Journal of Clinical Investigation
Vol. 42, No. 10, 1963

SECRETION RATE OF ALDOSTERONE IN NORMAL PREGNANCY *

By MAMORU WATANABE,† C. IRVING MEEKER,‡ MARY JANE GRAY, ETHAN A. H. SIMS, AND SAMUEL SOLOMON

(From the Department of Experimental Medicine, McGill University and the Royal Victoria Hospital, Montreal, Canada, and the Department of Obstetrics and Gynecology and the Metabolic Unit, Department of Medicine, College of Medicine, University of Vermont, Burlington, Vt.)

(Submitted for publication March 18, 1963; accepted June 27, 1963)

There is a progressive rise in excretion of the metabolites of the estrogens (1) and of progesterone [1] (2) during pregnancy in humans. In late pregnancy, these hormones are largely placental in origin (3–6). The physiological role of the adrenal gland in pregnancy is still not well understood. The high levels of blood hydrocortisone found in pregnancy were initially explained as a reflection of an increased secretion of adrenal cortical hormones (7). More recently, it has been shown that the high blood levels of hydrocortisone in pregnancy could be accounted for by the increased binding of this hormone to blood proteins (8). It has been reported that aldosterone is secreted in increased amounts during pregnancy (9, 10). From available evidence, it has been concluded that in humans aldosterone is not a secretory product of the placenta (11–14), but that it is formed in the adrenal gland.

In earlier studies (15–17), bioassay of the crude urinary extract revealed little or no increase in the salt-retaining factor in pregnancy as compared with the normal nonpregnant state. Barnes and Quilligan (18) reported the same findings with a bioassay of urinary extracts purified by three paper chromatographic systems. In 1956, Venning and Dyrenfurth (19) bioassayed extracts from acid-hydrolyzed urine purified by one paper chromatographic system and reported an increased level of aldosterone excretion in late pregnancy. At the same time, Martin and Mills (20) reported similar findings based on a physicochemical determination of aldosterone. Since then, several groups of investigators (21–25) have confirmed the earlier findings of an increased urinary excretion of acid-hydrolyzable conjugate of aldosterone in pregnancy.

Although much valuable information has been obtained from the urinary excretion studies, a more precise knowledge of aldosterone secretion has awaited the development of isotopic procedures for the estimation of the rate of secretion of the hormone. Jones and associates (9), using an isotope dilution method for aldosterone developed by Ayres and associates (26), studied six pregnant subjects in the third trimester and found that three had elevated levels, whereas the others were within the range for normal nonpregnant females. In a preliminary study by Van de Weile and associates (10), aldosterone secretion rates were found to be elevated in all three normal pregnant subjects studied.

In this study, aldosterone secretion rates were determined in a large number of subjects during the third trimester of pregnancy. It has been established that the secretory rate is consistently elevated in the third trimester and that it is responsive to changes in dietary sodium. The most striking alteration in the secretory rate can be elicited by lowering the dietary sodium.

METHODS

Subjects. All of the normal pregnant subjects were ambulatory. Most of the subjects were in a home for unwed mothers in Burlington, Vermont, under the supervision of the Department of Obstetrics and Gynecology of the University of Vermont. A small number of sub-

* Supported in part by U. S. Public Health Service research grants A-4329 and RG-3745 from the National Institute of Arthritis and Metabolic Diseases, and by Life Insurance Medical Research grant G-61-16.

† Fellow of the Medical Research Council of Canada.

‡ Research Trainee, U. S. Public Health Service.

[1] Trivial names used are: progesterone (4-pregnene-3,20-dione); hydrocortisone (17-hydroxycorticosterone or 11β,17α-21-trihydroxy-4-pregnene-3,20-dione); aldosterone (11β,21-dihydroxy-18-formyl-4-pregnene-3,20-dione); pregnanediol (pregnane-3α,20α-diol or 3α,20α-dihydroxy-5β-pregnane); tetrahydroaldosterone (3α,11β,21-trihydroxy-18-formyl-5β-pregnan-20-one); and desoxycorticosterone (21-hydroxy-4-pregnene-3,20-dione).

jects were in the Women's Pavilion of the Royal Victoria Hospital in Montreal. The gestation time of the pregnancy was calculated from the first day of the last normal menstrual period. When the date of delivery was more than 3 weeks from the expected date of confinement, gestation was calculated from the date of delivery, provided that the fetus showed no evidence of pre- or postmaturity and that labor was spontaneous. All subjects except two were between 26 and 40 weeks of gestation. One subject was studied at 15 weeks just prior to a therapeutic abortion. A second subject was studied at 20 and 22 weeks because of a diagnosis of intrauterine fetal death. She was an epileptic with a history of eight previous fetal deaths, but on this occasion, pregnancy was carried to term with spontaneous delivery of a normal living infant.

Dietary intake. The dietary sodium ingested by the pregnant subjects was determined in several ways. Nine of the subjects were given a diet containing 50 mEq or less of sodium per day. These subjects were placed on diets calculated to contain the amount of sodium required by the experimental design. For the 1 to 2 weeks of this restricted diet, no other food was ingested. In this group of nine subjects, three ingested less than 10 mEq of sodium per day, and six ingested 25 to 50 mEq. Urinary sodium excretion was determined for the 2 days of aldosterone secretion rate in order to assess the adequacy of control of dietary sodium. In the three subjects ingesting less than 10 mEq of sodium per day, the average sodium ingested was 8 mEq, and the average urinary sodium excretion was 17.5 mEq per day. For the six subjects receiving 25 to 50 mEq of sodium per day the urinary sodium paralleled the ingested sodium. An average of 84% of the ingested sodium was excreted in the urine.

For the rest of the subjects studied, the dietary sodium was estimated by the dietitian attached to the home for unwed mothers in Burlington, Vermont. Each subject was instructed to keep a daily record of the food intake for 1 month. The sodium content of the diet for each subject was estimated from the knowledge of amount ingested, the source of the foods, and the methods employed in its preparation. The sodium content of the basal home diet was estimated to be between 150 and 170 mEq per day. The dietitian estimated that the error of the dietary sodium calculated in this manner was 25 to 40 per cent. Consequently, we have placed 51 subjects into a group whose dietary sodium was estimated to be between 80 and 190 mEq with an over-all average of 150 mEq of sodium per day. In this group, the urinary sodium averaged 70% of the ingested sodium. The constant dietary sodium intakes reported (Tables VI and VII) were achieved by placing the subjects on the basal home diet as described above. During the period of study, the daily sodium intake was estimated by the dietitian.

In nine subjects, higher intake of sodium was attempted by the addition of sodium chloride (about 3 g per day) to the basal diet. A daily dietary sodium of over 190 mEq was achieved in this way in only four of these subjects; for them, the average sodium ingested was 209 mEq per day, and an average of 76% of this sodium was excreted in the urine.

