J. Physiol. (1981), **319**, *pp.* 153–164
With 6 text-figures
Printed in Great Britain

THE EFFECT OF PREGNANCY ON GLOMERULAR FILTRATION RATE AND SALT AND WATER REABSORPTION IN THE RAT

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(*Received 23 January 1981*)

SUMMARY

1. Glomerular filtration rate (G.F.R.) and salt and water reabsorption were measured in age-matched (12- to 13-week-old) virgin rats and rats at different stages of pregnancy and infused with saline at 37·5 and 200 μl./min.

2. G.F.R. and salt and water reabsorption were significantly higher in early pregnancy and remained at steady, high values until at least mid-pregnancy. G.F.R. was slightly lower close to term, but sodium and water reabsorption remained significantly enhanced.

3. Significant expansion of extracellular fluid volume (e.c.f.v) was first apparent during mid-pregnancy, indicating that e.c.f.v. expansion could not be directly responsible for the early increase in G.F.R.

4. Proximal tubules were significantly elongated 5–6 days after mating until term, indicating that an increased reabsorptive area in the proximal tubule may be at least partly responsible for the increased reabsorption in pregnancy.

INTRODUCTION

Changes in glomerular filtration rate (G.F.R.) in human pregnancy are well documented: a rapid early increase (Davison, 1974, 1978) to a level which is maintained throughout the major part of the gestational period followed by a small fall before parturition (Sims & Krantz, 1958; Davison & Hytten, 1974). Tubular reabsorption of salt and water also increases significantly during human pregnancy, particularly in the third trimester (Chesley, Valenti & Rein, 1958; Lindheimer & Katz, 1973; deWardener, 1973; Weinberger, Kramer, Grim & Peterson, 1977). The mechanisms involved in these changes are uncertain; and a detailed investigation would require direct glomerular and tubular micropuncture.

It has been suggested (Bishop & Green, 1980) that in view of similarities of many of the changes, the pregnant rat can be used as a useful model of changes in renal function during human pregnancy. Thus, in the rat, changes in G.F.R. and salt and water handling have been reported (Matthews & Taylor, 1960; Lichton, 1963; Lichton & Hugh, 1968; Lindheimer & Katz, 1971; Baylis, 1980*a*; Bishop & Green, 1980; Davison & Lindheimer, 1980). However, it must be stressed that information concerning some aspects of renal function in the pregnant rat is still controversial;

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and there is little agreement concerning the stage of gestation at which such changes are apparent (see Discussion).

Therefore, the main purpose of the present study was to determine a time course of changes in G.F.R. and salt and water handling during pregnancy in the rat. In addition, the possibility that the failure of some workers (see Discussion) to detect changes in renal function during pregnancy was consequent on the infusion protocol adopted in their experiments, was also examined by using two different rates of infusion in each virgin and pregnant rat. In order to investigate two of the possible mechanisms involved in the altered G.F.R. and salt and water reabsorption, measurements were also made of maternal extracellular fluid volume, which influences G.F.R. (Landwehr, Klose & Giebisch, 1967), and the length of the proximal tubules, which might influence the tubular area available for reabsorption (Garland, Green & Moriarty, 1978). A preliminary account of this work has been published (Atherton & Pirie, 1977).

METHODS

Experiments were performed on 12- to 13-week-old virgin and pregnant Sprague–Dawley rats. Day 0 of pregnancy was designated as the day on which mating had occurred (signified by the appearance of a plug of cervical mucus on the cage floor). All animals were deprived of food, but allowed free access to water for 18 hr before experimentation. They were weighed and then anaesthetized by an intraperitoneal injection of Inactin (sodium 5 ethyl-5-(1′-methyl-propyl)-2-thiobarbiturate; 120 mg/kg body weight; Promonta Corp., Hamburg). Body temperature was maintained at 37 °C using a thermostatically controlled heated table. A tracheostomy was performed to ensure a clear airway.

In each of the three series of experiments performed, the animals studied were virgin controls, 2–3 days pregnant, 5–6 days pregnant, 11–12 days pregnant and 19–20 days pregnant. The numbers of animals used in each series are given in the Tables and Figures.

