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A Comparison of the Metabolism of Radioactive 17-Isoaldosterone and Aldosterone Administered Intravenously and Orally to Normal Human Subjects *

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Summary. After intravenous and oral administration of radioactive aldosterone to normal subjects, 7.3 ± 0.4 (SE) and 5.4 ± 0.5 (SE) %, respectively, of the dose was recovered from a 48-hour collection of urine as aldosterone released by mild acid hydrolysis (from aldosterone 18-glucuronide), and 35 ± 5 (SE) and 39 ± 4 (SE) %, respectively, was recovered as tetrahydroaldosterone after incubation with β-glucuronidase.

After intravenous and oral administration of 17-isoaldosterone-4-^{14}C to a similar group of subjects, 35 ± 3 (SE) and 53 ± 4 (SE) %, respectively, of the dose was recovered as 17-isoaldosterone released by acid and less than 5% as total metabolites after incubation with β-glucuronidase. No detectable radioactivity (< 0.5%) could be recovered as tetrahydroaldosterone or as a compound with the expected chromatographic properties of tetrahydro-17-isoaldosterone.

The total radioactivity in the neutral extracts was also relatively small (< 2%) after administration of either labeled aldosterone or 17-isoaldosterone. The radioactivity as aldosterone in the neutral extract was much lower after oral [0.017 ± 0.003 (SE) %] than after intravenous [0.21 ± 0.04 (SE) %] administration of labeled aldosterone. The radioactivity as 17-isoaldosterone in the neutral extract was similar after intravenous [0.20 ± 0.02 (SE) %] and after oral [0.38 ± 0.18 (SE) %] administration of 17-isoaldosterone.

These results indicated that, due to lack of A-ring reduction of the molecule and the consequent slowing of hepatic clearance, 17-isoaldosterone is converted to an acid-labile conjugate (presumably 17-isoaldosterone 18-glucuronide) as the major metabolite. 17-Isoaldosterone was not secreted or converted to aldosterone to any significant extent in the normal subjects investigated.

Introduction

A batch of radioactive material obtained from a commercial source as aldosterone-4-^{14}C has been found to contain over 90% 17-isoaldosterone-4-^{14}C.[1] By preliminary investigation the radioac-

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[1] Trivial names: 17-isoaldosterone = 11β,21-dihydroxy-18-oxo-17-isopregn-4-en-20-one; tetrahydroaldosterone = 3α,11β,21-trihydroxy-18-oxo-5β-pregnan-20-one; tetrahydroaldosterone glucuronide = 3α,21-dihydroxy-11 : 18-oxido-5β-pregnan-20-one-18-yl-β,D-glucopyranosiduronic acid; corticosterone etioacid = 11β-hydroxyandrost-4-en-3-one-17β-carboxylic acid; tetrahydro-17-isoaldosterone = 3α,11β,21-trihydroxy-18-oxo-17-iso-5β-pregnan; tetrahydro-17-isoaldosterone glucuronide = 3α,21-dihydroxy-11 : 18-oxido-17-iso-5β-pregnan-20-one-18-yl-β,D-glucopyrano-

tive compound in the material had approximately the same running properties as aldosterone on both Kieselguhr column and paper chromatography using the Bush B5 solvent system (1). Only one major peak containing ^{14}C was found with either chromatographic method. No other peak in the column eluates had more than 2.5% of the total radioactivity. The radioactive compound in the pooled fractions of the major peak was administered orally with an intravenous injection of aldosterone-7-^{3}H to normal subjects. This was part of a study on the metabolism of aldosterone using an approach similar in principle to that employed by Bledsoe and co-workers (2).

It was found that the pattern of excretion of radioactivity in the various urinary metabolites after administration of the ^{14}C compound was markedly different from that previously found after oral administration of authentic aldosterone-4-^{14}C although the metabolism of aldosterone-7-^{3}H given intravenously to the same subjects was not unusual.

Subsequent investigation showed that the material supplied was over 90% 17-isoaldosterone-4-^{14}C and contained only about 2% aldosterone-4-^{14}C. The running speed of 17-isoaldosterone is slightly but definitely slower than that of aldosterone on column chromatography using the Bush B5 solvent system. Because of this difference in the properties of the two compounds on column chromatography, the initial purification had eliminated the trace of aldosterone-4-^{14}C originally present, and the administered material was pure 17-isoaldosterone-4-^{14}C. We have previously observed radioactivity with the properties of 17-isoaldosterone in preparations of radioactive aldosterone, particularly after prolonged storage (3). Schmidlin and co-workers (4) described its formation after hydrolysis of aldosterone 21-monoacetate with strongly alkaline solutions or after similar treatment of other aldosterone derivatives (4). However, in such impure preparations the 17-isoaldosterone is usually seen as a minor secondary peak close to but clearly separable from the primary peak of aldosterone in the Bush B5 column eluates. The predominance of 17-isoaldosterone in the material supplied actually prevented its immediate recognition by preliminary investigation, as the peak positions of a particular compound tend to vary slightly on different columns and, in order to obtain high specific activity material, we considered it undesirable to add carrier aldosterone as a marker for the purification.

Orally administered 17-isoaldosterone-4-^{14}C was excreted almost quantitatively as an acid–labile conjugate and to a negligible extent as metabolites released from the urine by incubation with β-glucuronidase. This is of interest because of the comparison with the metabolism of aldosterone, where the excretory pattern is almost reversed, and in view of the known difference in structure of the two 17-isomers.

siduronic acid aldosterone γ-lactone: 18-hydroxy-11β: 18-oxidoandrost-4-en-3-one-17β-carboxylic acid (18:20-lactone); 17-isoaldosterone etioacid = 11β,21-dihydroxy-18-oxo-androst-4-ene-17α-carboxylic acid.

