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Research Article

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After combining oral (¹⁴C) and intravenous (³H) administration of aldosterone, the ¹⁴C/³H ratios of urinary metabolites (free aldosterone, tetrahydroaldosterone glucuronide, and aldosterone 18-glucuronide) were measured and were expressed as a per cent of administered dose. From these data the splanchnic extraction of aldosterone was calculated. The splanchnic extraction was significantly lower in pregnant as compared to nonpregnant subjects, although previous work indicated no change in protein binding of aldosterone in pregnancy.

However, the data on the ¹⁴C/³H ratio of other metabolites suggested that a large part of the increased aldosterone 18-glucuronide metabolite in pregnancy is formed in the splanchnic circulation; also, there appeared to be increased tetrahydroaldosterone glucuronide formation extrasplanchnically.

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The Metabolism of Orally and Intravenously Administered Labeled Aldosterone in Pregnant Subjects

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ABSTRACT Aldosterone metabolism has been shown to be altered in pregnancy. The increased conversion of intravenously administered aldosterone- ^3H to the acid-labile conjugate in the urine (aldosterone 18-glucuronide) has again been observed. The urinary yield of intravenously administered aldosterone- ^3H as aldosterone 18-glucuronide in 16 pregnant subjects of 13.4 ± 0.9 (SE) % was significantly higher ($P < 0.001$) than in 11 nonpregnant subjects (seven males and four females) of 7.3 ± 0.5 (SE) %.

After combining oral (^{14}C) and intravenous (^3H) administration of aldosterone, the $^{14}\text{C}/^3\text{H}$ ratios of urinary metabolites (free aldosterone, tetrahydroaldosterone glucuronide, and aldosterone 18-glucuronide) were measured and were expressed as a per cent of administered dose. From these data the splanchnic extraction of aldosterone was calculated. The splanchnic extraction was significantly lower in pregnant as compared to nonpregnant subjects, although previous work indicated no change in protein binding of aldosterone in pregnancy.

However, the data on the $^{14}\text{C}/^3\text{H}$ ratio of other metabolites suggested that a large part of the increased aldosterone 18-glucuronide metabolite in pregnancy is formed in the splanchnic circulation; also, there appeared to be increased tetrahydro-

aldosterone glucuronide formation extrasplanchnically.

INTRODUCTION

Aldosterone metabolism was shown to be altered in pregnancy (1). The validity of this observation was questioned by Watanabe, Meeker, Gray, Sims, and Solomon (2). The present paper confirms the original observations and presents additional data to suggest possible mechanisms for this altered metabolism in pregnancy.

Jones, Lloyd-Jones, Riondel, Tait, Tait, Bulbrook, and Greenwood (1) reported a higher excretion of aldosterone released by mild acid treatment of urine (now presumed to be aldosterone 18-glucuronide) from pregnant compared with nonpregnant subjects. They suggested that this was partially due to the increased conversion of secreted aldosterone to this metabolite. Subsequently Watanabe and coworkers (2) studied the metabolism of aldosterone in four pregnant subjects but were unable to confirm this observation of altered conversion. We therefore reinvestigated this problem by administering intravenously aldosterone-7- ^3H to six pregnant subject and then measuring the radioactive yield to three urinary fractions: free aldosterone, aldosterone released from the acid-labile conjugate (hereafter presumed to be aldosterone 18-glucuronide [3]) and tetra-

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hydroaldosterone released by incubation with β -glucuronidase.¹ These studies, described herein, have confirmed the results of Jones and coworkers (1).

In addition, oral aldosterone-4-¹⁴C and intravenous aldosterone-7-³H were given simultaneously to 10 other pregnant subjects, and the radioactive yield to the three urinary fractions from both administered doses was observed. The aim of this approach was to determine the site of and possibly the mechanism for the increased formation of aldosterone 18-glucuronide in pregnancy. Also, from such data the splanchnic extraction of aldosterone in pregnancy could be calculated (4, 5).

