total of 0.945  $\mu$ c of the same steroid was given in physiological saline as a constant infusion over a period of 90 minutes at the rate of 0.191 ml (0.0105  $\mu$ c) per minute. Five ml of plasma was withdrawn 7.5 and 15 minutes after the single injection. These were analyzed without preliminary chromatographic separation and 10 ml of plasma (taken at 30, 105, 120 and 140 minutes after the first injection) was analyzed after chromatography of the diacetate as previously described.

## RESULTS

The values for the radioactivity present specifically as aldosterone and fully corrected for recovery, in plasma taken 7.5, 15, 22.5, 30, 50 and 70 minutes after injection of 0.1  $\mu$ g 7- $H^3$ -aldosterone, are shown in Figures 1, 2 and 3. They

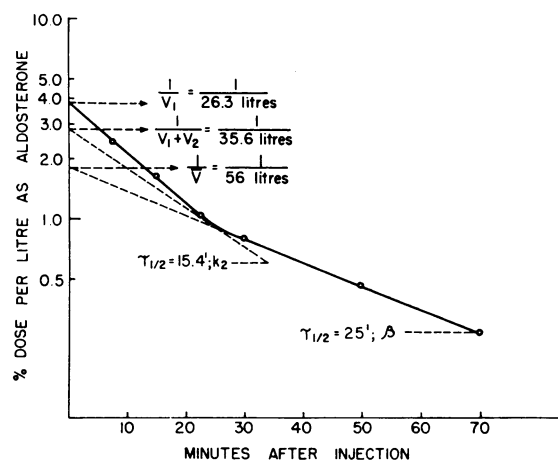


FIG. 1. DISAPPEARANCE OF 7- $H^3$ -ALDOSTERONE IN PLASMA OF NORMAL CLINIC SUBJECT, E. A.

<sup>3</sup> 1,4-bis-2-(5 phenyloxazolyl)-benzene.

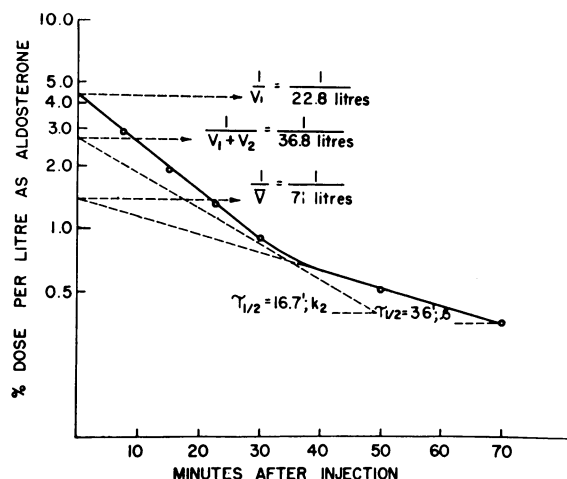


FIG. 2. DISAPPEARANCE OF 7-H<sup>3</sup>-ALDOSTERONE IN PLASMA OF NORMAL CLINIC SUBJECT, M. C.

are plotted as logarithm of per cent dose injected per liter of plasma against time after injection. These represent disappearance curves for the three clinic patients. For all these subjects, the volumes of plasma taken for analysis were 5 ml at 7.5, 15 and 22.5 minutes, 10 ml at 30 minutes, and 20 ml at 50 and 70 minutes. The disappearance curves obtained in the two volunteers are shown in Figures 4 and 5. The data were obtained as

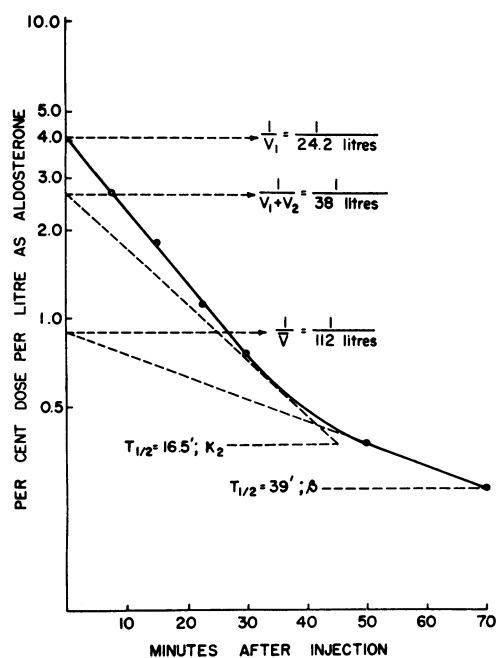


FIG. 3. DISAPPEARANCE OF 7-H<sup>3</sup>-ALDOSTERONE IN PLASMA OF NORMAL CLINIC SUBJECT, A. A.

for the other three subjects but, in addition, 50 ml plasma at 120 minutes and 100 ml at 180 minutes were also analyzed.