Series I: renal function

Animals received an I.V. infusion of 0·9% saline (containing [^{3}H]inulin to give an infusion rate of 6 μc/hr) via a catheter (PP10) placed in the left jugular vein. In each experiment two infusion rates were used: 37·5 μl./min during surgery and for 3 hr following the commencement of urine collection; thereafter at 200 μl./min for a further 3 hr. Catheters were also placed in the right carotid artery (PP50) for continuous recording of blood pressure (via a Statham p. 23 DC transducer and Grass 7 polygraph) and in the left ureter (PP10) to collect urine. Urine (collected under liquid paraffin in small pre-weighed tubes) and blood samples (obtained from the tail vein during, and by direct cardiac puncture at the end of, the experiment) were taken hourly for the 4 hr following commencement of urine collection and thereafter every 30 min for the remainder of the experiment.

Analyses

Urine and plasma [^{3}H]inulin was counted (10 μl. samples) in a liquid scintillation counter (Intertechnique model SL30, Paris) using P.C.S. (Amersham–Searle, Illinois, U.S.A.) diluted 1:1 with A.R. toluene as scintillant. Sodium concentration was determined by flame photometry (Corning-EEL Model 430); and chloride concentration by electrometric titration (Corning-EEL chloride meter Model 920).

Calculations

G.F.R. was calculated as the clearance of inulin, $C_{IN} = U_{IN}V/P_{IN}$; absolute reabsorption as $C_{IN} - V$ for water and $C_{IN}P_x - U_xV$ for solute x; fractional reabsorption as $1 - V/C_{IN}$ for water and $1 - [(U_xP_{IN})/(U_{IN}P_x)]$ for solute x, where U and P are the urinary and plasma concentrations, respectively, and V is urine flow.

Series II: extracellular fluid volume

To measure extracellular fluid volume (e.c.f.v.) the renal arteries and veins were exposed and ligated. 1 ml. 0·9 % saline (containing 20 μc [^{3}H]inulin/ml.) was injected via a catheter (PP10) in the left jugular vein, followed by 0·05 ml. 0·9 % saline to ensure complete delivery. Plasma [^{3}H]inulin concentrations in blood samples taken from the tail at the end of the 3rd and 4th hr after injection and also by direct cardiac puncture at the end of the 4th hr were not significantly different, indicating the occurrence of equilibration. E.c.f.v. was calculated as the volume of distribution of [^{3}H]inulin 4 hr after the injection of isotope. At the end of the experiments in the 11- to 12-day and 19- to 20-day-pregnant rats the uterine horns and contents were removed and weighed.