Methods

All the radioactive compounds administered[2] were finally purified on a 60-cm column 1 cm in diameter with 28 g Celite and 14 ml methanol–water (1:1 vol:vol) as the stationary phase. Benzene was the mobile phase. Details of the column and the source and purification of the solvents have previously been published (5). Five-ml fractions were collected, and, in all cases, the two fractions containing the major peak of radioactivity were pooled and the other eluted material was discarded.

After purification of aldosterone-7-^{3}H and aldosterone-4-^{14}C in this manner, nonradioactive aldosterone was added to a sample that was then run on a Bush B5 paper chromatogram and scanned for radioactivity with a Vanguard model 880 windowless flow counter both at high and low sensitivities. Another sample with carrier was acetylated and run on a Bush B3 paper system. In both cases, only one radioactive peak coincident with the carrier was observed, and less than 1% of the total radioactivity ran at the position of 17-isoaldosterone diacetate ($R_{\text{aldosterone diacetate}}$ 1.5)[3] on the Bush B3 paper.

In a similar investigation of the preparation of 17-isoaldosterone-4-^{14}C after column chromatography and ad-

[2] Aldosterone-7-^{3}H and aldosterone-4-^{14}C were obtained from the Endocrine Study Section, National Institutes of Health. 17-Isoaldosterone-4-^{14}C was obtained from New England Nuclear Corp., Boston, Mass., who also prepared the aldosterone-4-^{14}C for the Endocrine Study Section. The reason for the difference in the preparations seems to be the use of alkaline rather than enzymatic hydrolysis in preparing the batch that contained a high proportion of 17-isoaldosterone-4-^{14}C. However, individual vials may vary in the proportion of the isomers, so that storage conditions may also be important.

[3] R values were calculated as the following: paper chromatography, R_x 0.3 = R_F of compound of interest/R_F of standard compound x; column chromatography, R_x 0.3 = retention volume of compound x/retention volume of compound of interest.

dition of carrier 17-isoaldosterone[4] to a portion of the pooled peak fractions, we found that there was a single peak of radioactivity coincident with authentic 17-isoaldosterone, both as the free compound on the Bush B5 ($R_{aldosterone} = 0.77$) and as the diacetate on the Bush B3 paper. Another sample of the free radioactive compound with carrier was chromatographed on the propylene glycol–toluene paper system for 60 hours, and again the radioactivity ran with authentic 17-isoaldosterone ($R_{aldosterone} = 0.8$) (6). A scan of these three paper chromatograms at high sensitivity showed that less than 1% aldosterone-4-^{14}C was present. Examination of the crude supplied material by the same methods revealed about 2% aldosterone-4-^{14}C. About 2 μg of the 17-isoaldosterone-4-^{14}C or its acetyl derivatives was chromatographed on the same three systems without carrier and showed positive ultraviolet absorption at 254 mμ, soda fluorescence, and rapid tetrazolium reaction. All reacting areas were coincident with the radioactive peak and had equivalent running speeds to standards of 17-isoaldosterone and the corresponding derivative chromatographed in parallel.

After oxidation of the purified 17-isoaldosterone-4-^{14}C and partitioning of the product between an alkaline aqueous solution and methylene dichloride, only 2.1% of the radioactivity remained in the organic solvent (7). Under similar conditions, aldosterone-4-^{14}C was mainly converted to the γ-lactone (8), and 87% of the original radioactivity was extracted into the methylene dichloride. After neutralization of the alkaline solution containing the oxidation product of 17-isoaldosterone-4-^{14}C [presumably 17-isoaldosterone etioacid (7)], 89% of the original radioactivity was extracted with methylene dichloride. This radioactive compound had a running speed of R_f 0.04 ($R_{corticosterone\ etioacid} = 0.11$, and $R_{aldosterone\ \gamma\text{-}lactone} = 0.08$) in the paper system 75% glacial acetic acid/H_2O, cyclohexane/benzene 2:1 (9), and the resultant single radioactive peak found on scanning coincided with the soda fluorescence of the product from the authentic 17-isoaldosterone carrier. The product from about 3 μg of 17-isoaldosterone-4-^{14}C put through the same procedure without carrier gave ultraviolet absorption ($\lambda = 254$ mμ) and positive soda fluorescence that coincided with the radioactive peak, but only a slow and weak tetrazolium reaction. About 90% of the radioactivity (2% of the total) that remained in the methylene dichloride after the alkaline partition of the oxidation product of 17-isoaldosterone ran slower than aldosterone γ-lactone in the Bush B3 system but together with 17-isoaldosterone etioacid in the acid paper system. Only 0.2% of the total ran with aldosterone γ-lactone.

The counts per minute due to ^{3}H and ^{14}C in a mixture of the aldosterone-4-^{14}C and the aldosterone-7-^{3}H were measured before and after conversion to the diacetate and purification of the derivative by column chromatography. There was no significant difference in the measured ratios of ^{3}H to ^{14}C in the pooled fractions.

[4] We are grateful to Drs. J. Schmidlin and A. Wettstein, Ciba Pharmaceuticals, Basel, Switzerland, for a generous gift of nonisotopic 17-isoaldosterone.

We concluded, therefore, that aldosterone-4-^{14}C, aldosterone-7-^{3}H, and 17-isoaldosterone-4-^{14}C prepared after column chromatography were of satisfactory radiochemical purity for administration to the normal human subjects.

The 17-isoaldosterone-4-^{14}C (0.86 μc, 6.1 μg) or aldosterone-4-^{14}C (0.86 μc, 6.1 μg) was made up in a 10% ethanolic solution of water for oral administration. This dose was swallowed and then immediately washed down with 6 ml water. The aldosterone-7-^{3}H (4 μc, 0.044 μg) was administered intravenously in 10 ml 6% ethanolic solution of physiological saline. 17-Isoaldosterone-4-^{14}C was given in the same solution as the aldosterone-7-^{3}H when injected intravenously. The radioactive steroids were given to normal male and female volunteers, ranging in age from 25 to 40 years, who gave their informed verbal consents to the procedure and were on ad libitum electrolyte intakes. In all experiments, the aldosterone-7-^{3}H was given intravenously. Simultaneously, 17-isoaldosterone-4-^{14}C or aldosterone-4-^{14}C was given orally, or 17-isoaldosterone-4-^{14}C was given in the same intravenous solution (Table I).