Determination of radioactivity. Samples to be counted were evaporated under nitrogen in 5-dram vials [2] and dissolved in 5 ml of toluene containing 0.3% of 2,5-diphenyloxazole (PPO) and 0.01% of 1,4-bis-2-(5-phenyloxazolyl)benzene (POPOP). These samples were counted in a Packard Tri-Carb (model 314-X) liquid scintillation spectrophotometer with the counting chamber set at $-2°$ C. The photomultiplier voltage tap was set at 5 (1,000 volts), and the pulse height discriminators were set at 10 to 100 (channel 1) and 100 to ∞ (channel 2). The efficiency of tritium counting for channel 1 at these voltage settings was approximately 16%. The efficiency of C^{14} counting for channel 2 at these voltage settings was approximately 60%. When C^{14} and H^3 were counted simultaneously in the toluene: PPO: POPOP system, the discriminator ratio method of Okita, Kabara, Richardson, and Leroy (27) as modified by Ulick (28) was employed for separating the C^{14} and H^3 counts. The "a" ratio (H^3 counts in channel 2/H^3 counts in channel 1) was maintained between 0.018 and 0.024. The "b" ratio (C^{14} counts in channel 2/C^{14} counts in channel 1) was between 1.7 and 2.2.

Purification of aldosterone. Each lot of *d*-aldosterone-7α-H^3 (SA, 20 μc per μg) [3] was tested for radiochemical purity by the following procedure. To a sample containing a known number of counts per minute was added a weighed amount of *d*-aldosterone.[4] The aldosterone was then chromatographed on successive paper systems, and the specific activity of the eluted steroid was determined at each stage by the blue tetrazolium reaction (29) on a measured sample. When a constant activity had been obtained for free aldosterone, it was acetylated with acetic anhydride-1-C^{14}.[5] The diacetate was then chromatographed on at least two systems until its specific activity was also constant, as determined by the H^3/C^{14} ratio of the eluted steroid. The specific activities at the various stages of purification for the lots of aldosterone used in this study are shown in Table I.

Administration of aldosterone. The labeled aldosterone was dissolved in benzene: methanol (10:1) and refrigerated for storage. A sample containing a known amount of radioactivity (approximately 1 μc and 0.05 μg) was pipetted into a 5-dram vial, and the solvent was evaporated under nitrogen. The steroid was dissolved in 0.5 to 1.0 ml of absolute ethanol, and the solution was diluted with 10 to 15 ml of 5% glucose in water. The diluted solution was either injected intravenously over several minutes, or it was injected into the tubing of an intravenous infusion. All of the glassware used in the prepa-

[2] Wheaton Glass Company, Millville, N. J.

[3] Courtesy of the Endocrine Study Section, National Institutes of Health, Bethesda, Md.

[4] Courtesy of Dr. Walter Murphy, Ciba Limited, Montreal, Canada.

[5] New England Nuclear Corporation, Boston, Mass.

ration of the injected aldosterone had been previously sterilized. The syringe, needle, and vial were washed with saline, ether, and ethyl acetate. A neutral ethyl acetate extract of the washes was prepared, and a sample was counted. There was seldom more than 5,000 cpm remaining in all of the washings.

Distribution of radioactivity in the urinary metabolites of aldosterone. Normal nonpregnant females in the follicular phase of the menstrual cycle and normal young males served as control subjects for these experiments. Four normal pregnant females in the third trimester were also studied. Each subject was injected with 300,000 to 700,000 cpm of aldosterone-7α-H^3. Urine was collected for 3 days and processed as follows. Ten per cent of each day's urine was extracted three times with 100 ml of methylene chloride in order to remove the free aldosterone. The methylene chloride was washed with 0.05 N Na_2CO_3 and then with water until neutral. After the methylene chloride extract was dried over Na_2SO_4, the solvent was removed on a flash evaporator, and the residue was taken for counting. The residual aqueous phase was then adjusted to pH 1 in order to hydrolyze the 3-oxo-conjugate (30) of aldosterone. After standing for 24 hours at room temperature, the urine was extracted with methylene chloride (0.5, 0.25, and 0.25 of the equivalent urine volume), the organic phase was processed as described above, and 10% of the neutral extract was taken for counting. A second 10% of each day's urine was hydrolyzed with β-glucuronidase as previously described (28), and 10% was again taken for counting. The rest of the extracts from each day's urine after pH 1 hydrolysis and β-glucuronidase hydrolysis were chromatographed on paper. After pH 1 hydrolysis, the extract obtained was chromatographed in the chloroform: formamide system for 3 to 4 hours, and the area on the paper corresponding to the reference aldosterone was eluted and counted. After β-glucuronidase hydrolysis, the resulting extract was chromatographed in the ethylene dichloride: formamide system until the isatin dye (2,3-indolinedione), used as marker, had migrated to the bottom of the paper. Tetrahydroaldosterone under these conditions has a migration rate of 0.6 to 0.8 of tetrahydrocortisone. The tetrahydroaldosterone was eluted and counted. The counts obtained were not corrected for losses incurred in the procedure.

Aldosterone secretion rates (ASR). Aldosterone secretion rates were determined by the isotope dilution technique described by Ulick, Laragh, and Lieberman (31). Only minor modifications were made in the method as originally described. The specific activity of the tetrahydroaldosterone was obtained from the H^3/C^{14} ratio of the triacetate formed after acetylation of the metabolite with previously standardized acetic anhydride-1-C^{14}. Acetylation of desoxycorticosterone was used to standardize the acetic anhydride. Where a high ASR was anticipated, acetic anhydride assaying at 20 to 50 cpm per μg of desoxycorticosterone acetate was used, whereas for medium and low secretion rates, acetic anhydride having 75 and 100 cpm per μg, respectively, was more advantageous. Only when the H^3/C^{14} ratio in the peak fraction and the two adjacent fractions from the final Celite partition chromatogram differed by less than 10% was the determination acceptable. The Celite partition column (cyclohexane: 85% methanol) could be used three times, after which the hold-back volume of the triacetate of tetrahydroaldosterone was too small.

The precision of the results obtained depends on the purity of the administered aldosterone and of the isolated urinary tetrahydroaldosterone. Ulick (28) has presented data demonstrating that the specific activity of tetrahydroaldosterone diacetate is constant after chromatography on paper in the methylcyclohexane: formamide system followed by Celite partition chromatography. We have extended these observations by measuring the specific activity of tetrahydroaldosterone in ten experiments after two consecutive chromatograms on the Celite partition

TABLE I

Chromatographic purification of d-aldosterone-7α-H^3

Chromatographic systems	Specific activity of batch						
	A	B	C	D	E	F	G
	cpm/μmole $\times 10^5$						
Before chromatography					7.73	7.61	2.78
A Chloroform: formamide					8.03	7.62	
B Toluene: propylene glycol	9.00		2.41	7.70			
C Bush C	8.44	4.45	2.91	7.99			
D Bush B_5		4.49			7.69	7.91	2.85
Acetylation with acetic anhydride-1-C^{14}							
E Methylcyclohexane: toluene 4:1/methanol: water 4:1	9.32	4.68	2.53	8.32	8.54	8.16	2.94
F Methylcyclohexane: toluene 1:1/formamide: methanol 1:1	9.00	4.85	2.24	7.67	8.45	7.95	2.95
G Toluene:Skellysolve—C 2:3/ water: methanol 4:1		4.85					

