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CHANGES IN WATER AND ELECTROLYTE BALANCE, PLASMA VOLUME AND COMPOSITION DURING PREGNANCY IN THE RAT

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SUMMARY

1. Daily changes in water and electrolyte balance during pregnancy were investigated in rats housed in metabolism cages.

2. Fluid intake was significantly elevated above control values from day 13 of pregnancy, with urine output failing to be raised to the same extent. This would result in an extensive fluid retention if extrarenal fluid losses were not substantially altered.

3. Electrolyte intake increased from as early as the third day after mating with an accompanying increase in renal ionic excretion. A net retention of Na, Cl and K did not occur until the final week of pregnancy when the urinary output of these ions was reduced.

4. In a parallel study, changes in plasma volume and composition throughout pregnancy were investigated.

5. A significant increase in plasma volume occurred from day 6 of pregnancy at a time well before fluid intake or urine output were altered. This indicates either an altered extrarenal output or a shift of fluid between body fluid compartments.

6. Maternal plasma sodium and total osmolality were reduced during the last week of pregnancy despite the salt retention, suggesting an increased fetal usage.

7. Such findings are related to the known renal and endocrine changes of rat pregnancy.

INTRODUCTION

Recent studies have demonstrated increases in glomerular filtration rate (G.F.R.) and salt and water reabsorption at an early stage of pregnancy in the rat (Bishop & Green, 1980; Atherton, 1981; Atherton & Pirie, 1981; Garland & Green, 1982). Midterm changes in glomerular function appear to depend on an elevated renal plasma flow rate, resulting from plasma volume expansion (Baylis, 1980). In other studies the timing of the renal changes have been questioned (Lindheimer & Katz, 1971; Davison & Lindheimer, 1980).

Much of the above information has been obtained from saline-infused rats, and there is little data available for non-infused intact animals. Moreover, no attempt

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has been made in the above studies to investigate changes in fluid or electrolyte *intake* which might contribute to or result from the renal and cardiovascular changes.

The few previous attempts to quantify water and electrolyte balance in rat pregnancy (Lichton, 1961; Kirksey & Pike, 1962; Churchill, Bengele & Alexander, 1980) have been concerned primarily with the sodium retention of the third trimester and all have presented compounded weekly balance data. Because of the controversy surrounding the exact timing of the renal changes in pregnancy, the present study was undertaken in order to establish a daily pattern of water and electrolyte balance throughout the gestation period and to elucidate some of the factors that could be responsible for any plasma volume expansion. The possible link between G.F.R. and plasma volume was further investigated by determining a precise time course for plasma volume changes in pregnancy.

METHODS

Experiments were performed on virgin and pregnant female Sprague–Dawley rats aged 10–13 weeks at the start of the experiment. Animals were maintained under a constant 12 hr light photoperiod (08.00–20.00 hr) and a temperature of 21–23 °C. Three experimental studies were undertaken.

Series (I) balance studies

These were performed on animals housed individually in glass metabolism cages (Jencons 'Metabowls'; Jencons Scientific Ltd., Hemel Hempstead). Water (distilled) and food (CRM rat and mouse diet, averaging (m-mole g^{-1}): Na, 0·12; Cl, 0·11; K, 0·22) were available *ad libitum* throughout.

Animals were acclimatized to the metabolism cages for a period of 10–14 days before the experiment. They were then split into two weight-matched groups, and one group was mated ($n = 9$), the onset of pregnancy being determined by the appearance of a cervical plug of mucus on the cage floor. This was designated as day 1 of pregnancy. The other group of rats ($n = 10$) acted as virgin controls. Daily (24 hr) measurements were made of body weight, food and water intake, and urinary output over the subsequent three weeks. Measurements were always taken between 09.30 and 11.00 hr, and cages cleaned daily. Urine samples were stored at −20 °C until required for analyses.

Series (II) plasma volume studies

Age and weight-matched pairs of rats were used, one animal from each pair being mated, the other acting as a virgin control. Food and water were allowed *ad libitum*, and rats studied at the following times after mating: 3, 4, 9, 13, 16 or 20 days ($n = 8$ on each occasion).