Usually, urine was collected for 48 hours after administration of the radioactive compounds. A 1-L sample was extracted without pH adjustment with methylene dichloride (neutral extract), hydrolyzed by standing at room temperature (23° C) at pH 1 for 16 hours, and again extracted with methylene dichloride (acid extract) (5). Another 166-ml portion was hydrolyzed with β-glucuronidase and then extracted with ethyl acetate (enzyme glucuronide extract) (5). A portion of these total extracts was assayed for radioactivity with corrections for quenching by the internal standard method for both isotopes (5).

The residual neutral extract was acetylated after addition of carrier aldosterone and run on a partition column suitable for the purification of aldosterone diacetate (5). 17-Isoaldosterone diacetate ran faster than aldosterone diacetate in this system ($R_{aldosterone\ diacetate} = 1.8$). The remaining acid fraction without added carrier underwent column chromatography without acetylation on a system suitable for the purification of aldosterone. 17-Isoaldosterone was eluted later than aldosterone in this system ($R_{aldosterone} = 0.8$). The enzymic glucuronide extract was chromatographed on a column suitable for the purification of tetrahydroaldosterone (5). The characteristics of tetrahydro-17-isoaldosterone are unknown in this system, but it might be expected that the effective partition coefficient would be of the same order of magnitude as that of tetrahydroaldosterone. Fractions were examined with up to twice the retention volume of tetrahydroaldosterone. 17-Isoaldosterone had $R_{tetrahydroaldosterone} = 2.0$ on this column system, and aldosterone had $R_{tetrahydroaldosterone} = 2.5$. Samples of the column fractions were assayed for both ^{14}C and ^{3}H, and the nonisotopic compounds were located by paper chromatography and soda fluorescence or blue tetrazolium reaction. The per cent radioactivity as aldosterone and 17-isoaldosterone in the neutral and acid extracts and these compounds plus tetrahydroaldosterone or similar

TABLE I

Radioactivity from urine as aldosterone and 17-isoaldosterone in the neutral and acid extracts and as tetrahydroaldosterone released by β-glucuronidase after an intravenous dose of aldosterone-7-^{3}H and an oral dose of aldosterone-4-^{14}C or an oral or intravenous dose of 17-isoaldosterone-4-^{14}C

Subject (sex)	^{3}H per cent intravenous dose of aldosterone-7-^{3}H			^{14}C per cent oral dose of aldosterone-4-^{14}C		
	Aldosterone in neutral extract	Aldosterone in acid extract	Tetrahydroaldosterone in β-glucuronidase extract	Aldosterone in neutral extract	Aldosterone in acid extract	Tetrahydroaldosterone in β-glucuronidase extract
C.F. (M)	0.22	6.3	58	0.015	4.3	56
N.G. (M)	0.17	7.2	32	0.013	4.5	36
T.B. (M)	0.20	10.1	31	0.022	7.3	40
A.D. (M)	0.16	5.2		0.027	4.3	
A.G. (M)	0.10	5.4		0.003	4.3	
C.B. (M)	0.06	7.2	39	0.006	6.9	47
H.M. (M)	0.27	8.4	19	0.016	5.1	24
J.F. (F)	0.29	5.2	25	0.020	3.7	27
P.W. (F)	0.42	9.6	41	0.029	7.8	44
Mean ± SE	0.21 ± 0.04	7.2 ± 0.6	35 ± 5	0.017 ± 0.003	5.4 ± 0.5	39 ± 4
	Per cent intravenous dose of aldosterone-7-^{3}H			Per cent oral dose of 17-isoaldosterone-4-^{14}C		
	Aldosterone in neutral extract	Aldosterone in acid extract	Tetrahydroaldosterone in β-glucuronidase extract	17-Isoaldosterone in neutral extract	17-Isoaldosterone in acid extract	17-Isotetrahydroaldosterone in β-glucuronidase extract
D.P. (M)	0.17	6.9	37	0.34	63	<0.5
A.G. (M)	0.18	6.2	30	0.031	49	<0.5
J.M. (M)	0.20	8.3	28	0.10	63	<0.5
S.C. (F)	0.18	8.5	14	0.81	57	<0.5
J.P. (F)	0.20	8.4	33	0.47	48	<0.5
R.P. (F)	0.11	6.8 (0.96)*	26	0.19	36 (2.6)	<0.5
Mean ± SE	0.17 ± 0.01	7.5 ± 0.4	28 ± 3	0.32 ± 0.18	53 ± 4	<0.5
	Per cent intravenous dose of aldosterone-7-^{3}H			Per cent intravenous dose of 17-isoaldosterone-4-^{14}C		
	Aldosterone in neutral extract	Aldosterone in acid extract	Tetrahydroaldosterone in β-glucuronidase extract	17-Isoaldosterone in neutral extract	17-Isoaldosterone in acid extract	17-Isotetrahydroaldosterone in β-glucuronidase extract
D.P. (M)	0.33	7.4	38	0.22	45	<0.5
A.G. (M)	0.17	4.8	38	0.15	34	<0.5
A.D. (M)	0.14	4.0	38	0.16	26	<0.5
I.N. (M)	0.21	11.4	39	0.21	37	<0.5
S.K. (M)	0.44	7.7	29	0.26	35	<0.5
Mean ± SE	0.26 ± 0.06	7.1 ± 1.3	36 ± 2	0.20 ± 0.02	35 ± 3	<0.5
Total subjects mean	0.21 ± 0.02	7.3 ± 0.4	33 ± 2			

* Values in parentheses for subject R.P. are after a repeated 15–hour hydrolysis at pH 1 on the same sample.

compounds, such as tetrahydro-17-isoaldosterone, in the enzymic glucuronide extract was estimated.