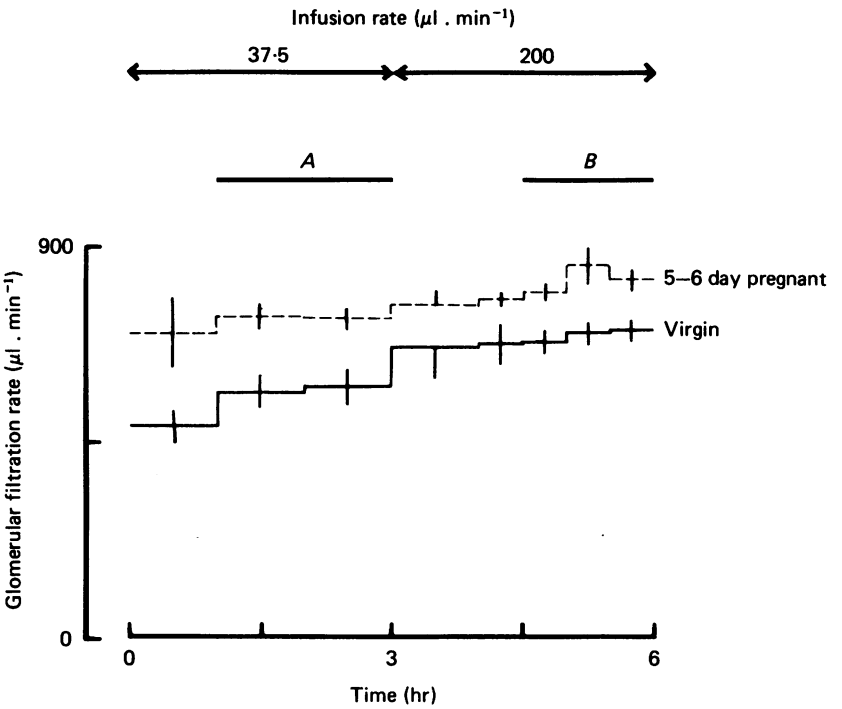

Fig. 1. Graphic presentation of the infusion protocol and treatment of results using as an example the time course of changes in G.F.R. (mean ± S.E. of mean) for virgin and 5- to 6-day-pregnant rats. During the 2 hr denoted by *A* and the 90 min denoted by *B* steady-state conditions prevailed. Hence, G.F.R. for the individual collection periods in *A* and in *B* within each experiment have been pooled. The over-all mean ± S.E. of mean for period *A* and *B* are presented in Fig. 2. Similar considerations were applied to all measurements which have been included in Table 2 and Figs. 2, 3 and 4.

Series III: proximal tubular length

The lengths of superficial proximal tubules were measured in virgin and pregnant rats using the method described by Garland *et al.* (1978).

In brief, animals were prepared for micropuncture as described by Bishop, Green & Thomas (1978). Up to ten superficial proximal tubules of the left kidney were filled with silicone rubber solution (Microfil-Canton Biomedical Products Inc., Colarado, U.S.A) using micropipettes 15–20 μm o.d. Following ligation of the renal vessels, the kidney was removed and stored in distilled water at 4 °C for 24 hr. Each proximal tubular cast including the glomerulus and pars recta was dissected out

after 60 min immersion in 10% sodium hydroxide and drawn using a camera lucida attachment to a stereo microscope.

Data are presented as the mean ± S.E. of mean. For series I experiments, two-way analysis of variance was performed to test whether steady-state conditions had been attained with respect to G.F.R. and the output and reabsorption of water, sodium and chloride within each infusion period. All measurements were shown to be at steady state during the final 2 hr of infusion at the low rate and at a higher value during the final 90 min of infusion at the high rate, During these times, in each infusion period, the results for the individual urine collections within each experiment have been pooled and an overall mean ± S.E. of mean calculated. An example of this is given in Fig. 1.

Statistical comparisons for all data were performed using Student's *t* test for unpaired samples. The significance of the difference between the means for the virgin and each group of pregnant rats is represented in the Figures and Tables by the symbols + $P < 0.05$; * $P < 0.01$; ● $P < 0.001$.

RESULTS

Series I: renal function

There were no significant differences in blood pressure between rats at different stages of pregnancy and virgin controls. Blood pressure never fell below 100 mmHg in any experiment. Changes in body weight in this series, as in series II and III were comparable to those reported by Garland *et al.* (1978), and are therefore not presented.

Plasma concentrations

Differences in plasma sodium concentrations (μmole/ml.) between virgin (low rate of infusion, 145 ± 2; high rate of infusion, 143 ± 3) and pregnant animals during both rates of infusion were small and not significantly different apart from the small increase at 5–6 days of pregnancy (low rate of infusion, 151 ± 2; high rate of infusion, 151 ± 1; $P < 0.05$ for both). Plasma chloride concentrations (μmole/ml.) were significantly lower than the virgin controls (low rate of infusion, 114 ± 1; high rate of infusion, 125 ± 1) during the high rate of infusion at 2–3 days (116 ± 2; $P < 0.01$) and 5–6 days (120 ± 1; $P < 0.01$) of pregnancy and during both rates of infusion at 19–20 days (low rate in infusion, 101 ± 2; $P < 0.001$; high rate of infusion, 107 ± 1; $P < 0.001$).

Glomerular filtration rate

G.F.R. (mean ± S.E. of mean) for the steady-state periods during both infusion rates are shown in Fig. 2. During the low rate of infusion, G.F.R. was significantly higher than the control value in 5- to 6-day and 11- to 12-day-pregnant animals. After the onset of the high rate of infusion, G.F.R. increased and stabilized at higher mean value in all groups after 90 min. During this steady-state period, G.F.R. was significantly higher than the control in animals pregnant for 2–3 days, 5–6 days and 11–12 days. For the 19- to 20-day-pregnant animals in both infusion periods, G.F.R. was not significantly different from virgin controls. It is considered that the difference in G.F.R. between animals pregnant for 11–12 days and 19–20 days, although not statistically significant is likely to be physiologically significant since (*a*) R. Green (unpublished observations) has shown significant differences between 11- to 12-day- and 19- to 20-day-pregnant rats, and (*b*) Atherton & Pirie (1978) showed that there were no differences between virgin and 19- to 20-day-preganant rats.