In all five subjects there was an extremely rapid initial drop in plasma radioactive concentration to about 4 per cent of the injected dose per L of plasma. The curve then became less steep (with a half-life of about 14 minutes) until some 30 minutes after injection. At this time, the plasma concentration was about 1 per cent of the injected dose per L of plasma. The curve then again became less steep in all cases and was a straight line for three of the five subjects. Because of the low concentration of radioactivity in plasma after the injection of a tracer amount of hormone requiring large amounts of blood to be assayed, the slope of the final part of the curve was estimated from plasma samples taken up to 70 minutes after injection in three patients. However, Figures 4 and 5 illustrate that the slope had become constant by that time. Statistical analysis of the data of these two experiments shows no significant difference in the curves between 30 to 70 minutes and 70 to 180 minutes after injection.

The values for the patient who was studied by constant infusion technique after a priming dose

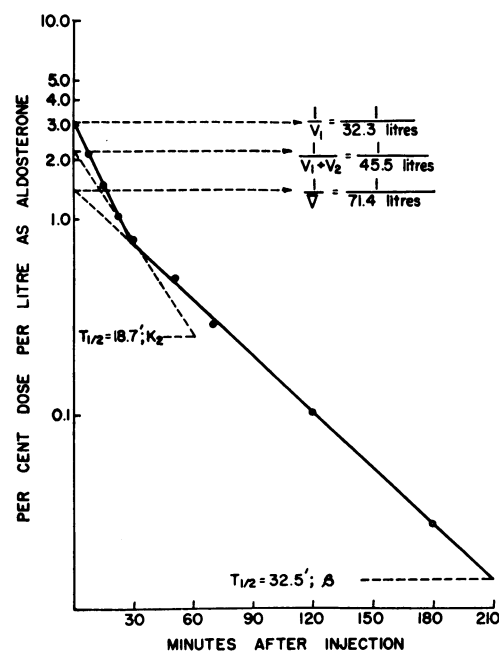


FIG. 4. DISAPPEARANCE OF 7-H<sup>3</sup>-ALDOSTERONE IN PLASMA OF NORMAL VOLUNTEER, J. D.

are shown in Figure 6. The following values were obtained, expressed as millimicrocuries aldosterone per liter of plasma: 29.9, 15, 9.9, 9.5 and 8.8, for the 7.5, 15, 30, 105 and 120 minute samples, respectively. Therefore, the radioactivity as aldosterone was constant from 30 to 120 minutes after the first injection within the limits of experimental error. The values as radioactive aldosterone 140 minutes after the single injection (20 minutes after the end of the constant infusion) was 6.4  $m\mu C$  per L plasma.

**Calculations.** From the characteristics of these disappearance curves the concentration of radioactivity in plasma as a function of time may be expressed as

$$x = Ae^{-\alpha T} + Be^{-\beta T}$$

where  $x$  = per cent injected radioactive dose per liter of plasma as aldosterone at time  $T$ .

$B$  is the intercept on the ordinate of the extrapolated part of the curve (semilog plot, Figure 7).  $A + B$  is the corresponding intercept from the earlier part of the curve.  $\beta$  is the slope of the final part of the curve.  $\beta = 1000/T_{1/2}$  (min) (units per day). Both  $B$  and  $\beta$  are calculated from the 50' and 70' values for Figures 2 and 3, from the 30',