In one experiment, a 1–L sample from a 48–hour collection after intravenous injection of aldosterone-7-^{3}H and oral administration of 17-isoaldosterone-4-^{14}C was hydrolyzed at pH 1 and at room temperature for 0 to 15 and 15 to 30 hours, and the extracts in the two time intervals were analyzed for the radioactive steroids (Table II). In two experiments, after intravenous administration of both aldosterone-7-^{3}H and 17-isoaldosterone-4-^{14}C, portions from 0– to 6–, 6– to 12–, and 0– to 48–hour samples of urine were analyzed for aldosterone in the acid extract (Table II). In two experiments, 17-isoaldosterone-4-^{14}C was added to some normal nonradioactive urine after extraction at neutral pH and carried through the column chromatography of the acid extract, and its over–all recovery was estimated.

In an experiment in which two subjects had been injected intravenously with 17-isoaldosterone-4-^{14}C, samples of their pooled fractions that contained 17-isoaldos-

TABLE II

Urinary excretion of intravenous doses of aldosterone-7-^{3}H or 17-isoaldosterone-4-^{14}C as compound released from 18-glucuronide in 0– to 6–, 6– to 12–, and 0– to 48–hour collections

Subject (sex)	Per cent intravenous dose of aldosterone-7-^{3}H as aldosterone in acid extract			Per cent intravenous dose of 17-isoaldosterone-4-^{14}C as 17-isoaldosterone in acid extract		
	0 to 48 hours	0 to 6 hours	6 to 12 hours	0 to 48 hours	0 to 6 hours	6 to 12 hours
I.N. (M)	11.4	9.8	0.1	37	36	0.5
S.K. (M)	7.7	8.2	0.1	35	31	0.6

terone after column chromatography of the acid extract were acetylated. The amounts of 17-isoaldosterone diacetate were then estimated after paper chromatography (Bush B3 system run for 3½ hours) by assaying soda fluorescence directly on paper against standards of 17-isoaldosterone diacetate (5). The 17-isoaldosterone acetylates quantitatively under these conditions, and the radioactivity as the conjugate is excreted completely in 48 hours; therefore, the specific activity and urinary production rate of the 17-isoaldosterone could be calculated. The production rate was corrected for the micrograms injected.

In all experiments, ^{14}C and ^{3}H counts per minute were estimated by liquid scintillation counting with channel discrimination as previously described (5). The vials were usually counted for 40 minutes. The counts per minute was usually high enough so that better than 5% statistical accuracy was achieved, except as discussed below. The ^{14}C counts per minute as tetrahydroaldosterone or tetrahydro-17-isoaldosterone after administration of 17-isoaldosterone-4-^{14}C was negligible, and a maximal yield of 0.5% was calculated from the usual recovery and counting efficiency. The ^{14}C counts per minute as aldosterone (peak fraction of aldosterone diacetate from the column) in the neutral extract was low, being on the average about 4 (^{3}H in the peak fraction averaged 200 cpm). The statistical accuracy of the ratio (K_F) of ^{14}C to ^{3}H for aldosterone in the neutral extract was therefore about 25%. The splanchnic extraction (H) can be calculated as $(1 - K_F)$ 100% (2). For normal subjects with greater than 90% extraction, the standard error in the estimation of H is therefore maximally 3%. For subjects with lower extractions, the ^{14}C counts per minute as aldosterone was higher, and the calculated per cent error in the estimation of H was about the same as for normal subjects.

Results

After intravenous injection of aldosterone-7-^{3}H, the mean per cent ^{3}H radioactive dose found as aldosterone (isolated as the diacetate after acetylation from the neutral extract) was 0.21 ± 0.02 (SE) %; as aldosterone from the acid extract, 7.3 ± 0.4 (SE) %; and as tetrahydroaldosterone from the enzymatic glucuronide extract, 33 ± 2 (SE) % (18 subjects) (Table I). These values were not significantly different in the three groups of subjects, i.e., those simultaneously given an oral dose of aldosterone-4-^{14}C or 17-isoaldosterone-4-^{14}C or an intravenous dose of 17-isoaldosterone-4-^{14}C (Table I). They were also similar to those reported for normal subjects in other studies (2, 5, 10, 11) if appropriate corrections are made for recoveries. The recovery of aldosterone added to the urine after the neutral extraction and then isolated from the acid extract under the conditions used for our studies is about 70% (12). The aldosterone released from the acid–labile conjugate by a 16–hour hydrolysis at pH 1, corrected for losses in the isolation of the released aldosterone but not for inefficiency of hydrolysis, was therefore about 10% of the injected intravenous dose.

After oral administration of aldosterone-4-^{14}C, the amount recovered from the urine as tetrahydroaldosterone after hydrolysis with β-glucuronidase ($39 \pm 4\%$) is similar to that found after intravenous administration of aldosterone-7-^{3}H ($35 \pm 5\%$) (Table I). However, the per cent of the oral dose recovered as aldosterone in the neutral extract ($0.017 \pm 0.003\%$) is greatly reduced (by a factor of about 12) compared to that found after intravenous administration ($0.21 \pm 0.04\%$); the per cent of the oral dose released by acid hydrolysis as aldosterone was $5.4 \pm 0.5\%$, which is also significantly lower ($p = 0.05$) than the corresponding value after intravenous administration (7.2 ± 0.6), but by a factor of only 1.3. After correction for losses, 7.7% of the oral dose of aldosterone-4-^{14}C was calculated to have been released by acid hydrolysis.