TABLE II

Excretion of radioactivity in urine after administration of aldosterone-7α-H³ to normal and to pregnant subjects

Subject	Day:	Free radioactivity 1	2	3	pH 1-hydrolyzable radioactivity 1	2	3	β-Glucuronidase-hydrolyzable radioactivity 1	2	3
					% of injected dose					
Normal										
A		1.4	0.1	*	19.0	0.9	0.2	45.2	3.2	0.5
B		1.6	0.1	0.1	20.9	1.2	0.5	54.0	7.1	2.5
C		1.3	0.1	*	18.8	1.4	0.3	54.0	5.7	1.2
D		1.7	0.2	0.1	17.5	0.8	0.2	44.7	2.8	0.4
E		1.5	0.4	0.1	8.8	2.6	1.0	36.0	8.1	3.2
Average		1.5	0.2	*	17.0	1.4	0.4	46.8	5.4	1.5
Pregnant										
A		1.0	*	*	20.2	0.1	*	52.7	0.8	*
B		0.3	0.2		19.4	0.4		22.2	1.1	
C		2.5	0.1	0.1	28.7	0.3	0.1	61.0	2.1	0.7
D		0.8	0.1	*	27.7	0.3	0.2	42.1	1.2	0.7
Average		1.1	0.1	*	24.0	0.3	0.1	44.5	1.3	0.5

* Less than 0.1% of injected dose.

column. The specific activity of tetrahydroaldosterone after the second chromatogram differed by less than 5%. Our counting error was not greater than 5%.

Creatinine determination. Creatinine in serum and urine was determined by a modification of the method of Folin adapted to an autoanalyzer as developed by Skeggs (32). In order to obtain adequate optical density readings from the low concentrations of serum creatinine encountered in pregnancy, the method was modified as follows. A sample rate of 20 per hour for serum and 40 per hour for urine was used in the autoanalyzer, and diluted serum was passed through two dialyzers in series. A wetting agent, Tween 20, was added to produce a more uniform rate of flow. The interfering chromogen encountered in serum is apparently bound to protein, since after dialysis in the autoanalyzer, excellent agreement is obtained with the Hare method (33), and a good recovery of creatinine added to synthetic sera is realized. Endogenous creatinine clearances were calculated from the two successive 24-hour urine collections used for the ASR.

Sodium determinations. Sodium in urine was measured by means of a Patwin flame photometer employing lithium as an internal standard.

RESULTS

Test of purity of the d-aldosterone-7α-H³. Although the aldosterone-7α-H³ was said to be 96% pure, it was tested for homogeneity in our laboratory. The specific activities obtained at the different stages of chromatography are shown in Table I. Because the tests of purity were done at different times over a 2-year period, the procedure used with each batch was not the same, and in batches A, B, C, and D, the specific activity just before chromatography was not determined. In subsequent lots, this was done, and there was a good agreement between the initial specific activity in lots E, F, and G and the final specific activtiy of the diacetate after chromatography in system F. In the earlier lots tested, there was a close correlation between the specific activity of the free aldosterone and its diacetate. This procedure demonstrated that the aldosterone-7α-H³ used was radiochemically homogeneous within the limits of our methods.

Urinary excretion and fractionation of radioactivity. Table II summarizes the results obtained from the fractionation of each 24-hour urine collection into unconjugated metabolites, metabolites released at pH 1 (3-oxo-conjugate) and metabolites resulting from β-glucuronidase hydrolysis. The values reported here have not been corrected for losses incurred in the extraction procedure. There was essentially no difference in the pattern of urinary excretion of radioactivity after an iv administration of aldosterone-7α-H³ in the pregnant as compared with the nonpregnant subjects. In the 3-day urine collection, the conjugated and unconjugated metabolites of aldosterone accounted for 74.2% of the injected radioactivity in normal subjects and 71.9% in pregnant subjects. In normal subjects, 1.7% of the injected dose was present in an unconjugated form, 18.8% was released by

acid hydrolysis, and 53.7% was released by β-glucuronidase hydrolysis, whereas in pregnant subjects the percentages of the administered dose were 1.2, 24.4, and 46.3, respectively. The radioactivity appearing in the urine in an unconjugated form was excreted mostly in the first 24 hours in both the pregnant and the nonpregnant subjects. Most of the radioactivity released after pH 1 hydrolysis was likewise excreted in the first 24 hours in both groups of subjects. After β-glucuronidase hydrolysis, the first 24-hour urine contained 87 and 96% of the radioactivity in the form of a glucosiduronidate in normal and pregant subjects, respectively.

In Table III are shown the results obtained after chromatography of the extracts resulting from the pH 1 and β-glucuronidase hydrolysis of the urinary conjugates. The aim of this study was to measure the radioactivity in the form of aldosterone and tetrahydroaldosterone excreted in the urine. Again, there was very little difference in the pattern of metabolism of aldosterone to urinary aldosterone released at pH 1 and tetrahydroaldosterone in pregnant as compared with nonpregnant subjects. In the nonpregnant, 8.3% of the injected dose was present as aldosterone and 19.8% as tetrahydroaldosterone, while in the pregnant subjects, the percentages were 9.5 and 21.3, respectively. In both groups of subjects, the radioactivity present as aldosterone was excreted mainly during the first 24 hours. The radioactivity present as tetrahydroaldosterone was also excreted mainly in the first 24 hours, 94.5 and 97.6% of that present in the 3-day urine appearing within the first 24 hours in normal subjects and in pregnant subjects, respectively. These data indicated that a 24-hour urine collection would suffice for the determination of aldosterone secretory rate, since 95% or more of the radioactivity present as tetrahydroaldosterone was excreted within this time. As a precaution, urine was collected for 2 days in all cases, although the first 24-hour urine collection was used for the determination of ASR.

In a large number of instances, crude urine counts were obtained on the second 24-hour urine. These did not reveal an appreciable excretion of radioactivity. In some instances, the metabolites in the second 24-hour urine were hydrolyzed, and tetrahydroaldosterone was isolated by paper chromatography. These experiments convinced us that at least 95% of the tetrahydroaldosterone was excreted in the first 24 hours, and since our estimation of the secretory rate was within 10%, it

TABLE III

Excretion of radioactivity in urine as aldosterone and tetrahydroaldosterone after injection of aldosterone-7α-H³ to normal and to pregnant subjects

Subject	Day:	Aldosterone from pH 1 hydrolysis 1	2	3	Tetrahydroaldosterone from β-glucuronidase hydrolysis 1	2	3
		% of injected dose					
Normal							
A		8.2	0.1	0.1	15.8	0.4	*
B		5.4	*	0.1	34.7	0.9	0.1
C		9.0	0.1	0.1	18.7	1.3	0.4
D		13.0	0.1	0.1	7.2	0.1	0.1
E		5.1	0.1	0.1	17.1	2.0	0.2
Average		8.1	0.1	0.1	18.7	0.9	0.2
Pregnant							
A		14.2	*	*	29.8	1.4	*
B		3.5	*		8.9	0.3	
C		11.7	0.1	*	14.0	0.4	*
D		8.3	0.2	*	30.7	0.1	0.1
Average		9.4	0.1	*	20.8	0.5	*

* Less than 0.1% of injected dose.