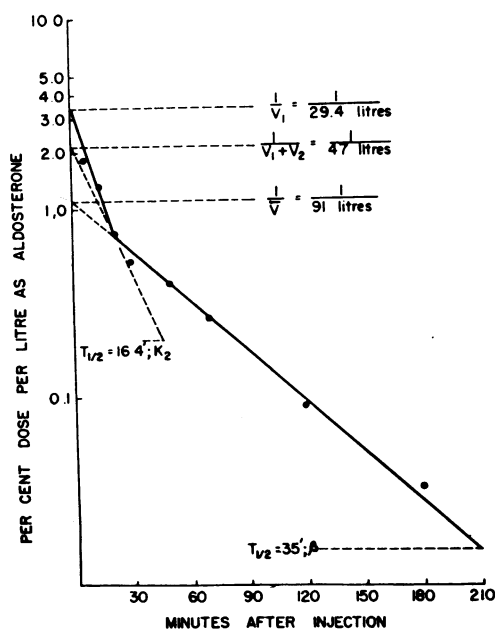


FIG. 5. DISAPPEARANCE OF  $7-H^3$ -ALDOSTERONE IN PLASMA OF NORMAL VOLUNTEER, A. M.

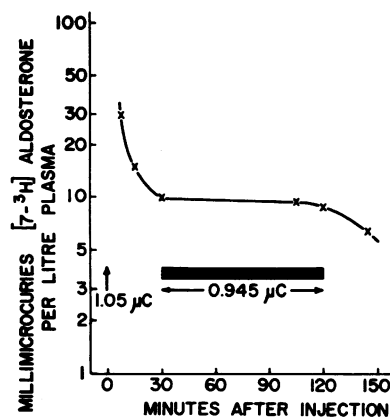


FIG. 6. CONCENTRATION OF  $7-H^3$ -ALDOSTERONE IN PLASMA OF NORMAL SUBJECT, Y. A. Priming dose of 1.05  $\mu C$  followed 30 minutes later by a constant infusion for 90 minutes of a total of 0.945  $\mu C$ .

50' and 70' values for Figure 1 and the 30', 50', 70', 120' and 180' values for Figures 4 and 5;  $\alpha$  is calculated as follows: the latter part of the curve is extrapolated (intercepting the ordinate at  $B$ , Fig. 7) and the resulting calculated plasma concentrations subtracted from the measured values. These corrected concentrations (from 0 to 30 minutes) are plotted semilogarithmically and the slope is  $\alpha$ . Table I shows the calculated values for  $A$ ,  $B$ ,  $\alpha$  and  $\beta$  for the five subjects described. Where possible (Figures 1, 4, 5) these were obtained by the method of least squares.

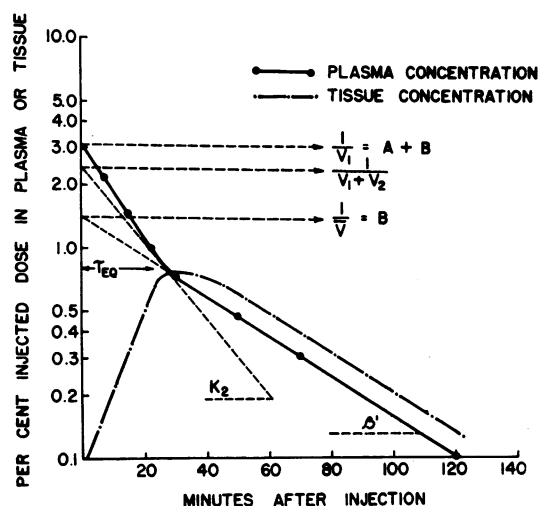


FIG. 7. CONCENTRATION OF RADIOACTIVITY IN INNER POOL (PLASMA) AND OUTER POOL (TISSUE) TO BE EXPECTED FROM MODEL IN FIGURE 8.

TABLE I  
Calculated transport and metabolism rate constants and volumes of distribution of aldosterone  
in five normal women

Subject	A	B	$\alpha$	$\beta$	$K_1$	$K_2$	$V_1$	$V_1+V_2$	$\bar{V}$
	% dose/L				units/day		liters		
Patient E. A.	2.0	1.8	143	40	31.4	65	26.3	35.6	55.5
Patient M. C.	3.0	1.4	128	27.8	37.0	60	22.8	36.8	71
Patient A. A.	3.2	0.89	100	25.6	47.3	60.5	24.2	38.0	112
J. D., normal	1.7	1.4	118	30.8	30.0	53.6	32.3	45.5	71
A. M., normal	2.3	1.1	133	28.5	37.0	61.1	29.4	47.0	91
Mean values	2.4	1.3	124	30.5	36.5	60.0	27.0	40.6	80.1