Water, sodium and chloride output (Table 1)

During the low rate of infusion differences between the control and pregnant groups for water, sodium and chloride outputs were usually small. Water, sodium and chloride outputs increased after the onset of the high rate of infusion. Compared to the control, the urinary outputs during the high rate of infusion were lower in all pregnant groups, particularly at 19–20 days, when they were considerably lower than those of the control and earlier pregnant groups.

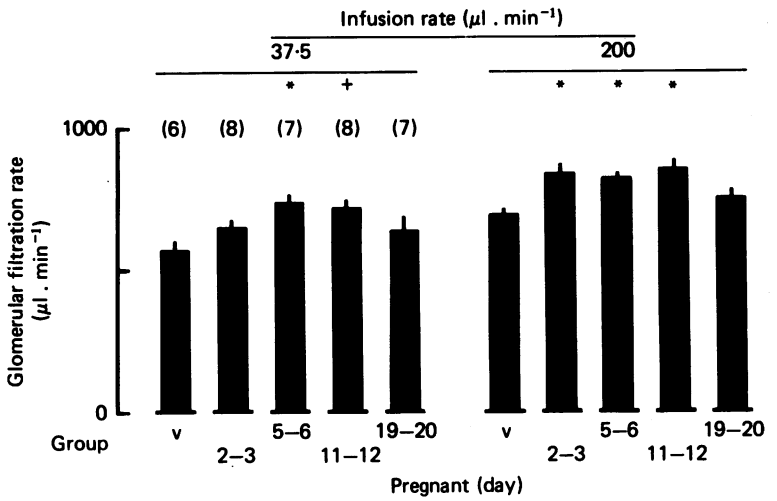

Fig. 2. G.F.R. (mean ± S.E. of mean) in virgin (v) and pregnant rats during the steady-state periods of the low and high rates of infusion. The numbers in parentheses represent the number of animals used in each group.

Water, sodium and chloride reabsorption

The absolute amounts of water, sodium and chloride reabsorbed (Fig. 3) during the low rate of infusion were significantly higher for 5- to 6-day- and 11- to 12-day-pregnant animals than for the virgin controls. During the high rate of infusion, not only did animals at all stages of pregnancy reabsorb more total water and sodium than the controls (Fig. 3), but also they reabsorbed a significantly greater percentage of the filtered loads of water, sodium and chloride (Fig. 4). During the low rate of infusion, since the amount excreted was only a small fraction of the filtered load (less than 1% for sodium, chloride and water), differences in percentage reabsorption between virgin and pregnant animals were very small and never significantly different.

Series II: Extracellular fluid volume

The volume of distribution of [^{3}H]inulin represents the true maternal e.c.f.v. since inulin does not cross the placenta (Lindheimer & Katz, 1971). In Fig. 5, e.c.f.v. has been expressed as an absolute value for all rats and as a percentage of the total body

TABLE 1. Urinary water, sodium and chloride outputs during the low and high rates of infusion

Infusion rate (μl. min^{-1})...		37·5			200		
			Output (μM/min)			Output (μmole/min)	
Animal group	n	Flow (μl./min)	Na	Cl	Flow (μl./min)	Na	Cl
Virgin	(6)	2·5±0·4	0·3±0·1	0·5±0·1	85·2±4·5	16·8±1·2	18·7±1·1
2–3 days pregnant	(8)	3·2±0·6	0·3±0·1	0·7±0·2$_+$	63·1*±4·6$_+$	12·7$^+$±0·8$_*$	13·8$^+$±0·8$_+$
5–6 days pregnant	(7)	4·2$^+$±0·5$_+$	0·6$^+$±0·1$_*$	1·2*±0·2$_●$	70·9$^+$±4·3$_*$	14·3±0·8$_*$	15·6$^+$±0·7$_●$
11–12 days pregnant	(8)	3·3±0·7	0·5±0·2$_+$	0·8±0·2$_+$	68·3$^+$±5·5$_+$	14·4±1·3$_*$	15·6±1·2$_*$
19–20 days pregnant	(7)	2·4±0·6	0·1±0·04	0·2$^+$±0·1	46·5$_●$±6·3	8·8$_●$±1·0	9·9$_●$±1·1

Values represent means±S.E. of mean. The numbers in parentheses represent the number of animals in each group.