TABLE IV

Aldosterone secretion rate (ASR) in normal subjects

Subject	Age	Dietary Na	Dietary K	ASR
	years	*mEq/day*		*μg/day*
Women*				
J.R.	26	100	100	216
		†		179
L.S.	24	†		144
		†		136
L.M.	24	†		119
M.L.	26	†		85
E.B.	31	†		159
Y.C.	20	†		143
J.Z.	28	†		138
R.S.	32	†		192
Range				85–216
Average				151 ±37 (SD)
Men				
J.D.	26	150	100	138
		†		164
M.W.	27	150	100	88
H.K.	35	100	100	180
A.C.	24	†		102
S.S.	35	†		135
H.G.	23	100	100	137
		10	100	448
Range				88–180
				135 ±32 (SD)

* Studied during the follicular phase of the menstrual cycle.
† Ad libitum.

was not necessary to process the second 24-hour urine, although it was routinely saved until a satisfactory count could be obtained on the neutral samples of the first 24-hour collection.

Aldosterone secretion rate in normal men and women. ASR measured in normal men and women are summarized in Table IV. In the women studied, ASR was found to be 85 to 216 μg per day, while in normal men the values were 88 to 180 μg per day. J.R. and J.D. were studied on two occasions, initially on a rigidly controlled intake of sodium and potassium, and again on an unrestricted diet; ASR in J.R. were 216 and 179 μg per day, respectively, and in J.D., 138 and 164 μg per day, respectively. L.S. was studied on two occasions on an unrestricted diet during the follicular phase of the menstrual cycle. ASR on these two occasions were 144 and 136 μg per day. In H.G., the dietary sodium was restricted from 100 to 10 mEq per day, resulting in a rise in ASR from 137 to 448 μg per day.

ASR in normal pregnant subjects. Table V summarizes ASR measured in 57 subjects from weeks 15 to 39 of gestation. Three subjects in this group were on a free diet, while the remainder ingested 80 to 190 mEq of sodium per day. As early as week 15 of pregnancy, there is a significant elevation in ASR above the normal range established in our laboratories. It would appear that there is a gradual increase in ASR until about week 28, and that this rate of secretion is maintained through week 35 of gestation. Thereafter, there seems to be a further rise in ASR to term. To date, we have not found an ASR in a normal pregnant subject during the third trimester within the range of normal values measured in the follicular phase of the menstrual cycle (Table IV). A wide range in ASR was noted in different subjects at the same stage of gestation.

TABLE V

Changes in aldosterone secretion rate with increasing gestation in subjects receiving 80 to 190 mEq per day dietary sodium and free diets

Weeks of gestation	No. of subjects	Aldosterone secretion rates	
		Range	Average
		μg/day	
15	1*	387	
20	1*	892	
22	1*	953	
26 + 27	3	618– 999	859
28 + 29	4†	321–2,912	1,115
30 + 31	11	307–2,473	1,024
32 + 33	14‡	529–1,505	961
34 + 35	12	392–1,739	1,131
36 + 37	7	549–2,641	1,321
38 + 39	3	724 2,056	1,586
Total	57		

* Studied on free diets.
† Two of these subjects were on free diets.
‡ One of the subjects was on a free diet.

TABLE VI

Serial measurements of aldosterone secretion rate (ASR) in two subjects with constant dietary sodium of 160 mEq per day

Subject M		Subject J	
Weeks of gestation	ASR	Weeks of gestation	ASR
	μg/day		μg/day
30	709	28	525
31	1,047	30	552
33	616	32	734
34	880	34	392
35	735	36	1,440
39	1,980	38	724

Serial determination of ASR in two pregnant subjects. In two subjects, ASR was measured throughout the third trimester of pregnancy, and the results are shown in Table VI. In both subjects, there was considerable fluctuation in ASR measured with added weeks of gestation. In subject J, a fall in ASR was noted near term, but subject M demonstrated a rise in ASR at 39 weeks.

ASR at 4-week intervals on a constant dietary sodium. Because of wide fluctuations in ASR from one subject to the next, we decided to measure ASR at intervals of 4 weeks, with the subjects maintained on a constant sodium intake so that each subject could serve as her own control. The results are shown in Table VII, which includes some ASR measured serially in the two subjects shown in Table VI. With dietary sodium maintained at a constant level, there was a net increase in ASR over the four weeks of study in 14 of the 16 subjects. In two subjects (J1 at 30 to 34 weeks and M1 at 31 to 35 weeks), there was a net fall in ASR at week 4 of the study. The largest changes were noted when the second determination of ASR was performed in the last 4 weeks of pregnancy. This agrees with our earlier observations shown in Table V, where the average ASR seemed to increase in the last 4 weeks of pregnancy.

TABLE VII

Aldosterone secretion rates at 4-week intervals in subjects on a constant sodium diet

Subject	Dietary Na	Weeks of gestation—determination		Aldosterone secretion rate			
				Determination			
		1	2	1	2	Δ	% Δ
	mEq/day			μg/day			
J1	160	28	32	525	734	+ 209	+ 39.8
J1	160	30	34	552	392	− 160	− 28.9
M1	160	30	34	709	880	+ 171	+ 24.1
H1	156	30	34	307	570	+ 263	+ 85.6
M2	152	31	35	768	856	+ 88	+ 11.4
P1	178	31	35	1,415	1,599	+ 184	+ 13.0
M1	160	31	35	1,047	735	− 312	− 29.8
S1	165	31	35	971	1,739	+ 763	+ 79.0
J2	130	32	36	935	1,589	+ 654	+ 69.9
E1	169	32	36	1,425	2,641	+1,216	+ 85.3
J1	160	32	36	734	1,440	+ 706	+ 96.1
E2	108	32	36	954	961	+ 7	+ 0.7
B1	139	33	37	804	893	+ 89	+ 11.0
E3	121	33	37	1,072	1,174	+ 102	+ 9.5
J1	160	34	38	392	724	+ 332	+ 84.7
M1	160	35	39	735	1,980	+1,245	+169.4

ASR after change in dietary sodium. In a study comparable to the one just described, four subjects were maintained on a known amount of dietary sodium, which was then increased over 4 weeks. These experiments were also planned so that each subject could serve as her own control. In three other subjects, dietary sodium was restricted for 2 weeks. In both groups, ASR were measured twice. The experimental design and results are shown in Table VIII. One of the four subjects had a net increase in ASR when sodium was added to the diet. In the other three subjects, the reverse effect was observed. In the three subjects studied at intervals of 2 weeks, when dietary sodium was markedly restricted, there was a large increase in ASR, to some of the highest values measured in our laboratory.