## DISCUSSION

*Theoretical.* A reasonable model for the transport and metabolism of a steroid in man consists of an inner pool which, for steroids weakly bound to plasma proteins, probably includes the plasma and extracellular volumes, and an outer pool (Figure 8). Transport between the spaces can be described by a rate constant  $K_1$ . If the liver volume is relatively small, and transport from plasma to metabolic cells proceeds at a reasonable rate, it can be considered as part of the inner pool as regards metabolic events.<sup>4</sup> This seems reasonable in view of the fact that molecules much larger than steroids such as albumin are metabolized in the liver and must be transported into the cells. The over-all metabolism of this pool, total volume  $V_1$ , can be described by the rate constant  $K_2$ . The total volume of the outer pool is  $V_2$ . In the present treatment, metabolism in the outer pool is taken as being negligible. This must be assumed, because analyses in the outer pool are at present impossible to obtain and otherwise, solutions of any mathematical treatment would be indeterminate. However, it has been shown that the liver is efficient in metabolizing aldosterone (5-8). Also Davis (9) has recently shown that the rate of metabolism of aldosterone is very low in the hepatectomized dog. Therefore, this assumption is probably reasonable as a first approach for this hormone. If all radioactivity is measured as aldosterone, then:  $K_1$  = fraction of injected dose per milliliter of plasma transported to the outer pool in unit time;  $K_2$  = fraction of injected dose per milliliter of plasma metabolized in the inner pool per unit time;  $x$  = fraction of injected dose

per milliliter of plasma at time  $T$ ;  $z$  = fraction of injected dose per milliliter of outer pool at time  $T$ .

$$V_1 \frac{dx}{dT} = -V_1 K_1 x - V_1 K_2 x + V_1 K_1 z$$

$$V_2 \frac{dz}{dT} = V_1 K_1 x - V_1 K_1 z$$

$$\text{and } x = Ae^{-\alpha T} + Be^{-\beta T}$$

$$z = D(e^{-\alpha T} - e^{-\beta T})$$

$$\text{where } A = \left( \frac{K_1 V_1}{V_2} - \alpha \right) \times \frac{1}{\beta - \alpha};$$

$$B = \left( \frac{\beta - \frac{K_1 V_1}{V_2}}{\beta - \alpha} \right) \times \frac{1}{V_1}$$

$$\text{and } D = \left( \frac{K_1}{V_2} \right) \times \frac{1}{\beta - \alpha}$$

$$\frac{K_1 V_1 \times K_2}{V_2} = \alpha \beta;$$

$$K_1 + K_2 + \frac{K_1 V_1}{V_2} = \alpha + \beta$$

$$\text{and } K_2 = \frac{\alpha \beta (A + B)}{A \beta + B \alpha};$$

$$V_1 + V_2 = \frac{\beta^2 A + B \alpha^2}{(A \beta + B \alpha)^2}$$

$$K_1 = V_2 (A \beta + B \alpha).$$

Figure 7 shows some hypothetical radioactive concentrations in plasma and tissue at various times after injection. As has been pointed out by several investigators in other fields (10-14), the radioactive concentration in the outer pool is lower than that in the inner pool for some time after injection. These concentrations then become equal when that in the outer pool is maximal

<sup>4</sup> However, any reversible spread of steroid into liver tissue after the first few minutes after injection would be considered as transport into the outer pool.

(14). From this time on, the radioactive concentration in the outer pool is greater than in the inner. The final slope of the plasma concentration ( $\beta$ ) is a result of a combination of the effects of metabolism and transport. It will be a flatter slope than would be expected from the effects of metabolism alone ( $\beta < K_2$ ) because continuous transport occurs from the outer pool into the inner pool. The slope of the curve will only be equal to  $K_2$  when the outer and inner radioactive concentrations are equal (at time  $T_{Eq}$ ). Similarly, the intercept on the ordinate from the extrapolated later curve (B) gives a volume ( $\bar{V}$ ) ( $\bar{V} = 1/B$ ) which is not the true volume of distribution. This volume multiplied by the radioactive concentration in the plasma will give the total radioactivity as the hormone at any particular time. However, the radioactive concentration in the outer pool is greater than in the inner, therefore the volume  $\bar{V}$  is greater than the more meaningful volume of distribution ( $V_1 + V_2$ ). This volume ( $V_1 + V_2$ ) multiplied by the blood concentration of steroid will be equal to the total body content of nonisotopic hormone.  $V_1 + V_2$  can be calculated from the expressions given. It is also the reciprocal of the intercept on the ordinate obtained by extrapolating, using the slope  $K_2$ , from the radioactive concentration at the equilibrium time as shown in Figure 7. The line in Figures 1–5 and 7 which intercepts the ordinate at  $1/(V_1 + V_2)$  and has a slope  $K_2$  has been drawn from the calculated values of  $1/(V_1 + V_2)$  and  $K_2$ .  $V_1$  is given by  $1/(A + B)$ . The calculated values of  $V_1$ ,  $V_1 + V_2$ ,  $\bar{V}$ ,  $K_1$  and  $K_2$  for five subjects are shown in Table I.