On the morning of the experiment, animals were anaesthetized with ether, and a catheter (pp50) inserted into the left carotid artery and exteriorized on the dorsal surface of the neck. This was subsequently used to monitor blood pressure and heart rate (Statham P23 DC Transducer; Grass 7 polygraph) and to remove blood samples. After a period of at least 3 hr, the rats were again lightly anaesthetized with ether and a second catheter (pp10) placed in a lateral tail vein. Animals were then comfortably positioned in metabolic restraining cages (Atherton, Hai & Thomas, 1968) and allowed to recover.

Mean arterial blood pressure and heart rate were monitored until stable values were obtained (usually 60–90 min after the second operation). 0·1 ml. Evans Blue dye (6 mg $ml.^{-1}$ in 0·9 % saline) was then injected via the tail vein catheter, and 15 min later a carotid arterial blood sample collected in heparinized microcentrifuge tubes (300 μl). The blood was centrifuged, haematocrit measured, and Evans Blue concentration determined on the separated plasma.

Series (III) plasma composition studies

Animals were studied at the same stages of pregnancy as for series (II) experiments. They were allowed free access to food and water prior to experimentation. Values for (n) are shown in Fig. 7. The rats were weighed and then decapitated using a small mammal guillotine, and blood collected

into heparinized tubes. These were kept on ice until centrifuged, and plasma immediately drawn off and stored frozen at −20 °C until analysed.

Analyses. For series (I) and (III) urine and plasma samples were analysed for sodium and potassium by flame photometry (Corning-EEL flame photometer 450) and for osmolality using a Roebling Osmometer. Series (I) urines were further analysed for chloride using a chloride meter (Corning-EEL 920).

For series (II), Evans Blue concentration was determined colorimetrically (Cecil Spectrophotometer 272, 620 nm) in both the injected solution and plasma. Its volume of distribution was then calculated.

Data are presented as means ± S.E. of mean. Statistical analysis was performed using a Student's *t* test for unpaired samples. Levels of significance are indicated by the symbols *, $P < 0.05$; †, $P < 0.01$; ‡, $P < 0.001$.

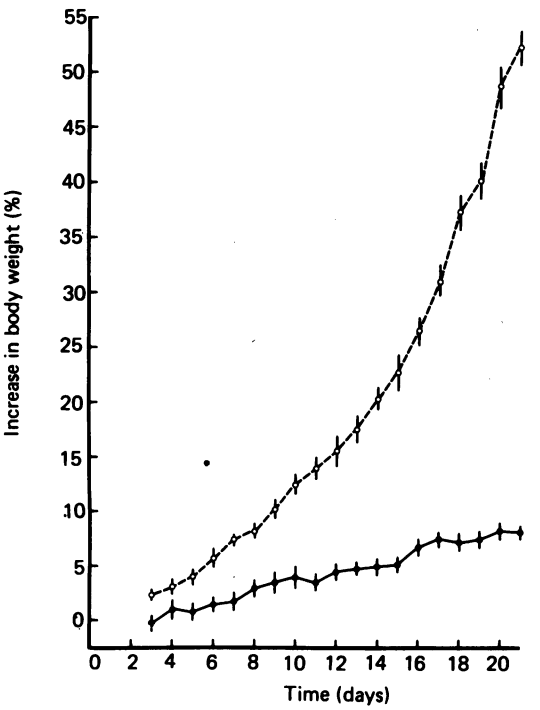

Fig. 1. Changes in body weight (expressed as percentages of that on day 2) for pregnant (○, $n = 9$) and virgin control (●, $n = 10$) animals housed in metabolism cages. Differences between the two groups were significant from day 5 onwards.

RESULTS

General data

Pregnant animals used in series (II) and (III) experiments showed similar changes in body weight to those previously described (Garland, Green & Moriarty, 1978). Body weight changes for the rats kept in metabolic cages (expressed as percentage of body weight on day 2) are shown in Fig. 1. They also indicate normal growth curves. Fetal numbers in the three experimental series ranged from nine to sixteen per pregnant female.