Summary of effects of change in dietary sodium and of gestation on ASR. Table IX summarizes and compares ASR measured at different stages of gestation in subjects ingesting various amounts of dietary sodium. This table was compiled with some of the data in previous tables. Sodium intake varied from 6 to 225 mEq per day. Subjects have been divided into four dietary groups, namely, those receiving less than 10 mEq of sodium per day, 25 to 50 mEq, 80 to 190 mEq, and over 190 mEq per day. Average ASR in the group receiving between 80 to 190 mEq (Table V) was 1,129 μg per day. Increase of dietary sodium

TABLE VIII

Effect of changes in dietary sodium on the aldosterone secretion rate

Subject	Weeks of gestation—determination		Dietary Na			Aldosterone secretion rate		
			Determination			Determination		
	1	2	1	2	Δ Na	1	2	% Δ
			mEq/day			μg/day		
Added salt								
M3	27	31	108	174	+ 66	999	1,208	+ 20.9
J3	32	36	178	219	+ 41	711	596	− 16.1
N1	32	36	165	206	+ 41	1,206	635	− 47.3
P2	32	36	91	161	+ 70	1,505	549	− 63.5
Restricted salt								
S2	27	29	150	9	−141	960	9,224	+860.8
G1	31	33	150	6	−144	2,473	4,058	+ 64.1
M4	35	37	150	8	−142	1,296	10,248	+690.7

TABLE IX

*Effect of dietary sodium on average aldosterone secretion rates in pregnancy**

Weeks of gestation	mEq per day of dietary sodium			
	<10	25–50	80–190	>190
		μg aldosterone/day		
26+27			859 [3]	
28+29	9,224 [1]		1,718 [2]	
30+31			1,024 [11]	
32+33	4,058 [1]	797 [1]	979 [13]	843 [2]
34+35		1,440 [2]	1,131 [12]	
36+37	10,248 [1]	1,970 [1]	1,321 [7]	615 [2]
38+39		2,886 [2]	1,586 [3]	
Average	7,843 [3]	1,903 [6]	1,129 [51]	729 [4]

* Number of determinations in each group is given in brackets.

resulted in a decrease in ASR. For the four subjects receiving over 190 mEq of sodium per day, average ASR was 729 μg per day. Restriction of dietary sodium to between 25 and 50 mEq per day resulted in higher ASR, averaging 1,903 μg per day for the six subjects. Restriction of dietary sodium to less than 10 mEq per day in three subjects markedly increased ASR to as much as 10 mg per day, for an average of 7,843 μg per day. Any individual period of gestation shows the same pattern.

Relationship between endogenous creatinine clearance and ASR. Figure 1 shows the relationship between endogenous creatinine clearance, taken as an index of the quantity of sodium presented to the renal tubules by glomerular filtration, and the simultaneous ASR. The subjects were studied from weeks 30 through 37 of gestation with a dietary sodium intake of 120 to 190 mEq per day. No correlation was evident between the two values, either for the group as a whole or for subjects selected on the basis of shorter periods of gestation. Likewise, in a smaller number of subjects whose intake of sodium was more restricted, no correlation was evident between the glomerular filtration rate, as measured by the endogenous creatinine clearance, and ASR.

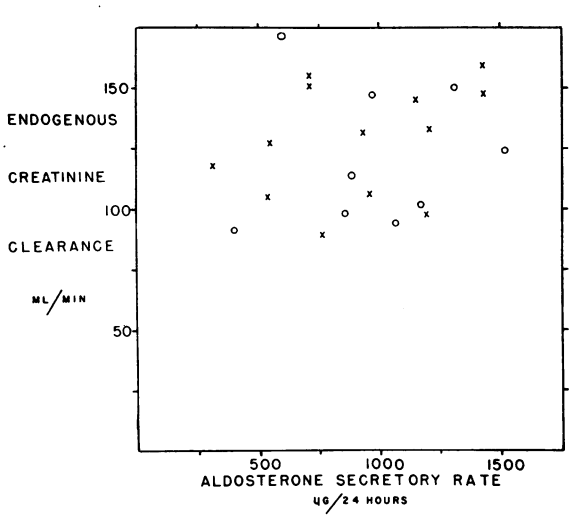

FIG. 1. THE RELATION BETWEEN ENDOGENOUS CREATININE CLEARANCE AND ALDOSTERONE SECRETION RATE IN PREGNANCY. Dietary sodium was 120 to 190 mEq per day. × = subjects between weeks 30 and 32 of gestation, and ○ = subjects between weeks 35 and 37, inclusive.

DISCUSSION

High urinary values of aldosterone have been reported in the last trimester of normal pregnancy by many investigators (19–25). High urinary excretion of aldosterone was assumed to reflect an increased secretion of aldosterone by the adrenal gland, but the findings of Jones and associates (9) suggested that this high urinary excretion may not reflect a proportionate increased secretion of the hormone. Although Jones and associates noted increased aldosterone secretion in half of the pregnant subjects in their study, they postulated that the increased urinary excretion of aldosterone in pregnancy was due in part to an alteration in the metabolism of the hormone from that in normal nonpregnant subjects. They reported an increased metabolism of aldosterone to the 3-oxo-conjugate and a decreased formation of the glucosiduronidates in pregnancy. The results obtained in our laboratory, however, do not support these findings. We cannot demonstrate any significant difference in the percentage of radioactivity released by acid hydrolysis or β-glucuronidase hydrolysis of aldosterone metabolites in the urine of pregnant and nonpregnant subjects (Table II). The radioactivity recovered as aldosterone and tetrahydroaldosterone after a single chromatographic separation on paper also showed no significant differences in the two groups (Table III).

The results obtained in our laboratory and by other investigators are compared in Table X, which shows that there are some differences. These may be related to the variability among individual subjects. Between 40 and 50% of the injected radioactivity appears to be recovered in

TABLE X

*Distribution of radioactivity in aldosterone metabolites reported in the literature**

First author	% of injected dose as			
	β-Glucuronidase-hydrolyzable	Tetrahydroaldosterone	pH 1-Hydrolyzable	Aldosterone
Normal subjects				
Watanabe (this paper)	36 –54 [46.8]	7.2–34.7 [18.7]	8.8–20.9 [17.0]	5.1–13 [8.1]
Jones (9)	37 –43 [39.0]		5.9–10.3 [8.0]	3.6– 6.8 [5.1]
Flood (34)	49 –54 [51]	30 –43 [36.5]	8.8–18.8 [11.8]	5.9– 9.5 [7.3]
Layne (35)		[36]		5.2– 9.7 [7.3]
Luetscher (36)	[43]			[10]†
Coppage (37)		28.7–50 [40.4]†		5.1–11.1 [8.5]
Pregnant subjects				
Watanabe (this paper)	22.2–61.0 [44.5]	8.9–30.7 [20.8]	19.4–27.7 [24.0]	3.5–14.2 [9.4]
Jones (9)	7 –41 [24.5]		12.6–24.2 [18.2]	7.8–19.4 [13.3]

* Average values are given in brackets.
† Corrected for recovery.

the first 24-hour urine after β-glucuronidase hydrolysis, with 30 to 40% present as tetrahydroaldosterone, and in both our pregnant and nonpregnant groups, our values compare with those of other investigators. Our values for radioactivity recovered as tetrahydroaldosterone, however, are lower than those of other investigators, perhaps because of our larger losses in the chromatographic procedure. The lower averages may also be related to the wide range of values within the small group studied. This condition also applies to the pregnant group of Jones and associates (9), in which radioactivity released by β-glucuronidase varied from 7 to 41%.