METHODS

The pregnant subjects (all in the last trimester) were patients at the Cleveland Metropolitan General Hospital or the Boston City Hospital. 10 pregnant women (Table I) aged 17-31 yr were admitted as volunteers to the

¹ Trivial names: 17-isoaldosterone = 11 β ,21-dihydroxy-18-oxo-17-isopregn-4-en-20-one; tetrahydroaldosterone = 3 α ,11 β ,21-trihydroxy-18-oxo-5 β -pregnan-20-one; aldosterone 18-glucuronide = 21-hydroxy-11:18-oxidopregn-4-ene-3,20-dion-18-yl- β -D-glucopyranosiduronic acid; 17-isoaldosterone-18-glucuronide = 21-hydroxy-11:18-oxido-17-isopregn-4-ene-3,20-dione-18-glucopyranosiduronic acid.

Perinatal Research Unit. The six other pregnant subjects were studied during a hospital admission for unrelated problems. The four nonpregnant female subjects were in the age range, 23-32 yr. M.E. and C.K. were both in the proliferative phase of their menstrual cycles at the time of investigation. J.F. was menstruating, and P.W. was at mid-ovarian cycle. All were on a normal uncontrolled diet (Table I).

The radioactive steroids were administered at 10 a.m. Thereafter the subjects were allowed to walk freely about the ward. Urine was collected for 48 hr after administration of labeled aldosterone. A single intravenous injection of 2.0 μ c of aldosterone-7-³H was given to six pregnant subjects. A combined oral and intravenous dose of aldosterone was given to an additional 10 pregnant and four nonpregnant women. The oral dose was 0.5 μ c of aldosterone-4-¹⁴C, and the intravenous injection was 2.0 μ c of aldosterone-7-³H as a single injection. The yields of administered radioactivity from aliquots of the 48-hr collections of urine as free aldosterone, as aldosterone released from aldosterone 18-glucuronide by mild acid hydrolysis, and as tetrahydroaldosterone released by β -glucuronidase incubation were measured. Details of the administration of the steroid and the analysis of the urine are as previously described by Flood, Pincus, Tait, Tait, Willoughby, Layne, Ramcharan, and Rossipal (5, 6) except for the measurement of the yield of administered radioactivity as urinary free aldosterone. In previous studies with normal and elderly subjects (including two of the nonpregnant females), the ¹⁴C/³H ratio was constant throughout the column fractions containing the measured metabolites including the free aldosterone,

TABLE I
Clinical Data

Subject	Age	Weight	Height	Hct	Blood pressure	Gestation at test	Estrogen excretion			Remarks
							Estrone	Estradiol	Estriol	
	yr	kg	cm	%	mm Hg	wk	μ g/24 hr			
Pregnant										
B. K.	26	52.0	155	34	126/70	31	220	100	4100	Wt gain 11.4 kg
V. M.	19	65.0	160	33	110/70	35	440	130	4800	Wt gain 5.2 kg
I.P.	29	77.5	167	31	134/80	37	300	120	8700	Wt gain 17.5 kg
J. S.	17	59.0	163	35	118/72	32	950	240	3000	Wt gain 6.8 kg
M. N.	21	57.3	157	40	130/80	34	360	160	3300	Wt gain 7.7 kg
A. N.	31	78.6	156	38	120/70	36	170	90	5800	Wt gain 13.6 kg
D. J.	30	99.8	155	36	130/80	36	900	300	9000	Wt gain unknown
R. C.	29	64.2	163	32	100/70	31	340	90	4100	Wt gain 10.0 kg
G. N.	18	48.0	152	39	102/79	36	1100	260	4300	Wt gain 3.2 kg
B. G.	22	63.2	160	37	100/70	32	730	210	3500	Wt gain 14.3 kg
Nonpregnant										
M. E.	32	66.5	155	34	122/88	—	—	—	—	Menstrual cycle day 7
C. K.	23	55.9	165	37	120/70	—	—	—	—	Menstrual cycle day 4
J. F.	36	68.2	172	—	—	—	—	—	—	Menstrual cycle day 10-15
P. W.	26	61.8	168	45	124/86	—	—	—	—	Menstrual cycle day 24-28