(I) Balance studies

Daily values of intake and urinary output are presented in Figs. 2–5 and cumulative weekly values shown in Table 1 to allow comparison with previous studies. In Table 1, week 1 consists of only 6 days in both groups since 24 hr collections were begun on day 1 of pregnancy.

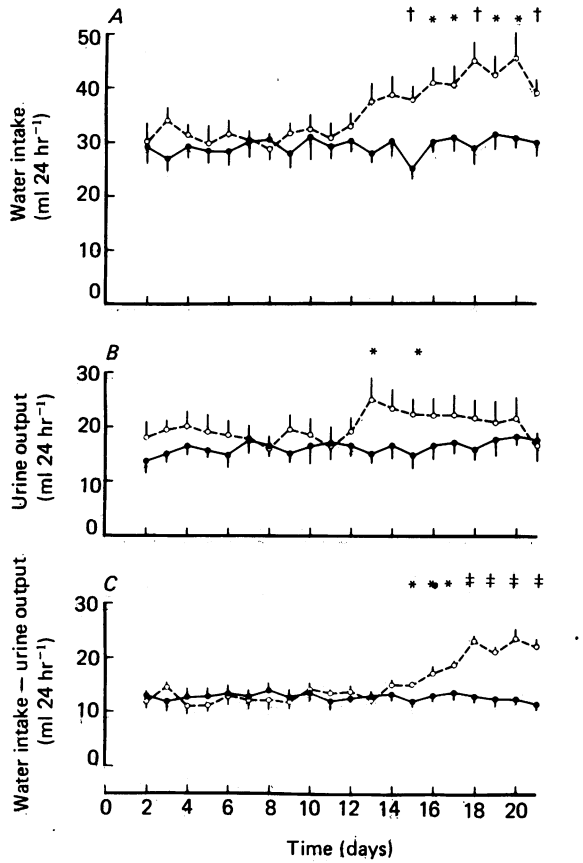

Fig. 2. Daily changes in *A*, water intake; *B*, urine output and *C*, water intake−urine output, for pregnant (○) and virgin control (●) animals. *n*, as in Fig. 1.

Water balance. Daily changes in water intake for the two groups of animals (Fig. 2*A*) show virgin controls to drink around 30 ml. per day during the experimental period. Water intake for pregnant rats was similar to controls for the first half of pregnancy, but was increased from day 13 onwards. Weekly data (Table 1) indicate a 37 % increase in water intake during the final week of pregnancy when compared to control values for the same period.

Urine output in controls was around 15 ml. per day (Fig. 2*B*). Pregnant animals showed a modest increase in urine output over control values during the second half of pregnancy, but this increase only achieved statistical significance on days 13 and 15. Cumulative weekly values (Table 1) showed no significant differences between the two groups.

The combined effects of changes in water intake and urine output are shown in Fig. 2*C*, where the daily mean difference between these two parameters is presented. This value increased progressively from day 14 onwards in the pregnant group, so that the last week of pregnancy showed a significant 56% increase compared to control values (Table 1).

TABLE 1. Cumulative weekly data for balance studies in virgin control (V, $n = 10$) and pregnant (P, $n = 9$) animals