In the nonpregnant group, radioactivity released by pH 1 hydrolysis is the highest in our group of subjects, whereas radioactivity present as aldosterone compares with the results of others. There does not seem to be any significant difference in the percentage of radioactivity released by pH 1 hydrolysis for our normal subjects, our pregnant subjects, and the pregnant subjects of Jones and associates (9). The radioactivity present as aldosterone is highest in this group (9).

In six pregnant subjects, Jones and associates (9) reported ASR of 248, 260, 336, 596, 950, and 1,100 μg per day. Only the last three values fall outside 2 SD from their average for nonpregnant subjects. In our group of pregnant subjects, ASR showed similar variations from one subject to another, even at comparable stages of gestation and with comparable intakes of sodium. ASR were two to twenty times those of nonpregnant women during the follicular phase of the menstrual cycle, with an average ASR in the third trimester of pregnancy of 1,100 μg per day. The lowest values recorded in the pregnant subjects were 307, 321, and 392 μg per day, but all of these values fell outside 3 SD from our average nonpregnant values. In all of our pregnant subjects, therefore, ASR increased. It appears to increase above nonpregnancy levels even as early as week 15 of gestation. From week 28, and perhaps from as early as week 20, ASR appears to be fairly constant at about 1,000 μg per day. It increases further from week 36 of gestation to term.

With the realization that urinary excretion of aldosterone in normal individuals varied considerably with the amount of dietary sodium (38), Kumar, Feltham, and Gornall (24) investigated aldosterone excretion under conditions in a metabolic ward in six normal pregnant subjects ingesting 1 and 8 g of sodium chloride per day. Salt restriction of 1 g per day doubled the aldosterone excretion in the same pregnant subject to an average of 100 μg per day. Our data demonstrate that dietary sodium influences ASR in pregnant subjects (Tables VII through IX). When dietary sodium was increased by 41 to 70 mEq per day, there was an average decrease of 26.5% in ASR. In contrast, when dietary sodium was unaltered, there was a net increase of 45% in ASR over 4 weeks of gestation. Marked restriction of sodium to less than 10 mEq per day increased ASR by an average of 538%. When ASR was tabulated against the actual ingested

sodium for the 64 pregnant subjects (Table IX), the effect of dietary sodium on ASR was again evident. For the diets containing 25 to 225 mEq of sodium per day, average ASR appears to be inversely related to the sodium ingested. For the subjects receiving less than 10 mEq of sodium per day, there is a marked increase in ASR. This increase is greater than that seen for normal nonpregnant individuals under the same degree of sodium restriction (39).

Evidence from many sources indicates that sodium is retained during pregnancy, and that this retained sodium is required to meet the needs of the fetus and secundinae. Sodium balance studies in the third trimester of pregnancy have pointed to a persistent retention of sodium (25, 40–45), variously estimated to be from 7 to 50 mEq per day. Chesley (45) has suggested 18 mEq per day as a representative figure for the amount of sodium retained in late pregnancy. Dieckmann and Pottinger (46) reported that even on a low-sodium intake of 9 mEq per day, two normal pregnant women accumulated 50 mEq of sodium per kg increase in body weight in the last trimester of pregnancy. Serial studies of Plentl and Gray (47) and of Davey, O'Sullivan, and Browne (48) indicated that there is a net increase in exchangeable sodium, averaging 510 mEq in the last two trimesters of pregnancy. This represented an average gain of 20 mEq of exchangeable sodium per week. The authors felt that this increment in exchangeable sodium could be accounted for in the products of gestation and the expanded maternal blood volume. Lichton (49), in a study of pregnant rats, showed an average net accumulation of approximately 83 mEq of sodium per kg of weight gain throughout gestation. Of the total sodium retained, 15, 23, and 62% occurred in each successive third of the gestation. Analysis of the postpartum sodium balance and of fetal sodium at term indicated that there was no net accumulation of sodium in the tissues of the dams. The amount of sodium retained per kg weight gain of the pregnant dams was identical with the amount of sodium found in 1 kg of gravid uterus plus contents at term. The absolute amount of sodium retained by the pregnant dams was also not significantly different from the total amount of sodium present in the fetal tissues.

The withdrawal of sodium from the mother for deposition in the fetus can be expected to stimulate the secretion of aldosterone. Under these circumstances, it would be reasonable for ASR to be influenced by the sodium available in the diet. When dietary sodium is restricted to about 10 mEq per day, the sodium sequestered into the growing fetus represents a larger portion of the ingested sodium. If at least 10 mEq of sodium per day is sequestered into the uterus and its contents, all of the ingested sodium would have to be retained when the diet contains 10 mEq or less of sodium per day. Under these circumstances, a near maximal stimulation of aldosterone secretion by the adrenal gland may be expected.

The deposition of sodium into the uterus and its contents can be regarded as loss of sodium from the maternal vascular compartment. The mechanism of stimulation of aldosterone in pregnancy thus seems to be analagous to that proposed for experimental formation of ascites by Davis and associates (50, 51). In those experiments, sequestration of sodium or fluid, or both, from the vascular compartment into the peritoneal cavity stimulated the secretion of aldosterone by the adrenal gland. Davis, Kliman, Yankopoulos, and Peterson (50) also found that the stimulation of aldosterone by the loss of fluid and electrolytes from the vascular tree in experimental ascites could not be offset by expansion of the intravascular volume. This might also explain the fact that increased secretion of aldosterone occurs in pregnancy despite increased plasma volume (52) and extracellular volume (53).

If the increased secretion of aldosterone seen in pregnancy is indeed a homeostatic mechanism for the conservation of sodium for both mother and fetus, the basal secretion of 1,100 μg per day would appear to be excessive for the task, since all subjects were receiving between 80 and 190 mEq of dietary sodium per day. Other factors must then be considered that are unique to pregnancy and that may stimulate the increased secretion observed. Two such factors are the large amounts of progesterone secreted by the placenta and the increased filtered load of sodium seen in pregnancy.

The natriuretic action of progesterone was reported in a series of articles by Landau and his co-workers (54–56). This action was demon-

strated to be due to an inhibition of aldosterone by progesterone, presumably at the renal tubular level. Martin and Mills (20) first suggested that this competitive interaction of the two steroids may explain the elevated excretion of aldosterone in pregnancy. A correlation between urinary pregnanediol excretion and aldosterone secretion was reported for normal pregnant subjects by Jones and associates (9). Laidlaw, Ruse, and Gornall (57) demonstrated that exogenous progesterone could stimulate aldosterone secretion in normal nonpregnant subjects. No convincing natriuresis could be demonstrated, however, when progesterone was administered to three normal pregnant subjects by Landau, Plotz, and Lugibihl (58). This lack of effect was attributed to the inability of the administered progesterone to inhibit the large amounts of aldosterone secreted in pregnancy.