*General.* After a rapid intravenous injection of 4-C<sup>14</sup>-cortisol into man, the radioactive hormone is distributed extremely quickly into a space of about 6 L (2). After this initial distribution, the radioactivity then appears to move more slowly into a total apparent volume ( $\bar{V}$ ) of about 16 L at 30 minutes after injection. The final slope ( $\beta$ ) can then be described by a half-life of about 80 minutes. Peterson has interpreted this curve as indicating a fairly rapid distribution of cortisol into about a 16 L volume and then assumes that this volume is a single compartment in which the hormone has a metabolic half-life of about 80 minutes. In terms of the model previously described, this

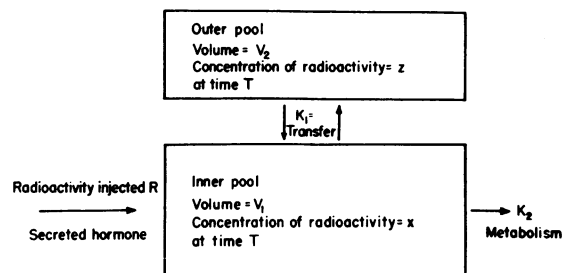


FIG. 8. MODEL DESCRIBING THE METABOLISM AND TRANSPORT OF STEROIDS.

curve could also be interpreted as indicating an extremely rapid distribution into a space of about 6 L ( $V_1$ ) and then a relatively slow movement into a total volume ( $V_1 + V_2$ ) of about 13 L. The smaller volume ( $V_1$ ) can be regarded as a single compartment which has a metabolic half-life of about 30 minutes.

In contrast, there is little doubt that the transport and metabolism of aldosterone requires at least a two-compartmental model for interpretation. For aldosterone there is an initial rapid distribution into a space of 20 to 30 L ( $V_1$ ), which is much greater than the corresponding volume for cortisol. After this there is a slower movement of radioactivity into a total apparent space of 50 to 120 L ( $\bar{V}$ ) or a calculated volume ( $V_1 + V_2$ ) of about 40 L. Whatever the interpretation of the disappearance curve of 4-C<sup>14</sup>-cortisol, undoubtedly aldosterone, both in the initial rapid and later slower phase of movement, distributes itself into much larger volumes than cortisol. Corticosterone behaves in a manner similar to cortisol in this regard (2). As cortisol and corticosterone are strongly bound to a specific plasma protein ("transcortin") (15–17) and aldosterone seems to be much more weakly bound (18), this may be one explanation for the difference in the distributions of the steroids. An analogous situation has been noted for the transport of triiodothyronine and thyroxine (19).

The slope ( $\beta$ ) of the latter part of the curve for the disappearance of 7-H<sup>3</sup>-aldosterone is about 30 ( $T_{1/2} = 33$  minutes) and this is much greater than for cortisol ( $\beta$  value 12.8,  $T_{1/2} = 78$  minutes). This indicates that the rate of metabolism of aldosterone is greater than that of cortisol. But the value does not represent metabolism alone. A more valid method of comparison of the metabo-

lism of steroids would be similar to that adopted by Pearlman (20). Thus, secretion rate =  $M \times$  plasma concentration, where  $M$  can be defined as the turnover rate of the plasma and  $M = V_1 \times K_2$ . This turnover rate ( $M$ ) seems a reasonable measure of the over-all metabolism of a steroid for the purposes of comparison.