After oral administration of 17-isoaldosterone-4-^{14}C to the same group of normal subjects, $53 \pm 4\%$ of the dose was recovered as 17-isoaldosterone from the acid extract (Table I). Of the 17-isoaldosterone-4-^{14}C added to the urine after the neutral extraction, 63 and 67% (mean 65) was recovered in the appropriate column fractions after chromatography of the acid extract. Therefore, after correction for losses, 82% of the oral dose was found as 17-isoaldosterone after acid hydrolysis of the urine. Negligible radioactivity appeared in the position of aldosterone in the column fractions. After acetylation of a sample of the mea-

sured eluted fractions, the radioactivity ran with 17-isoaldosterone diacetate on the Bush B3 paper system (run for 3½ hours). The total acid extract contained from 45 to 89% (mean 69) of the dose.

Little radioactivity (< 5% total) was found in the fractions from the column after chromatography of the enzymic glucuronide extract after oral administration of 17-isoaldosterone-4-^{14}C, and less than 0.5% was found in positions equivalent to or eluted more slowly than tetrahydroaldosterone (Table I). A sample of the total enzyme glucuronide extract also showed little radioactivity (1.8 to 3.7%, mean 2.8). These results showed that only very small amounts, if any, of the administered 17-isoaldosterone were excreted as tetrahydroaldosterone or the corresponding 17-tetrahydroisoaldosterone glucuronide.

After oral administration of 17-isoaldosterone-4-^{14}C, only small amounts of radioactivity as the original compound were found in the neutral extract (0.03 to 0.81%, mean 0.32 ± 0.18); however, these values are considerably higher than those obtained as aldosterone in the neutral extract after oral administration of aldosterone-4-^{14}C (0.017 ± 0.003%). The radioactivity was in the position of 17-isoaldosterone diacetate after chromatography of the acetylated extract. The ^{14}C in the total neutral extract was also small (< 1% for all samples).

After intravenous injection of 17-isoaldosterone-4-^{14}C, 0.20 ± 0.02% of the dose was 17-isoaldosterone in the neutral extract; this is similar to the amount found after oral administration of the same steroid (0.38 ± 0.18%) or the amount found as aldosterone in the neutral extract after intravenous injection of aldosterone (0.21 ± 0.02%). Less than 0.5% was detected as tetrahydroaldosterone or "17-isotetrahydroaldosterone" in the enzymic glucuronide extract after intravenous 17-isoaldosterone. The radioactivity in the corresponding total extracts was also low in the neutral (< 1%) and enzymatic glucuronide extracts (< 5%, mean 3.8%).

After intravenous injection of 17-isoaldosterone-4-^{14}C, the radioactivity as 17-isoaldosterone in the acid extract was high [35 ± 3 (SE) %] compared to that of aldosterone in the same extract after administration of aldosterone, but it was significantly ($p = 0.01$) lower than after oral 17-isoaldosterone (53 ± 4%).

The metabolism of a particular steroid was similar for both women and men (Table I).

Only a small amount of the dose was found as 17-isoaldosterone on column chromatography of the enzymatic glucuronide extract after oral administration of 17-isoaldosterone-4-^{14}C, compared to the amount released by acid hydrolysis from the same urine (4.1 ± 0.05%, 6 values). This indicates that the acid–labile metabolite of 17-isoaldosterone is not appreciably hydrolyzed by β-glucuronidase (< 6%) for all six urines under conditions that quantitatively split other glucuronides (13) and partially hydrolyzed (22 ± 2%, 6 values) the corresponding metabolite of aldosterone (i.e., aldosterone 18-glucuronide) under the same conditions (13).

Acid hydrolysis of one urine under the usual conditions for 16 hours gave 36% of an oral dose of 17-isoaldosterone-4-^{14}C as 17-isoaldosterone. A second hydrolysis for another 16 hours gave only 2.6% of the dose as the same compound (Table I). The corresponding values for the aldosterone from the acid extract as per cent of the aldosterone-^{3}H injected were 6.8 and 0.96%. This indicates that both the acid–labile conjugate of 17-isoaldosterone and aldosterone are rapidly hydrolyzed by the mild pH 1 and room temperature conditions for 16 hours.

After intravenous administration of both 17-isoaldosterone-4-^{14}C and aldosterone-7-^{3}H, the radioactivity as the appropriate acid–hydrolyzable conjugate was excreted nearly completely in 6 hours (Table II). In two urines, aldosterone-^{3}H and 17-isoaldosterone-^{14}C in the acid extract of 0– to 6–, 6– to 12–, and 0– to 48–hour samples were measured, and the radioactivity in the 6– to 12–hour samples was a relatively minor amount (Table II).

In two subjects, the urinary production rates of 17-isoaldosterone were found to be less than 5 μg per day.

Theory. If $\theta_a \equiv$ fractional rate of transfer of steroid from the extrasplanchnic to the splanchnic pool; $\theta_b \equiv$ fractional rate of transfer of steroid from the splanchnic to the extrasplanchnic pool; θ_d and $\theta_c \equiv$ fractional rates of total irreversible metabolism of steroid in the splanchnic and extrasplanchnic pools, respectively; θ_ϵ^S and $\theta_\epsilon^E \equiv$ fractional rates of metabolism of steroid to the particular metabolite in splanchnic and extrasplanch-

nic pools, respectively; $\theta_{c'}$ and $\theta_{d'} \equiv$ fractional rates of metabolism of steroid to compounds other than the particular metabolite in splanchnic and extrasplanchnic pools, respectively; E^S and $S^S \equiv$ total radioactivity (as a fraction of the injected radioactivity) in extrasplanchnic and splanchnic pools, respectively, after an oral dose; E^E and $S^E \equiv$ total radioactivity (as a fraction of the injected radioactivity) in extrasplanchnic and splanchnic pools, respectively, after an intravenous dose; and α_ϵ^S and $\alpha_\epsilon^E \equiv$ fractional rates of metabolism of radioactivity infused into splanchnic and extrasplanchnic pools to particular metabolite (rate of formation exclusively in the infused pool), then $\alpha_\epsilon^S = S^S\theta_\epsilon^S$ and $\alpha_\epsilon^E = E^E\theta_\epsilon^E$.