The significances of the differences between the means for the virgin and each pregnant groups and between the 19- to 20-day-pregnant rats and each of the other group of pregnant rats are presented by the upper and lower symbols respectively.

weight for virgin control, and for animals pregnant for 2–3 days, 5–6 days and 11–12 days. For 19- to 20-day-pregnant animals, however, e.c.f.v. has been expressed as a percentage of the non-conceptus body weight, since in these late-pregnant animals the weight of the conceptus was considerable ($14 \pm 0{\cdot}3$ % of total body wt). Non-conceptus body weight was not used for the 11- to 12-day-pregnant animals since the weight of the uterine horns and concepta were only a small percentage of body weight. ($1{\cdot}6 \pm 0{\cdot}05$ %). E.c.f.v., both as an absolute value and as a percentage of body

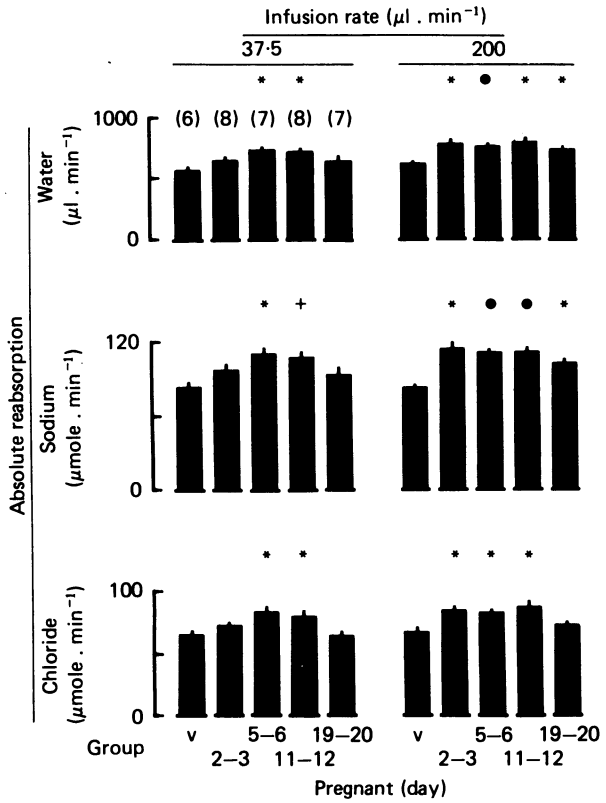

Fig. 3. Absolute reabsorption of water, sodium and chloride (mean ± S.E. of mean) in virgin (v) and pregnant rats during the steady-state periods of the low and high rates of infusion. The numbers in parentheses represent the number of animals used in each group.

weight, remained unchanged from the control value in 2- to 3-and 5- to 6-day-pregnant animals. However, significant expansion was apparent at 11–12 days and was even more marked at 19–20 days, for which the mean values were significantly greater than the control and also that for 11-to 12-day-pregnant animals.

Series III: proximal tubular length

The mean ± S.E. of mean lengths of superficial proximal tubules for the control and pregnant groups are shown in Fig. 6. Proximal tubular length remained similar to the control in 2- to 3-day-pregnant animals. However, at 5–6 days of pregnancy,