The  $M$  value is dependent upon whether a single- or double-compartment model is used for the calculation. If  $M_1$  is the value for the single-compartment model and  $M_2$  is the value for the two-compartment model, then

$$M_1 = \beta \times \bar{V} = \frac{\beta}{B};$$

$$M_2 = V_1 K_2 = \frac{\alpha\beta(A+B)}{A\beta+B\alpha} \times \frac{1}{A+B}$$

$$M_2 = \frac{\alpha\beta}{A\beta+B\alpha} = \frac{\beta}{B} \times \frac{1}{1 + \frac{A}{B} \times \frac{\beta}{\alpha}}$$

$$M_2 = M_1 \times \frac{1}{1 + \frac{A}{B} \times \frac{\beta}{\alpha}}$$

For cortisol the values of  $M_1$  and  $M_2$ , calculated from the data of Peterson (2), are 200 and 173 L per day, respectively.  $M_1$  and  $M_2$  values for aldosterone differ by a much larger factor. Using the mean value for normal subjects (Table I),  $M_2 = 1,620$  and  $M_1 = 2,370$ . It is therefore necessary to use the  $M_2$  value for calculations of the turnover rate of aldosterone.

However, whatever model is used, there is little doubt that the turnover rate for aldosterone is much greater than that of cortisol. The turnover rates of cortisol and corticosterone are similar (2). As pointed out by Bartter and Mills (21), the difference in the turnover rates of the three hormones could be related to the lower binding of aldosterone to plasma proteins compared with that of the other two hormones. This may result in a greater speed of movement of aldosterone from the plasma into the metabolic cells of the liver. In addition, aldosterone is metabolized to the "3-oxo conjugate" which may be more rapidly formed than the usual tetrahydro metabolites of cortisol.

A knowledge of the turnover rate can be used to calculate the mean plasma concentration from the secretion rate of the hormone, obtained from

TABLE II

*Calculated values for turnover rates ( $M_2$ ), secretion rates and mean plasma concentration in five normal women*

Subject	$M_2$	Secretion rate	Mean plasma conc.
		L/day	$\mu\text{g}/100 \text{ ml plasma}$
Patient E.A.	1,710	255	0.0149
Patient M.C.	1,370	59	0.0043
Patient A.A.	1,480	103	0.0069
J.D., normal	1,730	45	0.0026
A.M., normal	1,810	179	0.0099
Mean value	1,620	128	0.0077

the specific activity of a urinary metabolite. Table II shows the values for the secretion rates, turnover rates and the calculated mean daily plasma concentration for the five subjects investigated. The average plasma concentration was 0.0077 (0.0026 to 0.015)  $\mu\text{g}$  per 100 ml plasma. The values for  $M_2$  are fairly constant compared with the variation in secretion rates. In previous studies of 11 normal women the average secretion rates have been estimated as 140 (50 to 235)  $\mu\text{g}$  per day (3, 22). Using the mean value for  $M_2$  obtained in the present studies, the mean plasma concentration can be estimated as 0.009  $\mu\text{g}$  per 100 ml plasma. The plasma concentrations by direct methods (1) have given higher values; but these samples were all drawn in the morning and it is now known that there is a diurnal variation in the excretion of aldosterone (23) for subjects engaged in normal activity. Hence, 0.01  $\mu\text{g}$  per 100 ml plasma may be a reasonable mean daily concentration. In a preliminary study on one patient (1) an assumption was made that the volume of distribution of aldosterone was of the same order as that of cortisol. The  $M_1$  value was calculated to be 800. The present studies using two compartments show this to be in error. The difference between the calculated  $M$  values of 800 and the present 1,620 emphasize the importance of calculating the volumes of distribution in more than one compartment.

The values of the turnover rate are not dependent (to within 40 per cent error) on whether a single or more complex model is used to interpret the results. But the penetration of steroids into various tissues, which is important in considering their mode of action, is critically dependent upon which model is adopted. Thus, the vol-

time of distribution of aldosterone according to the single-compartment model is about 80 L ( $\bar{V}$ ) for some subjects (Table I). As this is greater than the total body water volume, the data suggest that a considerable amount of steroid is held in tissues at a concentration greater than in the plasma. However, the appropriate volume given by the two-compartment model is 40 L ( $V_1 + V_2$ ). This smaller value does not require this explanation. Similar considerations hold for calculated volumes of distribution of cortisol, where  $\bar{V}$  is 16 but  $V_1 + V_2$  is 13 L. The dangers of regarding the final slope of the curve as being entirely due to metabolism are also illustrated in this study, as the calculated  $K_2$  value for aldosterone is about one-half the final slope  $\beta$ .