		Week 1	Week 2	Week 3
Water intake (ml.)	V	172·3 ± 10·8	207·1 ± 12·7	207·2 ± 13·3
	P	183·6 ± 11·8	222·3 ± 17·8	284·1 ± 21·1†
Urine output (ml.)	V	93·2 ± 10·2	113·1 ± 12·5	117·3 ± 13·0
	P	109·2 ± 14·4	128·0 ± 19·7	144·9 ± 22·3
Water intake − Urine output (ml.)	V	76·6 ± 6·0	93·2 ± 6·8	89·0 ± 7·2
	P	74·3 ± 5·3	94·3 ± 6·1	139·2 ± 6·4‡
Food intake (g)	V	106·0 ± 3·6	135·6 ± 5·0	133·8 ± 5·2
	P	117·7 ± 2·8*	161·6 ± 4·5†	168·8 ± 4·7‡
Urinary sodium output (m-mole)	V	8·8 ± 0·4	11·1 ± 0·5	11·8 ± 0·4
	P	10·7 ± 0·4†	12·8 ± 0·5*	11·7 ± 0·5
Sodium intake − urinary sodium output (m-mole)	V	3·9 ± 0·6	5·2 ± 0·5	4·3 ± 0·5
	P	3·5 ± 0·3	5·9 ± 0·5	8·9 ± 0·7‡
Urinary chloride output (m-mole)	V	11·4 ± 0·5	14·2 ± 0·7	14·4 ± 0·7
	P	12·8 ± 0·4*	16·6 ± 0·5*	14·7 ± 0·6
Chloride intake − urinary chloride output (m-mole)	V	0·3 ± 0·4	0·8 ± 0·5	0·3 ± 0·5
	P	0·5 ± 0·3	0·9 ± 0·5	3·9 ± 0·6†
Urinary potassium output (m-mole)	V	13·5 ± 0·7	17·3 ± 0·9	18·3 ± 0·8
	P	15·3 ± 0·4*	20·3 ± 1·0*	22·0 ± 0·8†
Potassium intake − urinary potassium output (m-mole)	V	9·9 ± 1·2	12·9 ± 1·1	11·2 ± 0·9
	P	10·6 ± 0·7	14·6 ± 1·0	14·9 ± 1·2*

Sodium balance. Daily changes in food intake for both groups (Fig. 3*A*) show pregnant animals to eat significantly more food from as early as day 3 of pregnancy. Cumulative weekly values (Table 1) show increases of 11, 19 and 26% for pregnant animals compared to virgin controls during the 3 weeks studied. Since the food is the only source of sodium and other electrolytes, changes in their intake will parallel food intake, and consequently be identical to Fig. 3*A*. Similarly, cumulative electrolyte intake will parallel cumulative food intake, their percentage increases during pregnancy being the same as those quoted above.

Mean daily values for urinary osmolality in pregnant animals were lower than in controls, but the differences did not achieve statistical significance. There were no significant differences in mean daily values for urinary sodium concentration between the two groups. For controls, values ranged from 86·7–132·2 m-mole $l.^{-1}$; for pregnant animals the range was 79·3–129·1 m-mole $l.^{-1}$. Urinary sodium *output*, however, did alter during pregnancy, and daily changes are shown in Fig. 3*B*. Pregnant animals excreted significantly more sodium than virgin controls during the first two weeks of pregnancy, cumulative weekly values (Table 1) indicating increases of 21% and 15% respectively. During the third week, however, urinary sodium output decreased

in pregnant animals to finish significantly lower than control values on day 21 (Fig. 3*B*). Cumulative values for week 3 (Table 1), showed no significant difference between the two groups.

An assessment of sodium balance (excluding extrarenal losses) is shown in Fig. 3*C* and Table 1, obtained from daily mean differences between sodium input and output. An increase in sodium retention is evident in pregnant animals during the last week of pregnancy, cumulative values for these animals being over twice the value obtained from virgin controls over the same period.