A second factor requires consideration. The glomerular filtration rate and consequently the filtered sodium presented to the renal tubules for reabsorption may increase as much as 50% in pregnancy (59). There is apparently no anatomical hypertrophy of the kidney in pregnancy (60), and unless factors not yet defined increase sodium reabsorption under these conditions, the increase in filtered sodium may require an increase in the secretion of aldosterone for adequate retention. In order to determine whether there was a direct relationship in pregnancy between the filtered load of sodium and the aldosterone secreted, we measured these two parameters simultaneously in a number of pregnant subjects (Figure 1). No correlation was observed between the glomerular filtration rate, as reflected by the endogenous creatinine clearance, and the aldosterone secretion rate. These findings do not exclude the possibility, however, that the increased glomerular filtration rate contributes to the increased secretion of aldosterone observed in pregnancy. The optimal experimental design for demonstrating such a relationship would be the measurement of these two parameters in pregnant subjects at comparable weeks of gestation, with comparable sodium ingestion and progesterone secretion.

SUMMARY

All of the pregnant subjects studied had increased aldosterone secretion rates during the third trimester of pregnancy. Our data indicate that the metabolism of aldosterone is not altered in the pregnant as compared with the nonpregnant subject. The increased secretion of aldosterone may be seen as early as week 15 of pregnancy. Wide fluctuations in secretory rate were noted among individual subjects for comparable periods of gestation. Average secretory rates suggested that from weeks 28 to 35 secretion of aldosterone was relatively uniform at about 1,000 μg per day. There was a further increase in the secretion from week 36 to term.

The data indicate that the secretion of aldosterone in pregnancy is influenced by dietary sodium. For diets containing 25 to 225 mEq of sodium per day, the aldosterone secreted appears to be inversely related to the ingested sodium. For diets containing less than 10 mEq of sodium per day, there is a marked stimulation in the secretion of aldosterone.

No correlation could be established between the glomerular filtration rate, as measured by the endogenous creatinine clearance, and the aldosterone secretion rate in pregnant subjects.

ACKNOWLEDGMENTS

The authors are indebted to Mrs. Ene Purre, Miss Nandita Sens Gupta, Mrs. Rosa Schwarz, and Miss Doreen Dewar for their expert technical assistance; to Drs. J. C. Beck and E. McGarry for the use of the Metabolic Ward and to grant MT-631 of the Medical Research Council of Canada for support of the Metabolic Ward, Royal Victoria Hospital. We thank Drs. J. Ruse and D. W. Killinger for their helpful criticism of the manuscript.

REFERENCES

1. Cohen, S. L., G. F. Marrian, and M. Watson. Excretion of œstrin during pregnancy. Lancet 1935, 1, 674.
2. Venning, E. H. Further studies on the estimation of small amounts of sodium pregnanedial glucuronidate in urine. J. biol. Chem. 1938, **126**, 595.
3. Westerfeld, W. W., D. W. MacCorquodale, S. A. Thayer, and E. A. Doisy. The isolation of theelin from human placenta. J. biol. Chem. 1938, **126**, 195.
4. Huffman, M. N., S. A. Thayer, and E. A. Doisy. The isolation of α-dihydrotheelin from human placenta. J. biol. Chem. 1940, **133**, 567.
5. Browne, J. S. L. Cited by J. B. Collip. Calif. Med. 1930, **1**, 38.
6. Pearlman, W. H., and E. Cerceo. The isolation of progesterone from human placenta. J. biol. Chem. 1952, **198**, 79.

7. Gemzell, C. A. Blood levels of 17-hydroxycorticosteroids in normal pregnancy. J. clin. Endocr. 1953, **13**, 898.
8. Slaunwhite, W. R., Jr., and A. A. Sandberg. Transcortin: a corticosteroid-binding protein of plasma. J. clin. Invest. 1959, **38**, 384.
9. Jones, K. M., R. Lloyd-Jones, A Riondel, J. F. Tait, S. A. S. Tait, R. D. Bulbrook, and F. C. Greenwood. Aldosterone secretion and metabolism in normal men and women and in pregnancy. Acta Endocr. (Kbh.) 1959, **30**, 321.
10. Van de Wiele, R. L., E. Gurpide, W. G. Kelly, J. R. Laragh, and S. Lieberman. The secretory rate of progesterone and aldosterone in normal and abnormal late pregnancy. Advanced Astracts of Short Communications, 1st Int. Congr. Endocr., E. Fuchs, Ed. Copenhagen, Periodica, 1960, p. 159.
11. Baulieu, E.-E., M. De Vigan, H. Bricaire, and M-F. Jayle. Lack of plasma cortisol and urinary aldosterone in a pregnant woman with Addison's disease. J. clin. Endocr. 1957, **17**, 1478.
12. Laidlaw, J. C., M. Cohen, and A. G. Gornall. Studies on the origin of aldosterone during human pregnancy. J. clin. Endocr. 1958, **18**, 222.
13. Christy, N. P., and J. W. Jailer. Failure to demonstrate hydrocortisone and aldosterone during pregnancy in Addison's disease. J. clin. Endocr. 1959, **19**, 263.
14. Venning, E. H., S. Sybulski, V. E. Pollak, and R. J. Ryan. Aldosterone excretion in two adrenalectomized pregnant women. J. clin. Endocr. 1959, **19**, 1486.
15. Chart, J. J., E. G. Shipley, and E. S. Gordon. Evidence for a salt retaining factor in toxemia of pregnancy. Proc. Soc. exp. Biol. (N. Y.) 1951, **78**, 244.
16. Gordon, E. S., J. J. Chart, D. Hagedorn, and E. G. Shipley. Mechanism of sodium retention in preeclamptic toxemia. Obstet. and Gynec. 1954, **4**, 39.
17. Venning, E. H., B. Singer, and G. A. Simpson. Adrenocortical function in toxemia of pregnancy. Amer. J. Obstet. Gynec. 1954, **67**, 542.
18. Barnes, A. C., and E. J. Quilligan. Measurements of aldosterone in the eclamptogenic toxemias of pregnancy. Amer. J. Obstet. Gynec. 1956, **71**, 670.
19. Venning, E. H., and I. Dyrenfurth. Aldosterone excretion in pregnancy. J. clin. Endocr. 1956, **16**, 426.
20. Martin, J. D., and L. H. Mills. Aldosterone excretion in normal and toxæmic pregnancies. Brit. Med. J. 1956, **2**, 571.
21. Venning, E. H., T. Primrose, L. C. S. Caligaris, and I. Dyrenfurth. Aldosterone excretion in pregnancy. J. clin. Endocr. 1957, **17**, 473.
22. Koczorek, K. R., H. P. Wolff, and M-L. Beer. Über die Aldosteronausscheidung bei Schwangerschaften und bei Schwangerschaftstoxikosen. Klin. Wschr. 1957, **35**, 497.
23. Rinsler, M. G., and B. Rigby. Function of aldosterone in the metabolism of sodium and water in pregnancy. Brit. Med. J. 1957, **2**, 966.
24. Kumar, D., L. A. W. Feltham, and A. G. Gornall. Aldosterone excretion and tissue electrolytes in normal pregnancy and pre-eclampsia. Lancet 1959, **1**, 541.
25. Venning, E. H., I. Dyrenfurth, L. Lowenstein, and J. Beck. Metabolic studies in pregnancy and the puerperium. J. clin. Endocr. 1959, **19**, 403.
26. Ayres, P. J., O. Garrod, S. A. S. Tait, J. F. Tait, and G. Walker. The use of 16^3-H aldosterone in studies on human peripheral blood. Ciba Found. Colloq. Endocr., G. E. W. Wolstenholme and E. C. P. Millar, Eds. 1957, **11**, 309.
27. Okita, G., J. J. Kabara, F. Richardson, and G. V. Leroy. Assaying compounds containing H^3 and C^{14}. Nucleonics 1957, **15**, no. 6, 111.
28. Ulick, S. Stereospecificity in the metabolism of aldosterone in man. J. biol. Chem. 1961, **236**, 680.
29. Nowaczynski, W., M. Goldner, and J. Genest. Microdetermination of corticosteroids with tetrazolium derivatives. J. Lab. clin. Med. 1955, **45**, 818.
30. Ayres, P. J., J. Barlow, O. Garrod, A. E. Kellie, S. A. S. Tait, J. F. Tait, and G. Walker. The metabolism of (16^3-H) aldosterone in man *in* An International Symposium of Aldosterone, A. F. Muller and C. M. O'Connor, Eds. London, Churchill, 1958, pp. 73–99.
31. Ulick, S., J. H. Laragh, and S. Lieberman. The isolation of a urinary metabolite of aldosterone and its use to measure the rate of secretion of aldosterone by the adrenal cortex of man. Trans. Ass. Amer. Phycns 1958, **71**, 225.
32. Skeggs, L. T., Jr. An automatic method for colorimetric analysis. Amer. J. clin. Path. 1957, **28**, 311.
33. Hare, R. S. Endogenous creatinine in serum and urine. Proc. Soc. exp. Biol. (N. Y.) 1950, **74**, 148.
34. Flood, C., D. S. Layne, S. Ramcharan, E. Rossipal, J. F. Tait, and S. A. S. Tait. An investigation of the urinary metabolites and secretion rates of aldosterone and cortisol in man and a description of methods for their measurement. Acta Endocr. (Kbh.) 1961, **36**, 237.
35. Layne, D. S., C. J. Meyer, P. S. Vaishwanar, and G. Pincus. The secretion and metabolism of cortisol and aldosterone in normal and steroid-treated women. J. clin. Endocr. 1962, **22**, 107.
36. Luetscher, J. A., A. J. Dowdy, A. M. Callaghan, and A. P. Cohn. Studies of secretion and metabolism of aldosterone and cortisol. Trans. Ass. Amer. Phycns 1962, **75**, 293.
37. Coppage, W. S., Jr., D. P. Island, A. E. Cooner, and G. W. Liddle. The metabolism of aldosterone in normal subjects and in patients with hepatic cirrhosis. J. clin. Invest. 1962, **41**, 1672.
38. Luetscher, J. A., Jr., and B. J. Axelrad. Increased aldosterone output during sodium deprivation in normal men. Proc. Soc. exp. Biol. (N. Y.) 1954, **87**, 650.