However, although the two-compartment model has the advantage of giving determinate values for the volumes of distribution and also for  $K_1$  and  $K_2$ , it is entirely possible that a more complex model is appropriate. It could be that there are additional compartments (such as the cerebrospinal fluid) into which the steroid slowly diffuses. In this case, the curve would change in slope with time even after 50 minutes. There is no evidence that this occurs from the data obtained (Figures 4 and 5). If such diffusion did occur, the calculated  $K_2$  values and turnover rate would be too high. Also, if metabolism occurred in the outer pool, the turnover rate calculated from the single-injection data would again be inaccurate, and the  $\beta$  value would more truly reflect the over-all metabolic processes.

The validity of the calculations for a two-compartment system have been tested independently by the results observed from a constant infusion experiment. After the initial priming dose, steroid has been continuously infused. The radioactivity in the plasma measured specifically as the steroid remained constant.

Considering  $i \equiv$  the final constant radioactivity of the steroid per liter of plasma and  $C \equiv$  rate of infusion of radioactivity as steroid per day, then  $C/i \equiv$  turnover rate  $M$ . During the constant infusion of the radioactive steroid for 90 minutes after a priming dose, the radioactivity was found to be constant within the limits of experimental error (Figure 6). The  $M$  value calculated from the final plasma radioactive concentrations was

found to be 1,700 L per day which agrees with the  $M_2$  value (mean 1,620 L per day) calculated from the single-injection data using the two-compartment model as previously specified.

It therefore appears that the value of the turnover rate calculated from the data obtained after a single injection of radioactive hormone

$$\left( M_2 = V_1 K_2 = \frac{\alpha \beta}{A \beta + B \alpha} \right)$$

is valid. It should now be possible by using the methods described here to compare the mean concentrations and over-all rates of metabolism of aldosterone in various clinical conditions.

#### SUMMARY

Studies are described on the disappearance of the radioactivity in plasma after the injection of 0.1  $\mu$ g, 2  $\mu$ c of 7-H<sup>3</sup>-*d*-aldosterone into five normal woman. The radioactivity was measured specifically as aldosterone and followed for 70 minutes in three subjects and for 180 minutes in two subjects.

The disappearance curve was similar in all normal subjects studied. There was an extremely rapid initial drop in plasma radioactive concentration to less than 5 per cent of the dose per L of plasma immediately after injection. The curve then became less steep, with a half-life of about 14 minutes, until about 30 minutes after injection. At this time, the plasma concentration was about 1 per cent of the dose per L of plasma. The curve then flattened again and became a straight line (semilog plot) at 30 minutes for most subjects. The half-life of this slope was about 35 minutes. From these data it can be concluded that aldosterone spreads into a much greater volume and its rate of metabolism is higher than that of cortisol and corticosterone.

As a result of mathematical analysis in terms of a two-compartment model, the data have been interpreted to mean that aldosterone spreads rapidly into a volume of 25 L and then more slowly to 40 L. The initial volume has a metabolic half-life of about 15 minutes.

The mean turnover rate of aldosterone in unit volume of plasma, 1,620 L per day, has been calculated from these data. Combining this value with the concomitant mean secretion rate estimates



(urinary metabolite method) of 128  $\mu\text{g}$  per day, the mean plasma concentration of aldosterone for the five normal subjects has been calculated to be 0.0077  $\mu\text{g}$  per 100 ml of plasma.

The turnover rate has been confirmed by a constant infusion experiment on one woman.