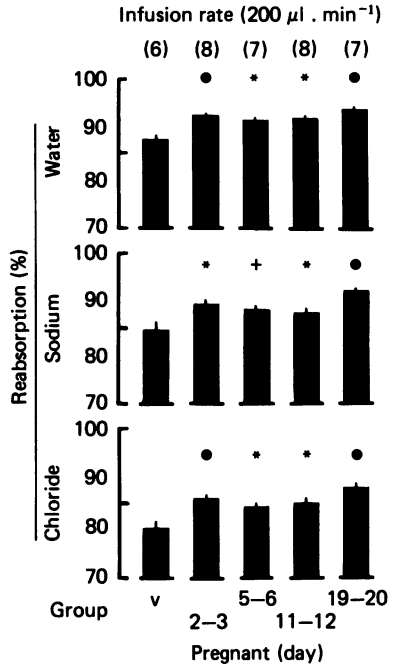

Fig. 4. Percentage reabsorption of water, sodium and chloride (mean ± S.E. of mean) in virgin (v) and pregnant rats during steady-state period of the high rate of infusion. The numbers in parentheses represent the number of animals used in each group.

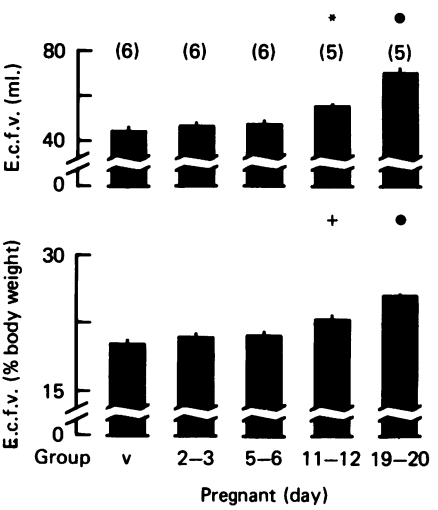

Fig. 5. Extracellular fluid volume (mean ± S.E. of mean) in virgin (v) and pregnant rats. The numbers in parentheses represent the number of animals used in each group.

proximal tubules were significantly longer compared to values from both the controls and 2- to 3-day-pregnant animals. Animals pregnant for 11–12 days and 19–20 days had mean proximal tubular lengths similar to that for animals pregnant for 5–6 days.

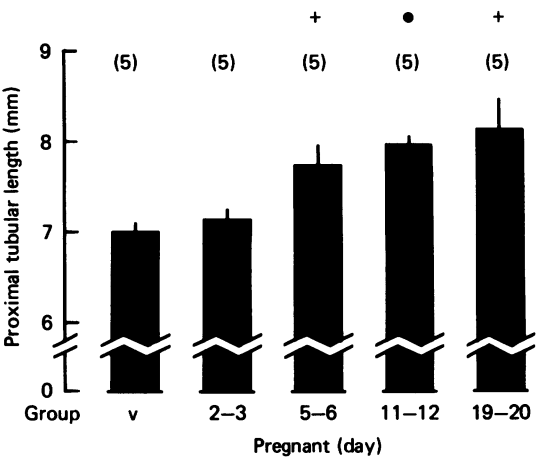

Fig. 6. Proximal tubular length (mean ± S.E. of mean) in virgin (v) and pregnant rats. The numbers in parentheses represent the numbers of animals used in each group.

DISCUSSION

The results from this study indicate that there are changes in G.F.R. and salt and water reabsorption during pregnancy in the rat.

The data, therefore, confirm and extend the observations of some workers; namely an increased G.F.R. at the end of the first week of pregnancy (Bishop & Green, 1980) and mid-pregnancy (Matthews & Taylor, 1960; Baylis, 1980*a*) with a decrease close to term (Matthews & Taylor, 1960; Lichton & Hugh, 1968). Others, however, have failed to demonstrate an increased G.F.R. during the first (Baylis, 1980*b*) and second (Lichton, 1963; Lindheimer & Katz, 1971; Davison & Lindheimer, 1980) week of pregnancy. The reasons for these differences are uncertain. It is unlikely that they are related to the extent of preparatory surgery involved and/or the use of anaesthetic (Davison & Lindheimer, 1980) since Atherton (1981) using conscious virgin and 9-day-pregnant rats demonstrated differences in G.F.R. qualitatively similar to the present results. The possibility that these differences may be related to the strain of rat used appears also to be inappropriate since Baylis (1980*a*) found G.F.R. to be higher in 12-day-pregnant than in virgin Munich–Wistar rats. It is possible, however, that the detection of differences very early in gestation may be related to the rate of fluid administration since in the present work differences were apparent when using an infusion rate of 200 μl./min but not with 37·5 μl./min in 2- to 3-day-pregnant rats.