which indicated the radiochemical purity of the isolated material. However, when free aldosterone from the urine from pregnant subjects was examined by this method, the $^{14}\text{C}/^3\text{H}$ ratio was not uniform in the fractions from the column containing free aldosterone. Therefore all these fractions were taken to dryness, acetylated, and the aldosterone diacetate purified on another column (60 cm long, 1 cm diameter toluene: Skellysolve (Skelly Oil Co., Tulsa, Okla.), 2:3.5; mobile phase; methanol: water, 4:1 stationary phase). The $^{14}\text{C}/^3\text{H}$ ratios in the fractions containing aldosterone diacetate were then satisfactorily constant. After administration of intravenous aldosterone-7- ^3H and oral aldosterone-4- ^{14}C to two nonpregnant female subjects, the $^{14}\text{C}/^3\text{H}$ ratios of the urinary free aldosterone, measured finally as the diacetate by the same method, was 0.110 and 0.010, which were not significantly different than the values previously estimated

for normal subjects (mean 0.037, range 0.014–0.063 for seven male subjects, 0.07 and 0.07 for two nonpregnant females) when only one column was used for purification without acetylation.

RESULTS

For the six pregnant subjects given an intravenous injection of labeled aldosterone without simultaneous oral administration, the 48 hr urinary yield of administered dose as aldosterone 18-glucuronide was 13.1 ± 1.8 (SE) % compared with 7.6 ± 1.0 (SE) % for four nonpregnant females and 7.1 ± 0.7 (SE) % for 7 males (Table II). The urinary yield was therefore significantly higher for pregnant compared with either group of non-

TABLE II
Conversion of 2.0 μC of Intravenous Aldosterone-7- ^3H and 0.5 μC of Oral Aldosterone-4- ^{14}C to Urinary Free Aldosterone, Acid-Released Aldosterone, and β -Glucuronidase-Released Tetrahydroaldosterone for Pregnant and Nonpregnant Female and Male Subjects

Subjects	Free aldosterone		Acid-released aldosterone		β -Glucuronidase-released tetrahydroaldosterone	
	$^{14}\text{C}/^3\text{H}$ $\times 100\%^*$ (r)	Hepatic extraction per cent (l-r)	^3H per cent of intravenous dose	$^{14}\text{C}/^3\text{H}$ $\times 100\%^*$	^3H per cent of intravenous dose	$^{14}\text{C}/^3\text{H}$ $\times 100\%^*$
Female						
Pregnant						
B. K.	12	88	12.9	98	33	95
V. M.	25	75	16.1	106	41	89
I. P.	16	84	10.8	90	32	98
J. S.	24	76	13.6	99	25	93
M. N.	24	76	10.4	102	33	100
A. N.	24	76	18.0	89	39	72
D. J.	13	87	19.6	92	19	118
R. C.	28	72	14.4	110	22	95
G. N.	55	45	7.7	134	21	95
B. G.	5	95	11.6	86	25	86
Mean \pm SE	23 \pm 4	77 \pm 4	13.5 \pm 1.2 \ddagger	101 \pm 4.5	29 \pm 2.5	94 \pm 4
Nonpregnant						
M. E. \S	11	89	6.5	69	42	98
C. K. \S	0	100	8.9	61	32	97
J. F. \parallel	7	93	5.2	71	25	108
P. W. \parallel	7	93	9.6	81	41	107
Mean \pm SE	6 \pm 2	94 \pm 2	7.6 \pm 1.0	71 \pm 4	35 \pm 4	103 \pm 3
Male						
Seven values \parallel (five for tetrahydroaldosterone)						
Mean \pm SE	3.7 \pm 0.6	96.3 \pm 0.6	7.1 \pm 0.7	75 \pm 5	36 \pm 7	117 \pm 6

* ^{14}C and ^3H expressed as per cent of oral and intravenous doses, respectively.

\ddagger Six other pregnant subjects in a different previously unpublished series gave 13.1 ± 1.8 (SE) % for this value.

\S Determined in same series and by same methods as urine from pregnant subjects.