Then, K = fraction of oral dose in particular metabolite/fraction of intravenous dose in particular metabolite =

$$K = \frac{\theta_b\theta_\epsilon^E + (\theta_a + \theta_c)\theta_\epsilon^S}{\theta_a\theta_\epsilon^S + (\theta_b + \theta_d)\theta_\epsilon^E} = \frac{\theta_b\theta_\epsilon^E + (\theta_a + \theta_{c'})\theta_\epsilon^S + \theta_\epsilon^S\theta_\epsilon^E}{(\theta_b + \theta_{d'})\theta_\epsilon^E + \theta_a\theta_\epsilon^S + \theta_\epsilon^S\theta_\epsilon^E}. \quad [1]$$

Also,

$$K = \frac{\alpha_\epsilon^S + \frac{\theta_b}{\theta_b + \theta_d}\alpha_\epsilon^E}{\alpha_\epsilon^E + \frac{\theta_a}{\theta_a + \theta_c}\cdot\alpha_\epsilon^S}. \quad [2]$$

Equation 2 can be derived intuitively, as $\theta_b/(\theta_b + \theta_d)$ is the fraction of the oral dose that reaches the extrasplanchnic circulation, and $\theta_a/(\theta_a + \theta_c)$ is the fraction of the intravenous dose that reaches the splanchnic circulation.

Urinary free aldosterone or 17-isoaldosterone. Provided that there is no hydrolysis of metabolites formed in the splanchnic circulation in the extrasplanchnic pool (including the urine), the metabolite considered can be the free aldosterone, and this arises from the extrasplanchnic pool only (θ_ϵ^S or $\alpha_\epsilon^S = 0$). Then,

$$K_F = \frac{\theta_b}{\theta_b + \theta_d}. \quad [3]$$

The splanchnic extraction is $1 - K_F$.

Urinary tetrahydroaldosterone or tetrahydro-17-isoaldosterone. If tetrahydroaldosterone is formed only in the splanchnic circulation,

then θ_ϵ^E or $\alpha_\epsilon^E = 0$,

$$K_T = \frac{\theta_a + \theta_{c'}}{\theta_a}. \quad [4]$$

If $\theta_b = 0$ (splanchnic extraction = 100%), $1 - K_T = \theta_{c'}/\theta_a$ will give the ratio of extrasplanchnic metabolism (to compounds other than tetrahydroaldosterone, such as the 18-glucuronide) to total splanchnic metabolism (including conversion to tetrahydroaldosterone) of an intravenous dose.

Urinary aldosterone or 17-isoaldosterone 18-glucuronide. For this metabolite, θ_ϵ^E, α_ϵ^E, θ_ϵ^S, and α_ϵ^S must be greater than zero.

Then,

$$K_A = \frac{\theta_b\theta_\epsilon^E + (\theta_a + \theta_{c'})\theta_\epsilon^S + \theta_\epsilon^S\theta_\epsilon^E}{(\theta_b + \theta_{d'})\theta_\epsilon^E + \theta_a\theta_\epsilon^S + \theta_\epsilon^S\theta_\epsilon^E} = \frac{\alpha_\epsilon^S + \frac{\theta_b}{\theta_b + \theta_d}\alpha_\epsilon^E}{\alpha_\epsilon^E + \frac{\theta_a}{\theta_a + \theta_c}\alpha_\epsilon^S}. \quad [6]$$

In general, K_A can only be greater than 1 if $\theta_{c'} > 0$.

If $\theta_b = 0$ (splanchnic extraction = 100%), then

$$K_A = \frac{(\theta_a + \theta_c)\theta_\epsilon^S}{\theta_d\theta_\epsilon^E + (\theta_a)\theta_\epsilon^S} = \frac{\alpha_\epsilon^S}{\alpha_\epsilon^E + \alpha_\epsilon^S\left(\frac{\theta_a}{\theta_a + \theta_c}\right)}. \quad [7]$$

The proportion (κ) of the total 18-glucuronide formed in the splanchnic circulation produced from secreted hormone =

$$\kappa = \frac{\alpha_\epsilon^S\left(\frac{\theta_a}{\theta_a + \theta_c}\right)}{\alpha_\epsilon^E + \alpha_\epsilon^S\left(\frac{\theta_c}{\theta_a + \theta_c}\right)}. \quad [8]$$

Then, $\kappa = K_A/K_T$, [9]

assuming no formation of tetrahydroaldosterone in the extrasplanchnic pool.

Therefore, κ can be calculated if K_A and K_T are known and it is assumed that the splanchnic extraction is 100% and tetrahydroaldosterone is formed exclusively in the splanchnic circulation.

If oral absorption of the steroid is incomplete, then the numerators in Equations 1 to 7 will be reduced correspondingly. The estimate of splanchnic extraction or the proportion of extrasplanchnic to splanchnic metabolism from the ratio of isotopes in the urinary free aldosterone or tetrahydroaldosterone will also be correspondingly too low. However, the estimate of the total proportion of the 18-glucuronide formed in the splanchnic circulation from the secreted hormone (Equation 9) is not dependent on the amount of oral absorption, as K_A and K_T will be affected equally.

Discussion

After oral administration of 17-isoaldosterone to normal human subjects, about 80% (corrected for recovery but not for efficiency of hydrolysis) is recovered as an acid–labile conjugate from a 48–hour urine collection. Only small amounts ($< 1\%$) are found in the neutral extract or as metabolites released by incubation with β-glucuronidase ($< 4\%$). These metabolites would include 17-isoaldosterone itself, tetrahydro-17-isoaldosterone, or tetrahydroaldosterone. In contrast, after both oral and intravenous administration of aldosterone, about 35% is recovered as tetrahydroaldosterone after incubation of the urine with β-glucuronidase. Also, after oral administration of the natural hormone, only 8% is released by mild acid hydrolysis as aldosterone (corrected for recovery of the released aldosterone), and after intravenous injection, the corresponding quantity is 10% (2, 5, 10). The difference in excretion of aldosterone in the acid extract after oral compared to intravenous injection of aldosterone has been postulated as being due to high hepatic extraction of aldosterone with some extrahepatic conversion of the intravenous aldosterone to the acid–labile conjugate (2, 14).