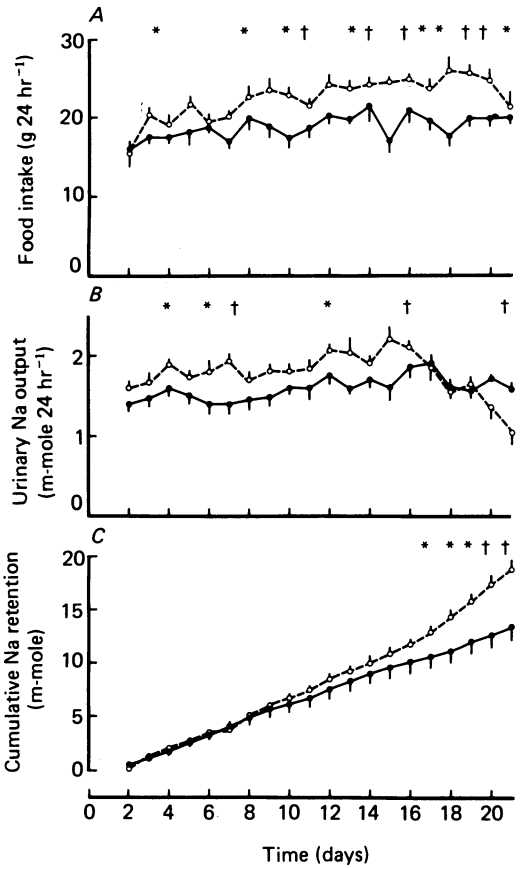

Fig. 3. Daily changes in *A*, food intake; *B*, urinary sodium output; and *C*, cumulative sodium retention, for pregnant (○) and virgin control (●) animals. *n* as in Fig. 1.

Chloride balance. Chloride intake increased significantly during each of the three weeks of pregnancy (Table 1). Urinary chloride concentrations were similar in both experimental groups. Daily mean values for controls ranged from 115·3–153·8 m-mole $l.^{-1}$; for pregnant rats the range was 108·3–163·3 m-mole l^{-1}. Daily changes in urinary chloride output (Fig. 4*A* and Table 1) indicate a similar pattern to that seen for sodium, with a significantly elevated output during the first and second weeks of pregnancy. Again during the third week, urinary chloride output decreased in the pregnant group so that values for days 20 and 21 were significantly lower than

controls on these days. Cumulative values for week 3 (Table 1) show no significant differences between the two groups.

Chloride balance data (Fig. 4*B* and Table 1), calculated as for sodium, demonstrate a retention of this ion during the last week of pregnancy.

Potassium balance. Potassium intake was elevated during pregnancy (Table 1). There were no significant differences in mean daily values for urinary potassium concentrations. For controls, values ranged from 123·9–185·1 m-mole $l.^{-1}$; for pregnant

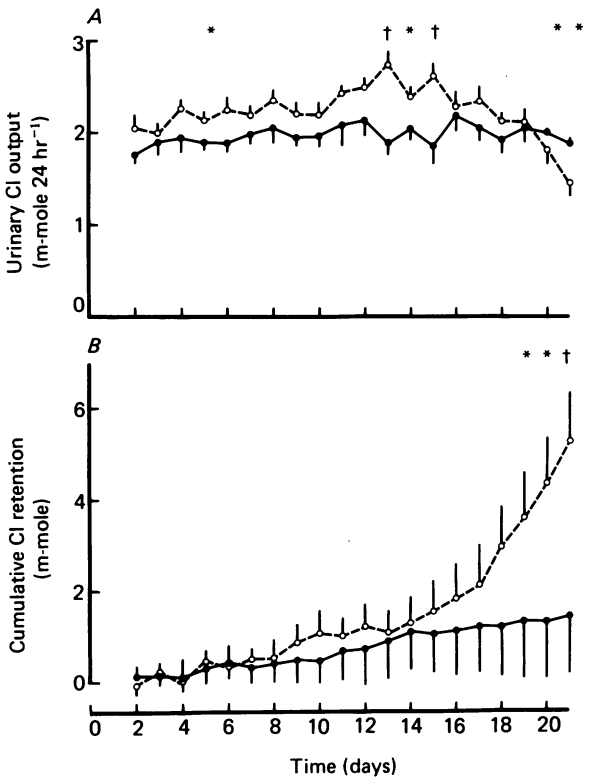

Fig. 4. Daily changes in *A*, urinary chloride output, and *B*, cumulative chloride retention, in pregnant (○) and virgin control (●) animals. *n* as in Fig. 1.

animals, the range was 143·4–189·4 m-mole $l.^{-1}$. Daily changes in urinary potassium output showed significantly elevated values during all three weeks of pregnancy when compared to control groups (Fig. 5*A*, Table 1). The decreased urinary potassium output seen towards term (Fig. 5*A*) was not as acute as for sodium or chloride, and values obtained from pregnant rats on days 20 and 21 were not significantly different from controls. As a consequence, Fig. 5*B* shows no significant difference in the retention of this ion between the two groups. The weekly cumulative data in Table 1, however, indicates a greater potassium retention during the third week of pregnancy, and in absolute amounts, the retention of this ion is greater than either sodium or chloride.