#### REFERENCES

1. Ayres, P. J., Garrod, O., Tait, S. A. S., Tait, J. F., Walker, G., and Pearlman, W. H. The use of [ $16\text{-}^3\text{H}$ ] aldosterone in studies on human peripheral blood. *Ciba Found. Coll. Endocr.* 1957, **11**, 309.
2. Peterson, R. E. The miscible pool and turnover rate of adrenocortical steroids in man. *Recent Progr. Hormone Res.* 1959, **15**, 231.
3. Flood, C., Layne, D. S., Ramcharan, S., Rossipal, E., Tait, J. F., and Tait, S. A. S. An investigation of the urinary metabolites and secretion rates of aldosterone and cortisol in man and a description of methods for their measurements. *Acta endocr. (Kbh.)* In press.
4. Bush, I. E. Methods of paper chromatography of steroids applicable to the studies of steroids in mammalian blood and tissues. *Biochem. J.* 1952, **50**, 370.
5. Albeaux-Fernet, M., and Romani, J. D. Sur la transformation de l'aldostérone en métabolites polaires sous l'action d'homogénats de foie. *C. R. Soc. Biol. (Paris)* 1958, **152**, 428.
6. Albeaux-Fernet, M., and Romani, J. D. Identification préliminaire des dérivés de l'aldostérone; confrontation des données de l'incubation *in vitro* et de celles des fractionnements des corticoïdes urinaires. *Ann. Endocr. (Paris)* 1958, **19**, 384.
7. Reaven, G. M. A comparison of the action of human and rat liver slices on several corticosteroids. *Endocrinology* 1955, **57**, 580.
8. Romani, J. D., Bessard, C., Sosa-Costellanos, J., and Keller, A. Nouvelles investigations sur la métabolisation *in vitro* de l'aldostérone. *Ann. Endocr. (Paris)* 1959, **20**, 299.
9. Davis, J. O. Mechanisms regulating the secretion and metabolism of aldosterone in experimental secondary hyperaldosteronism. *Recent Progr. Hormone Res.* In press.
10. Campbell, R. M., Cuthbertson, D. P., Matthews, C. M., and McFarlane, A. S. Behaviour of  $^{14}\text{C}$ - and  $^{131}\text{I}$ -labelled plasma proteins in the rat. *Int. J. appl. Radiat.* 1956, **1**, 66.
11. Dominguez, R. Kinetics of elimination, absorption and volume of distribution in the organism in *Medical Physics*, O. Glasser, Ed. Chicago, Year Book Publishers, 1950, vol. 2, p. 476.
12. Skinner, S. M., Clark, R. E., Baker, N., and Shipley, R. A. Complete solution of the three-compartment model in steady state after single injection of radioactive tracer. *Amer. J. Physiol.* 1959, **196**, 238.
13. Veall, N., and Vetter, H. Radioisotope Techniques in Clinical Research and Diagnosis. London, Butterworth, 1958, p. 184.
14. Zilversmit, D. B., Entenman, C., and Fishler, M. C. On calculation of "turnover time" and "turnover rate" from experiments involving use of labeling agents. *J. gen. Physiol.* 1943, **26**, 325.
15. Bush, I. E. The physiochemical state of cortisol in the blood. *Ciba Found. Coll. Endocr.* 1957, **11**, 263.
16. Slaunwhite, W. R., Jr., and Sandberg, A. A. Transcortin: A corticosteroid-binding protein of plasma. *J. clin. Invest.* 1959, **38**, 384.
17. Daughaday, W. H. Binding of corticosteroids by plasma proteins. V. Corticosteroid-binding globulin activity in normal human beings and in certain disease states. *A.M.A. Arch. intern. Med.* 1958, **101**, 286.
18. Mills, I. H. Discussion in Peterson, R. E. The miscible pool and turnover rate of adrenocortical steroids in man. *Recent Progr. Hormone Res.* 1959, **15**, 261.
19. Pitt-Rivers, R., and Tata, J. R. The Thyroid Hormones. London, Pergamon Press, 1959, p. 133.
20. Pearlman, W. H. Circulating steroid hormone levels in relation to steroid hormone production. *Ciba Found. Coll. Endocr.* 1957, **11**, 233.
21. Bartter, F. C., and Mills, I. H. Personal communication, 1959.
22. Jones, K. M., Lloyd-Jones, R., Riondel, A., Tait, J. F., Tait, S. A. S., Bulbrook, R. D., and Greenwood, F. C. Aldosterone secretion and metabolism in normal men and women and in pregnancy. *Acta endocr. (Kbh.)* 1959, **30**, 321.
23. Muller, A. F., Manning, E. L., and Riondel, A. M. Diurnal variation of aldosterone related to position and activity in normal subjects and patients with pituitary insufficiency in *An International Symposium on Aldosterone*, A. F. Muller and C. M. O'Connor, Eds. London, J. & A. Churchill, 1958, p. 111.