Because of the rather unique and similar properties of the acid–labile conjugates of aldosterone and 17-isoaldosterone, particularly their ease of hydrolysis at pH 1 and room temperature, their resistance to hydrolysis with β-glucuronidase, and their rapid renal excretion, considered together with the equivalence of the structure of the two steroids, except for the configuration of the 20-21 carbon side chain, it seems highly likely that both conjugates are similar in nature and therefore have a glucuronide residue at position 18 (13, 15). It could be postulated that the preferential excretion of this type of conjugate in the metabolism of 17-isoaldosterone given either intravenously or orally is due to its increased rate of formation because of decreased hindrance by the side chain in the 17-iso position. On the other hand, the negligible excretion as tetrahydro-17-isoaldosterone or other metabolites released by β-glucuronidase could be due to specificity of the A–ring–reducing enzyme. It is presumably not due to specificity in the mechanism for conjugating after reduction of the ketone at position 3 in the A–ring, as the amount of radioactivity in the total neutral extract is also small. The increase in the excretion of the acid–labile conjugate could be due to lowered competition from the route of metabolism involving A–ring reduction. Similarly, the negligible excretion of the tetrahydro-17-isoaldosterone glucuronide could be due to specificity of the A–ring–reducing mechanism or to increased competition from the 18-glucuronide route.

However, the negligible formation of the tetrahydro derivative does suggest some primary specificity in reduction of the A–ring. It would then be expected that the decreased rate of metabolism of the orally administered 17-isoaldosterone in the liver, due to lack of reduction of the A–ring, would lead to lowered hepatic extraction. Some of the orally administered steroid would then reach the kidney, which can form the acid–labile conjugate (14). The proportion of 17-isoaldosterone metabolized to the conjugate would then be expected to be much higher than that of oral aldosterone. Since tritium–labeled 17-isoaldosterone is not yet available, it has not been possible to compare directly in the same subjects the amounts of radioactivity as 17-isoaldosterone in the neutral urinary extract after simultaneous oral and intravenous administration of the labeled steroid, but the similarity in the values found [0.32 ± 0.18 (SE) % oral, and 0.20 ± 0.02 (SE) % intravenous] lends support to the hypothesis. This assumes that a negligible proportion of the 17-isoaldosterone in the neutral extract arises from hydrolysis of the conjugate in the body or urine, as seems to be the case with aldosterone (16). In contrast, the ^{14}C to ^{3}H ratio (K_F) of aldosterone from the neutral extract (as the total column fractions) after ^{14}C–labeled oral and ^{3}H–labeled intravenous aldosterone is 8 ± 1 (SE) %, giving a calculated splanchnic extraction for aldosterone of $(1 - K_F)$ $100\% = 92 \pm 1$ (SE) % for normal subjects (Equation 4).

It would be expected, if this hypothesis concerning the metabolism of 17-isoaldosterone were correct, that the proportion of 17-isoaldosterone excreted as the acid–labile conjugate would not be markedly lower after oral than after intravenous administration, unlike the corresponding situation for aldosterone. This was found to be so. However, unexpectedly, the per cent of the dose excreted as the acid–labile conjugate was significantly

higher after oral than after intravenous administration of 17-isoaldosterone-4-^{14}C. The mean values were significantly different (53 ± 4 and $35 \pm 3\%$, respectively). Also, two subjects (D.P. and A.G., Table I) were given both oral and intravenous 17-isoaldosterone-4-^{14}C on separate occasions, and the per cent of the dose excreted as the 17-isoaldosterone 18-glucuronide was greater (140 and 144%, respectively) after the oral than after the intravenous administration.

One explanation of this, from Equation 6, is that there is considerable extrasplanchnic metabolism of 17-isoaldosterone to compounds other than the acid–labile conjugate or metabolites released from urine by incubation with β-glucuronidase or unconjugated metabolites. Some of the radioactivity in the urine after intravenous injection cannot be accounted for in terms of these known metabolites. Another, perhaps more likely, explanation is that orally administered 17-isoaldosterone is converted to the acid–labile conjugate before reaching the liver, i.e., in the intestinal lumen, which can form glucuronides of other steroids (17).

The ratio (K_A) of aldosterone in the acid extract after oral and intravenous administration of labeled aldosterone was 75 ± 4 (SE) % (nine subjects). This is significantly higher than the ratio found by Bledsoe and co-workers (2), 58 ± 2 (SE) % (seven subjects), who simultaneously administered nonisotopic aldosterone by one route (orally in three and intravenously in four subjects) and aldosterone-^{3}H by the other. We have no explanation for this discrepancy. Cheville and co-workers (14), from analysis of renal venous blood and urinary excretion of conjugate in control subjects and patients with early heart failure, concluded that 61% (with confidence limits $\pm$ 20%) of the acid–labile conjugate is formed in the kidney. From the isotopic ratios obtained in the present studies for aldosterone from the 18-glucuronide (0.75 ± 0.04) and tetrahydroaldosterone (1.14 ± 0.04), the proportion of the total 18-glucuronide formed in the splanchnic circulation produced from secreted hormone can be calculated (Equation 9) as 0.66 ± 0.05 or $34 \pm 5\%$ of the total conjugate is formed from secreted hormone extrasplanchnically. This value is probably significantly lower than the corresponding estimate of Cheville and associates (14) ($61 \pm 20\%$ formed extrasplanchnically).

A possible reason for the discrepancies in the conclusions reached by using the two approaches could be similar to that proposed to explain the higher proportion of acid–labile conjugate of 17-isoaldosterone excreted after oral than after intravenous administration, i.e., formation of the acid–labile conjugate of aldosterone in the extrahepatic splanchnic tract.