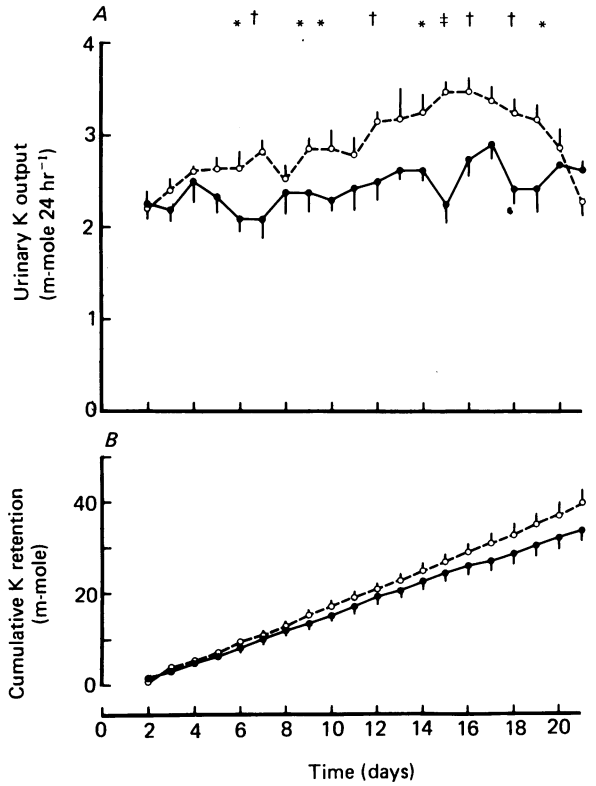

Fig. 5. Daily changes in *A*, urinary potassium output, and *B*, cumulative potassium retention, in pregnant (○) and virgin control (●) animals. *n* as in Fig. 1.

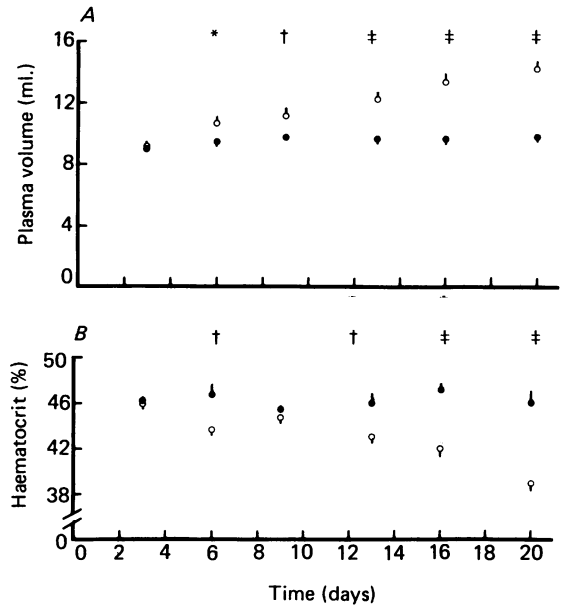

Fig. 6. Changes in *A*, plasma volume, and *B*, haematocrit in pregnant (○) and virgin control (●) animals. $n = 8$ for each group at each stage of pregnancy.

(II) Plasma volume studies

There were no significant differences in either blood pressure or heart rate between pregnant animals and their respective controls at any experimental stage. Mean values for all groups of rats for blood pressure were 120 ± 1 mmHg (virgin) and 118 ± 1 mmHg (pregnant). Corresponding values for heart rate were 449 ± 5 beats min^{-1} (virgin) and 456 ± 6 beats min^{-1} (pregnant).