\parallel Previously published as part of another series (5).

pregnant subjects ($P = 0.05$ and 0.01 for pregnant compared with nonpregnant female and male values, respectively). The values for the two nonpregnant groups were not significantly different. The mean value for these six pregnant subjects of 13.1 ± 1.8 (SE) % was in good agreement with the corresponding value obtained by Jones and coworkers (1) for six pregnant women (13.3 ± 1.7 [SE] as per cent of intravenous dose) and 13.5 ± 1.2 (SE) as per cent of intravenous dose for the 10 pregnant subjects in the present series who were given both oral and intravenous aldosterone. The combined value for the 16 pregnant subjects investigated in the present series was 13.4 ± 0.9 (SE) %, which was significantly higher ($P < 0.001$) than the 11 combined nonpregnant value of 7.3 ± 0.5 (SE) %. The recovery of labeled aldosterone added to the urine after neutral extraction and carried through the procedure was 56 and 57% in normal urine and 59 and 67% from pregnant urine. In previous work, Underwood and Tait (3) determined recoveries of 69 and 68% for urine from nonpregnant subjects by the same procedure. The recoveries from pregnancy urine are therefore not significantly different from normal urine and cannot account for differences observed. The values quoted are all uncorrected for losses. These losses cannot be corrected for in a rigorous manner without the use of labeled conjugate as indicator. This is not generally available in suitable amounts. However, comparisons of estimates carried out in the same laboratory will be valid.

The proportion of oral (^{14}C fraction of administered dose) compared with the intravenous (^3H fraction of administered dose) dose excreted ($^{14}\text{C}/^3\text{H}$ ratio) gives the relative contributions of the oral and the intravenously administered aldosterone to the urinary metabolites. This gives a measure of the proportionate influence of the splanchnic bed on administered aldosterone. When the $^{14}\text{C}/^3\text{H}$ ratio (r) of urinary free aldosterone is measured, the splanchnic extraction ($1-r$) may be calculated. The splanchnic extraction is that portion of aldosterone either metabolized or removed from the splanchnic circulation in one passage. The $^{14}\text{C}/^3\text{H}$ ratio (r) (both isotopic yields expressed as a per cent of administered dose) of urinary free aldosterone was 22.6 ± 4.3 (SE) % for the 10 pregnant subjects, which is significantly

higher than the corresponding values for the four nonpregnant females (6.3 ± 2.3 [SE] % ($P = 0.05$) and the seven males investigated previously (3.7 ± 0.6 [SE] % ($P < 0.01$)) (4, 5). The value for the 10 pregnant subjects was significantly higher ($P = 0.001$) than the combined 11 nonpregnant value of 4.6 ± 0.9 (SE) %. Similarly the calculated splanchnic extraction of 77 ± 4 (SE) % for the pregnant subjects (given by per cent $(1-r)$) (4, 5) was significantly lower than the corresponding value calculated for the nonpregnant subjects of 93.8 ± 2.3 (SE) % for the four females and 96.3 ± 0.6 (SE) % for the seven males or 95.4 ± 0.9 (SE) % for the combined 11 nonpregnant values.

Altered aldosterone metabolism might also appear as a changed proportion of intravenous aldosterone- ^3H appearing as tetrahydroaldosterone glucuronide in the urine. However the per cent of the administered intravenous dose of aldosterone- ^3H as tetrahydroaldosterone was 29 ± 2.4 (SE) % for the 10 pregnant subjects which was not significantly lower than the combined mean for the corresponding values in the nine male and female value of 35.4 ± 3.8 (SE) % (P value of 0.1). The male and nonpregnant female values were also not significantly different.

The $^{14}\text{C}/^3\text{H}$ ratio of the urinary tetrahydroaldosterone gives an indication of the relative contribution of splanchnic and extrasplanchnic contribution to this urinary metabolite. The $^{14}\text{C}/^3\text{H}$ ratio of the tetrahydroaldosterone was 94.1 ± 3.7 (SE) % for the 10 pregnant subjects which is significantly lower than the combined results for the nine (two samples were lost) nonpregnant values of 110.6 ± 4.1 (SE) % ($0.05 > P > 0.01$). The male and nonpregnant female values were not significantly different (Table II).