39. Mills, J. N. Aldosterone secretion in man. Brit. med. Bull. 1962, **18**, 170.
40. Thompson, H. E., Jr., and W. T. Pommerenke. Electrolyte and nitrogen metabolism in pregnancy. J. Nutr. 1939, **17**, 383.
41. Taylor, H. C., Jr., R. C. Warner, and C. A. Welsh. The relationship of the estrogens and other placental hormones to sodium and potassium balance at the end of pregnancy and in the puerperium. Amer. J. Obstet. Gynec. 1939, **38**, 748.
42. Hummel, F. C., H. R. Sternberger, H. A. Hunscher, and I. G. Macy. Metabolism of women during the reproductive cycle. VII. Utilization of inorganic elements (a continuous case study of a multipara). J. Nutr. 1936, **11**, 235.
43. Coons, C. M., R. R. Coons, and A. T. Schiefelbusch. The acid-base balance of the minerals retained during human pregnancy. J. biol. Chem. 1934, **104**, 757.
44. Freyberg, R. H., R. D. Reekie, and C. Folsome. A study of water, sodium, and energy exchange during the latter part of pregnancy. Amer. J. Obstet. Gynec. 1938, **36**, 200.
45. Chesley, L. C. Weight changes and water balance in normal and toxic pregnancy. Amer. J. Obstet. Gynec. 1944, **48**, 565.
46. Dieckmann, W. J., and R. E. Pottinger. Etiology of preeclampsia-eclampsia. V. Extra- and intracellular fluid changes and electrolyte balances. Amer. J. Obstet. Gynec. 1955, **70**, 822.
47. Plentl, A. A., and M. J. Gray. Total body water, sodium space, and total exchangeable sodium in normal and toxemic pregnant women. Amer. J. Obstet. Gynec. 1959, **78**, 472.
48. Davey, D. A., W. J. O'Sullivan, and J. C. M. Browne. Total exchangeable sodium in normal pregnancy and in preeclampsia. Lancet 1961, **1**, 519.
49. Lichton, I. J. Salt saving in the pregnant rat. Amer. J. Physiol. 1961, **201**, 765.
50. Davis, J. O., B. Kliman, N. A. Yankopoulos, and R. E. Peterson. Increased aldosterone secretion following acute constriction of the inferior vena cava. J. clin. Invest. 1958, **37**, 1783.
51. Davis, J. O., and W. C. Ball, Jr. Effects of a body cast on aldosterone and sodium excretion in dogs with experimental ascites. Amer. J. Physiol. 1958, **192**, 538.
52. Tysoe, F. W., and L. Lowenstein. Blood volume and hematologic studies in pregnancy and the puerperium. Amer. J. Obstet. Gynec. 1950, **60**, 1187.
53. Chesley, L. C. A study of extracellular water changes in pregnancy. Surg. Gynec. Obstet. 1943, **76**, 589.
54. Landau, R. L., D. M. Bergenstal, K. Lugibihl, and M. E. Kascht. The metabolic effects of progesterone in man. J. clin. Endocr. 1955, **15**, 1194.
55. Landau, R. L., K. Lugibihl, D. M. Bergenstal, and D. F. Dimick. The metabolic effects of progesterone in man: dose response relationship. J. Lab. clin. Med. 1957, **50**, 613.
56. Landau, R. L., and K. Lugibihl. Inhibition of the sodium-retaining influence of aldosterone by progesterone. J. clin. Endocr. 1958, **18**, 1237.
57. Laidlaw, J. C., J. L. Ruse, and A. G. Gornall. The influence of estrogen and progesterone on aldosterone excretion. J. clin. Endocr. 1962, **22**, 161.
58. Landau, R. L., E. J. Plotz, and K. Lugibihl. Effect of pregnancy on the metabolic influence of administered progesterone. J. clin. Endocr. 1960, **20**, 1561.
59. Sims, E. A. H., and K. E. Krantz. Serial studies of renal function during pregnancy and the puerperium in normal women. J. clin. Invest. 1958, **37**, 1764.
60. Sims, E. A. H. Chapter 28 *in* The Kidney in Pregnancy in Diseases of the Kidney, M. B. Strauss and L. J. Welt, Eds. Little, Brown, in press.