Values for plasma volume and haematocrit are shown in Fig. 6*A* and *B*. A significant increase in plasma volume was detected as early as day 6 of pregnancy, and, close to term, pregnant animals had a plasma volume approximately 30 % higher than their respective controls. Haematocrit was lower in the pregnant group and approximately 39 % close to term.

(III) Plasma composition studies

A significant decrease in plasma osmolality was first seen in 13 day pregnant rats when compared to both virgin controls and earlier stages of pregnancy (Fig. 7). There was little further subsequent change. Changes in plasma sodium concentrations followed a similar pattern.

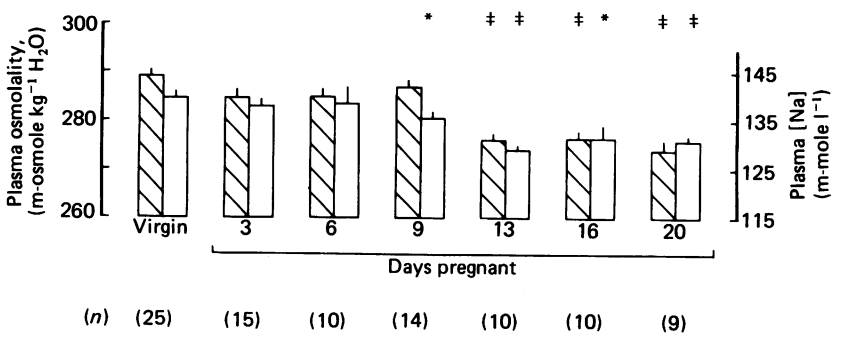

Fig. 7. Changes in plasma sodium (□) and total osmolality (▨) in virgin control and pregnant animals. The numbers in parentheses represent the numbers of animals used in each group.

DISCUSSION

The present data extended the few previous studies in rats (Lichton, 1961; Kirksey & Pike, 1962; Churchill *et al.* 1980) and provide a detailed background for the more specific investigations of renal functional changes during pregnancy. Pregnant animals housed in metabolism cages show weight changes comparable with those housed under more conventional conditions (Garland *et al.* 1978).

Water balance

The data demonstrate a progressively increasing difference between water intake and urine output from day 15 of pregnancy resulting from a large increase in fluid intake with urine output failing to be elevated to the same extent. If extra-renal fluid losses do not change appreciably, this would indicate an extensive fluid retention

during the final week of pregnancy. Churchill *et al.* (1980) showed a similar retention in their animals, although fluid intake and urine output were found to be elevated earlier in pregnancy.

The cause of the increased drinking is not known. Certainly there is no increase in plasma osmolality (Fig. 7 present study) or decrease in extracellular fluid volume (e.c.f.v.) (Atherton & Pirie, 1981) although unpublished data from our laboratory indicates a midterm increase in plasma renin activity. Angiotensin, if in turn stimulated, is known to have a potent dipsogenic action in the rat (Epstein, Fitzsimons & Rolls, 1970). Prolactin may also be involved here as it is elevated early in pregnancy (Butcher, Fugo & Collins, 1972; Smith & Neill, 1976) and can act synergistically with angiotensin to increase drinking in rats (Kaufman, 1981).

The extensive fluid retention must contribute to the continuing plasma volume expansion (Fig. 6*A*) as well as to the expanded e.c.f.v. (Churchill *et al.* 1980; Atherton & Pirie, 1981) and reduced plasma osmolality. However, the significant increase in plasma volume during the first week of pregnancy appears not to be due to an increased fluid intake or decreased urine output, since neither of these parameters are yet altered significantly. This implies either an altered extrarenal loss or an internal shift between body fluid compartments. The latter would explain the failure to demonstrate e.c.f.v. expansion at this stage of pregnancy (Atherton & Pirie, 1981). Thus it appears likely, that the increase in G.F.R. detected during the first week of pregnancy (Bishop & Green, 1980; Atherton & Pirie, 1981; Garland & Green, 1982) is the result, at least in part, of the increase in plasma volume. This relationship between plasma volume (Fig. 6*A*) and G.F.R. is also evident during mid-pregnancy (Baylis, 1980; Atherton & Pirie, 1981; Garland & Green, 1982) but not at term, since G.F.R. in the anaesthetized rat decreases whereas the present study shows plasma volume expansion to be maintained.