The effect of this possibility on the validity of the method for obtaining splanchnic extractions of aldosterone without hepatic catheterization by simultaneous oral and intravenous injection of labeled aldosterone (18) must be considered. From the data presented here and those of Cheville and co-workers (14), a maximum of one–half of the aldosterone 18-glucuronide formed after oral administration of aldosterone could be made in extrahepatic splanchnic tissues. However, only an over–all maximum of 20% of aldosterone is converted to this conjugate (13, 14), and, therefore, this route of metabolism of oral aldosterone could account at the most for 10% of the over–all metabolism of aldosterone. Thus, this metabolism of orally administered aldosterone before it reached the liver would lower the ^{14}C to ^{3}H ratio (K_F) as free aldosterone by a maximum of 10%. The hepatic extraction is calculated as $1 - {}^{14}C/{}^{3}H$, and the ^{14}C to ^{3}H ratio in normal subjects is usually < 0.1 and in subjects with abnormal metabolism, maximally 0.5. The error in the calculation of hepatic extractions would, therefore, be negligible, ($< 1\%$) in normal subjects and less than 10% in patients with cirrhosis. Nevertheless, conclusions reached from the ^{14}C to ^{3}H ratio (K_A) of aldosterone released from the aldosterone 18-glucuronide in the urine of a particular group of subjects could be significantly affected by abnormal conversion of aldosterone to the conjugate in the extrahepatic splanchnic tissues.

The increased formation of 18-glucuronide after intravenous injection of 17-isoaldosterone compared with aldosterone, as was discussed previously for the orally administered isomer, could be due to the lowered clearance of 17-isoaldosterone by the liver and the consequent increased relative formation of the conjugate by the kidney. However, after intravenous injection, the hepatic and renal circulations are in parallel for metabo-

lism of the steroid, whereas after oral administration they are in series, and the hepatic extraction of aldosterone, but not of 17-isoaldosterone, is complete. The relative increase in acid–labile conjugate formation for 17-isoaldosterone compared with aldosterone would be expected to be greater when they are both given by the oral rather than the intravenous route.[5] The relative conversion of 17-isoaldosterone and aldosterone to their respective acid–labile conjugates was ten to one when both were given orally and five to one when they were given intravenously.

An analogous explanation has been given by Bledsoe and co-workers (2) for the increased excretion of aldosterone 18-glucuronide (expressed as a per cent of the dose of intravenously administered labeled aldosterone) in cirrhosis of the liver (19). The rate of reduction of the A–ring of the aldosterone molecule may be lowered in cirrhosis. Therefore, hepatic clearance is slowed, and, as a consequence, a greater proportion of secreted (or injected) aldosterone is available for extrasplanchnic conversion to aldosterone 18-glucuronide.

The stereoisomerism of the side chain does not inhibit formation of the acid–labile conjugate nor does it appear to alter appreciably other characteristics, such as ease of acid hydrolysis and resistance of β-glucuronidase hydrolysis. It is not known with certainty whether the attachment of the glucuronide to the 18 position of aldosterone is α or β. The resistance to hydrolysis of the aldosterone 18-glucuronide by β-glucuronidase may be due to the attachment being α, or to hindrance from the side chain or other features of the structure, such as the acetal at 11,18 of the steroid molecule. Pasqualini, Uhrich, and Jayle (15) have reported that hydrolysis of the aldosterone conjugate by high concentrations of enzyme of the succus entericus of *Helix pomatia* (which appears to be more efficient for this purpose than bacterial or liver β-glucuronidase) is inhibited by potassium D-saccharate and D-saccharodilactone, which suggests that the attachment is β. The characteristics of the corresponding 17-isoaldosterone conjugate suggest that the resistance to β-glucuronidase is not due to side chain hindrance but to another feature of structure, such as the acetal at 11,18. The acid lability may be due to a similar structural feature.

The 17-isomer of aldosterone is converted to its corresponding acid–labile conjugate to a fivefold greater extent than aldosterone when both are given intravenously. Thus, if the isomers are not separated on chromatography of the acid extract, a 5% contamination of the aldosterone injected with its isomer could result in a 25% error in a determination of the secretion rate of aldosterone. Paper chromatography of the aldosterone as the free compound on the Bush B5 system would usually not separate the isomers completely. Column chromatography with the Bush B5 system and paper or column chromatography of the diacetate in the Bush B3 system would be expected to be successful in this regard. If the tetrahydroaldosterone is used as the urinary metabolite for the secretion rate estimation, any per cent of contamination of the injected aldosterone with its 17-isomer would lead to the same per cent of error in the determination of secretion rate, as would also be the case if the acid–labile conjugate were used and the isomers separated. The problem would not then be so serious, as the effect would not be magnified.

The negligible radioactivity as aldosterone or tetrahydroaldosterone in the urinary extracts after administration of 17-isoaldosterone indicates that it is not appreciably converted to aldosterone *in vivo*. Some tritium as 17-isoaldosterone could be detected in the acid extract after intravenous injection of aldosterone-^{3}H. This was at most 10% of the radioactivity as aldosterone in the same extract. Since the 17-isoaldosterone converted from aldosterone will be preferentially excreted as this type of conjugate, this result indicates very small ($< 2\%$) conversion of aldosterone to 17–isoaldosterone.

The secretion rate of 17-isoaldosterone was also found to be negligible in two subjects, so it is unlikely that significant amounts of aldosterone originate from this compound *in vivo*.

It may be that 17-isomers of other steroids with the $^4\Delta$ 3 ketonic structure are also not metabolized by reduction of the A–ring *in vivo*. If this is so, investigation of their metabolism may be useful in revealing other routes and sites of catabolism of the natural hormones.

[5] From Equation 6, K_A will increase with higher values of $\theta_b/(\theta_b + \theta_d)$ if α_e^S, α_e^E, and $\theta_a/(\theta_a + \theta_c)$ (and the proportion of the 18-glucuronide formed in the extrasplanchnic and splanchnic tissues) are similar for the two compounds.

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