The proportion of oral compared to intravenous dose excreted as aldosterone released from the acid-labile conjugate which is the major extrahepatic route of metabolism indicates the proportionate change taking place in this route of metabolism. The $^{14}\text{C}/^3\text{H}$ ratio of the aldosterone released by mild acid hydrolysis was 100.6 ± 4.5 (SE) % which was significantly higher ($P < 0.01$) than the 11 combined nonpregnant value of 73.1 ± 3.3 (SE) %. The male and nonpregnant female values were again not significantly different (Table II).

DISCUSSION

This study confirms the work of Jones et al. (1) that there is an increased conversion of aldosterone to urinary aldosterone 18-glucuronide in pregnancy. The results of Watanabe et al. (2) who could not demonstrate this effect may be explained by failure to study a large enough group of subjects.

The calculated splanchnic extraction of aldosterone in pregnancy is 77% which is significantly below the observed 90% in nonpregnant patients (measured both by direct hepatic vein catheterization [7] and indirect calculation as in the present study). Theoretically this lowered splanchnic extraction could be the result of increased aldosterone binding to plasma proteins other than to albumin (splanchnic extraction of steroids is not usually lowered by albumin binding [8]). However, although aldosterone does demonstrate increased plasma protein binding following unusually high doses of estrogen, there is no increase in pregnancy (8). Abnormally elevated estrogen secretion in the present pregnancy studies was ruled out by studying urinary estrogen excretion by the method of Brown² (9). All values were in the normal range, and no correlation could be demonstrated between the excretion of estrogen and calculated splanchnic extraction (Tables I and II). This unaltered binding would therefore suggest a reduction of the hepatic metabolism of aldosterone. This could be similar to cortisol disappearance in pregnancy and after estrogen treatment, in which the calculated metabolic clearance rate of free and albumin-bound cortisol is reduced (8, 10) without alteration in hepatic blood flow (11). This is apparently a hepatic cellular effect.

The metabolic clearance rate of aldosterone is not significantly different in pregnancy from that of nonpregnant subjects (12). The criticism by Braunsberg and James (13) that a different model should have been used overlooked that the metabolic clearance rate was measured after prolonged continuous infusion, which gave the same result. This finding indicated that additional possible compartments in pregnancy did not affect the determination in pregnancy. This has been discussed in detail by one of us (14). The combined results

² These determinations were kindly carried out by Dr. J. E. Roberts, Worcester Foundation.

for 13 nonpregnant subjects by both single injection and continuous infusion was 1631 ± 106 (SE) liters of plasma per day and 1543 ± 113 (SE) liters of plasma per day (15 pregnant subjects). The expected decrease in metabolic clearance rate due to the reduced splanchnic extraction (from 95 to 77%) with unchanged hepatic blood flow (11) would have been about 300 liters/day and it seems probably would have been detected. This finding suggests that there is a decrease in splanchnic clearance (unchanged total metabolic clearance) and an increase in extrasplanchnic clearance of aldosterone in pregnancy. This increase in extrasplanchnic clearance cannot be compensatory to changes in splanchnic clearance. However, renal blood flow is increased in pregnancy (15) and production of the 18-glucuronide metabolite from aldosterone by the kidney might thereby be raised. The fetus and the placenta could also be responsible for the increase in extrasplanchnic clearance; however, no relevant direct data are available to prove or disprove either possibility.

The increase in formation of aldosterone 18-glucuronide could be due to the increased production of this metabolite by extrasplanchnic tissues. The kidney is now known to contribute an appreciable portion to the total production of this metabolite (16). In cirrhosis the increased formation of this metabolite has been proposed as due to lowered splanchnic clearance with proportionate increase in production of the metabolite by the kidney. However, in pregnancy, the splanchnic clearance is probably lowered by only 20%, and the proportion of the aldosterone 18-glucuronide made from an intravenous dose of hormone is doubled (7-13%) (Table II). It is unlikely therefore that this is the major reason for this effect.