Electrolyte balance

During the first week of pregnancy, the animals significantly increased their food intake. This may be due to a central action of progesterone (Hervey, 1964; Hervey & Hervey, 1964), since the levels of this hormone are elevated within 2–3 days of mating (Morishige, Pepe & Rothchild, 1973). The resulting increased intake of sodium and other electrolytes is accompanied by an increase in their urinary excretion (Table 1), so that at this stage of pregnancy there is no net retention and neither plasma sodium nor osmolality are different from control values (Fig. 7). However, a significant increase in sodium, potassium and chloride retention is seen during the final week of pregnancy. This is the result not only of an increase in electrolyte intake but also of a significant reduction in urinary output. The salt retention during the later stages of pregnancy confirms previous reports, at least for sodium and potassium (Lichton, 1961; Kirksey & Pike, 1962; Churchill *et al.* 1980) although the latter study showed only a slight increase in urinary sodium output during the first half of pregnancy. Intrinsic differences between rat strains may exist, and the diets used have been different. For example, the rat food used in the present study contained twice as much sodium as Kirksey & Pike's 'normal diet' but has a lower salt content than that used by Lichton or Churchill *et al.* This may account for the earlier increase in fluid intake seen in the latter study.

Despite the salt retention in the later stages of pregnancy, maternal plasma sodium levels remain depressed, probably owing to at least 60 % of the retained sodium being sequestered by the fetuses (Lichton, 1961; Churchill *et al.* 1980). If pregnant rats are maintained on a very low sodium diet (0·014 m-mole g^{-1}), maternal plasma sodium levels fall still further by term in order to maintain normal fetal plasma sodium levels (Kirksey, Pike & Callahan, 1962).

Increased salt and water reabsorption throughout pregnancy have been demonstrated using infusion techniques in several studies (Lichton, 1963; Lichton & Hugh, 1968; Lichton, Rasa & Hugh, 1968; Lindheimer & Katz, 1971; Atherton & Pirie, 1981; Garland & Green, 1982), but the factors responsible are poorly understood.

The increased reabsorption of salt and water during the first two weeks of pregnancy is likely to be the result of increases in filtered load, fraction of the filtered load that is reabsorbed (fractional reabsorption) and proximal tubular reabsorptive area (Garland *et al.* 1978; Bishop & Green, 1980; Atherton & Pirie, 1981). Prolactin has been implicated in the altered filtered load and reabsorptive area (Garland, 1979; ElKarib & Green, 1981). Support for an increased fractional reabsorption is seen in the present study which indicates an unchanged urine volume in the face of an increased G.F.R.

The increased reabsorption close to term occurred despite a reduction in G.F.R. Thus, factors other than changes in the filtered load must be involved. However, plasma ADH levels and the ability to elaborate concentrated urine are similar in late-pregnant and virgin rats (Durr, Stamoutsos & Lindheimer, 1981) despite a lowered plasma osmolality in the second half of pregnancy (Durr *et al.* 1981; present study). A role for aldosterone is also uncertain since, although increased plasma levels have been observed in late pregnant rats (Whipp, Coghlan, Shulkes, Skinner & Wintour, 1978), surgical adrenalectomy and treatment with spironolactone have consistently failed to alter sodium retention at this stage (Lichton & Hugh, 1968; Lichton *et al.* 1968; Churchill, Bengele, Melby & Alexander, 1981).

In summary, the balance studies described here have quantified the water and electrolyte retention of the final week of pregnancy in terms of daily changes in input and urinary output. In a parallel study an increasing plasma volume expansion beginning before any apparent changes in fluid intake or urinary output, and a lowered plasma osmolality from midterm have been found. The former observation could be accounted for by an internal shift of fluid between body fluid compartments early in pregnancy, and may in turn be at least partly responsible for some of the specific renal changes occurring at this time.

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