Expansion of maternal e.c.f.v. detected at mid-pregnancy (Churchill, Bengele & Alexander, 1978; present study), in particular plasma volume (Baylis, 1980*b*), may

in part account for the observed increase in renal plasma flow (Matthews & Taylor, 1960; Baylis, 1980*a*). Furthermore, it is likely that the changes in G.F.R. are a direct consequence of changes in renal plasma flow at this stage in gestation (Baylis, 1980*a*). However, such considerations appear not to apply to the initial rise in G.F.R. early after mating since it occurred when e.c.f.v. was not expanded.

Enhanced tubular reabsorption of sodium has been reported 7–8 days (Bishop & Green, 1980) and 12 days (Baylis, 1980*a*) after mating; and of sodium and water close to term (Lichton, 1963; Lichton & Hugh, 1968; Lindheimer & Katz, 1971). The present data extend these observations in that sodium, chloride and water reabsorption were shown to be elevated from as early as 2–3 days after mating (high rate of infusion only) and throughout gestation (both rates of infusion) to 19–20 days (high rate of infusion only). It could be argued that if as is generally accepted glomerulotubular balance operates within the rat kidney, these changes in reabsorption are just a consequence of the increase in filtered load (resulting primarily from an increased G.F.R., since plasma sodium and chloride concentrations were essentially similar in all groups). However, that this is not the only mechanism involved is indicated by the observations that (*a*) there was an increase in the fraction of the filtered load reabsorbed in all groups when infused at the high rate, and (*b*) there was an increase in reabsorption of sodium and water in the 19– to 20-day-pregnant group in which the G.F.R. (hence filtered load) was lower.

We agree with others (Garland *et al.* 1978) that the enhanced whole kidney reabsorption of salt and water may be accounted for, in part, by the increase in length of the proximal tubule observed as early as 5–6 days after mating without any further significant change to term. Thus, given a constant proximal tubular diameter, an increased length would represent an increased area for reabsorption. However, that this is not the sole cause of the increased reabsorption is indicated by the observation that proximal tubular elongation was not detected at 2–3 days of pregnancy, when both the absolute and fractional amounts of salt and water reabsorbed were significantly greater than control values during the high rate of infusion. This latter observation adds support to the conclusions of Atherton & Pirie (1978) that the altered reabsorption in post-partum rats need not correlate well with changes in proximal tubular length.

The present data give no evidence as to the involvement of more distal sites of the nephron in the increased reabsorption of salt and water during pregnancy. However, it has been proposed that the high level of aldosterone observed during mid- and late-pregnancy (Whipp, Coghlan, Shulkes, Skinner & Wintour, 1978) is an important contributory factor in the increased sodium reabsorption in the second half of gestation in rats (Lichton, Rasa & Hugh, 1968). However, any involvement of aldosterone in the changes in the reabsorption of sodium in early pregnancy remains to be elucidated.

Finally, it is tempting to draw comparisons between the results of the present study with those found in pregnant women, namely, in women there is (*a*) an early rapid increase in G.F.R. (Davison, 1974, 1978) which is maintained for the major part of pregnancy with a decrease close to term (e.g. Davison & Hytten, 1974); (*b*) enhanced tubular reabsorption of salt and water (Chesley *et al.* 1958; Weinberger *et al.* 1977); and (*c*) e.c.f.v. expansion from mid-pregnancy onwards (Pitkin, 1977).

In summary, the present experiments on anaesthetized, saline-loaded rats have shown that G.F.R. is significantly elevated above virgin control values soon after mating and is maintained at a high, steady level through mid-pregnancy, falling slightly before parturition. Salt and water reabsorption is significantly enhanced from a very early stage after mating until parturition. The mechanisms for the early increase in G.F.R. remain unclear, whereas an expanded e.c.f.v. probably contributes to the elevated G.F.R. during the second half of gestation. An increased reabsorptive area in the proximal tubule may be at least partly responsible for the enhanced salt and water reabsorption, but does not account for the increased reabsorptive capacity demonstrable 2–3 days after mating. It is concluded that other factors, not yet determined, must be involved in the increased G.F.R. and salt and water reabsorption observed during pregnancy in the rat.

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