Oral aldosterone is converted less efficiently to urinary 18-glucuronide than intravenously administered hormone in nonpregnant subjects (17). This finding was confirmed experimentally and theoretically by Flood et al. (6). Oral aldosterone appears to be nearly completely extracted by the liver, whereas the intravenous aldosterone is also converted to the 18-glucuronide extrasplanchnically in the kidney. If extrasplanchnic formation of this conjugate were increased in the pregnant woman, and the other factors were unaltered, the ¹⁴C/³H ratio of the urinary aldosterone 18-glucuronide would be reduced. The isotope ratio was,

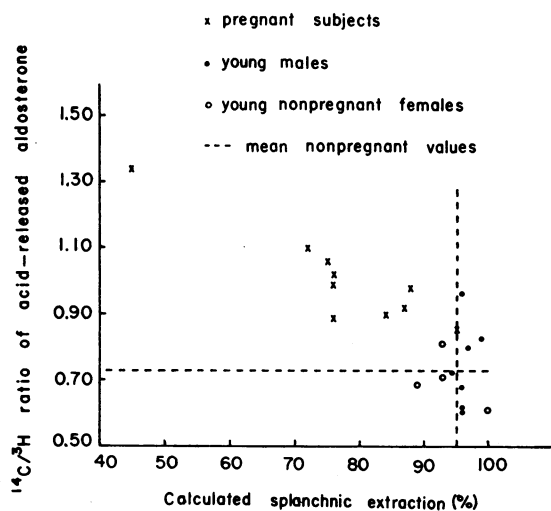


FIGURE 1 $^{14}\text{C}/^3\text{H}$ ratio of acid-released aldosterone (from oral ^{14}C and intravenous aldosterone- ^3H) plotted against the calculated splanchnic extraction for pregnant and nonpregnant subjects.

however, higher in the pregnant than the nonpregnant subjects. This could be accounted for by reduced splanchnic extraction. But it can be seen that the $^{14}\text{C}/^3\text{H}$ ratio when plotted against calculated splanchnic extraction is linearly related (Fig. 1); and if this is extrapolated to normal nonpregnant values, the ratio would still be high. Therefore lowered splanchnic extraction cannot entirely explain this increase in the isotope ratio, or as previously discussed, in the urinary ^3H yield, of the aldosterone 18-glucuronide. It seems therefore that at least some of the increased yield is due to a higher rate of formation of the conjugate in the liver.

These conclusions from the $^{14}\text{C}/^3\text{H}$ ratio assume that the extent of formation of aldosterone glucuronide from the oral dose of aldosterone before the hormone reaches the liver is the same in pregnant and nonpregnant subjects. Both aldosterone 18-glucuronide and 17-isoaldosterone 18-glucuronide are formed in the splanchnic bed before oral doses of aldosterone and 17-isoaldosterone reach the liver (5). If this occurs, the splanchnic extraction would be expected to be reduced, but the $^{14}\text{C}/^3\text{H}$ ratio in the urinary aldosterone 18-glucuronide would be increased, findings which were also demonstrated for 17-isoaldosterone (5). One subject (G. N.) whose splanchnic extraction was low (45%) had a high (134%) $^{14}\text{C}/^3\text{H}$ ratio for the acid-labile conjugate, a fact which tends to

confirm this conclusion. We have no other reasonable explanations for such ratios than that there is metabolism of the orally administered compounds to the 18-glucuronides before they reach the liver. If such formation were increased in pregnancy the ratio would be raised for this reason and not necessarily because of increased hepatic formation. The present experimental approach cannot definitely decide this point.

Excretion of tetrahydroaldosterone (as a per cent of intravenously injected aldosterone- ^3H) is decreased by administration of estrogens to nonpregnant females (18). It might be expected that the hepatic formation of tetrahydroaldosterone would be reduced in pregnancy, with a compensatory increase in the aldosterone 18-glucuronide by the liver. The splanchnic extraction is decreased, but the conversion of intravenously administered aldosterone- ^3H to tetrahydroaldosterone is not lowered (Table II). Formation of tetrahydroaldosterone may be reduced by the liver and increased by extrasplanchnic sites. This possibility would also explain the lowered $^{14}\text{C}/^3\text{H}$ ratio of this metabolite observed. As the kidney does not form appreciable amounts of tetrahydroaldosterone, this increase in extrasplanchnic production most likely reflects fetoplacental contribution.

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