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MICROPUNCTURE STUDY OF CHANGES IN GLOMERULAR FILTRATION AND ION AND WATER HANDLING BY THE RAT KIDNEY DURING PREGNANCY

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SUMMARY

1. In rats receiving 200 μl. min^{-1} sodium chloride, glomerular filtration rate, single nephron glomerular filtration rate, and fractional reabsorption were measured at various points along the nephron, and ionic concentrations measured in early distal tubular fluid in virgin and 6, 12 and 19 day pregnant rats.

2. Glomerular filtration increased progressively until the twelfth day of pregnancy. At 19 days of pregnancy the glomerular filtration rate, while still above virgin levels, was reduced below 12 day pregnant levels.

3. Single nephron glomerular filtration rates measured in proximal and distal tubules were different at both 12 and 19 days of pregnancy, indicating an alteration of tubuloglomerular feed-back.

4. A change in the ratio of glomerular filtration rate : distal single nephron filtration rate indicated a redistribution of glomerular filtrate to juxtamedullary nephrons by the sixth day of pregnancy.

5. Fluid reabsorption is similar up to the early distal tubule but it is not possible to say whether reabsorption is the same in proximal tubules. More fluid is reabsorbed by late distal tubules and collecting ducts in 12 and 19 day pregnant animals than in the virgin and 6 day pregnant animals.

6. Changes in ion reabsorption by the loop of Henle occurred during pregnancy; sodium reabsorption was increased by the sixth day of pregnancy and potassium reabsorption by the twelfth day.

INTRODUCTION

Changes in renal function during pregnancy in humans have been well documented clinically. These changes include increases in glomerular filtration rate (G.F.R.), glucose excretion and tubular reabsorption of salt and water and are associated with an increased extracellular volume (Hytten & Leitch, 1971; Davidson & Hytten, 1974, 1975; Davison & Noble, 1981; Dunlop, 1981). Appropriate investigations of single nephrons or parts of nephrons to elucidate the mechanisms underlying these gross changes cannot be performed in humans, however. It has been suggested (Bishop & Green, 1980) that the rat can serve as a useful model for the renal changes occurring

in human pregnancy. To date, changes in G.F.R., salt and water handling (Matthews & Taylor, 1960; Bishop & Green, 1980, 1981; Baylis, 1980; Atherton & Pirie, 1981), glucose excretion (Bishop & Green, 1980, 1981) and extracellular fluid volume (Churchill, Bengele & Alexander, 1980; Atherton & Pirie, 1981) all support this suggestion.

Although the rat has been used extensively for investigating renal function at the single nephron level in non-pregnant animals there have been only a few reports of single nephron function in pregnant animals, and these invariably deal with one particular aspect, usually at a single stage of pregnancy (Churchill, Bengele & Alexander, 1978; Baylis, 1980; Bishop & Green, 1981). There has been little attempt to determine the mechanisms underlying the changes or the site at which they occur. The present study was therefore designed to investigate single nephron glomerular filtration rate and reabsorption at different sites in the nephron at different stages of pregnancy. A high rate of saline infusion was used since it has been shown (Atherton & Pirie, 1981) that while this may slightly reduce the changes in G.F.R. that occur, changes in salt and water handling are considerably enhanced.

METHODS

All experiments were performed on female Sprague-Dawley rats aged 12–13 weeks. Animals were fed on standard rat cake (CRM diet, Na content 0·12 m-mole g^{-1}) until 16 hr before the start of the experiment and allowed water *ad lib* until they were anaesthetized; daily Na intake was about 2·2 m-mole. Four groups of rats were used; virgin controls and animals 6, 12 and 19 days pregnant. The onset of pregnancy was determined by the appearance of a cervical plug of mucus on the floor of the cage after mating. Pregnancy was confirmed at the completion of each experiment.

Animals were anaesthetized with an intraperitoneal injection of 110 mg kg^{-1} body weight of Inactin (5-ethyl-5-[1′-methyl-propyl]-2-thiobarbiturate) and prepared for renal micropuncture as previously described (Bishop, Green & Thomas, 1978). Briefly, they were placed on a heated operating table set to maintain body temperature at 37–38 °C, and catheters were placed in the left jugular vein for injection and infusion of solutions, and the right carotid artery for recording blood pressure; a tracheostomy ensured a clear airway. The left kidney was exposed through a flank incision and placed in a perspex cup, taking great care that the blood supply was not impaired, and the surface was covered with liquid paraffin; the left ureter was catheterized and the kidney surface illuminated through a fibre optic guide from a light source (Barr & Stroud, Anniesland, Glasgow). In all pregnant animals, especially those 19 days pregnant, the uterus with its contained fetuses was retained within the abdominal cavity and care was taken not to compromise its blood supply. After completion of surgery, the tubular transit time was measured with Lissamine Green (Steinhausen, 1963); only animals with a proximal transit time of less than 14 sec were used. Two types of experiment were performed.

(*A*) *Free flow studies*

In this series a loading dose of 1 ml. of 0·9 % saline (containing 20 μc [^{3}H]inulin) was followed by a continuous infusion of 0·9 % saline (containing 5 μc $ml.^{-1}$ of [^{3}H]inulin) at 200 $\mu l.\ min^{-1}$. Urine samples were collected hourly into weighed containers commencing one hour after the start of the infusion, and hourly blood samples (100 μl.) were collected from a tail vein into heparinized microhaematocrit tubes. Micropuncture was only performed in the second and third hours of collection, i.e. after the animals had been infused for at least two hours. Both proximal and distal tubules were punctured in the same animal.

Proximal tubules. In each animal three to six proximal tubules selected at random were punctured with pipettes of tip diameter 10–12 μm; there were thirty punctures in eight virgin animals and thirty-eight (eight animals), thirty-eight (seven animals) and thirty-four (seven animals) punctures in 6, 12 and 19 day animals respectively. A bolus of castor oil stained with Sudan Black was injected

into the tubule until the length of the bolus was at least 5 × the tubular diameter; a small negative pressure was then applied to overcome surface tension at the tip and collection was initiated. Pressure applied to the pipette was varied to maintain the position of the distally placed oil block constant. After collection of fluid for 3–8 min, the pipette was rapidly withdrawn into the layer of oil over the surface of the kidney and a small amount of paraffin was drawn into the tip of the pipette to prevent evaporation of the collected fluid. The tubule was drawn for later reference.

Distal tubules. In each animal, one to three early distal tubules were punctured. To identify distal tubules, 50 μl. of 10% Lissamine Green was injected intravenously and its progress through the tubules monitored. After traversing the proximal tubule, the dye disappears from the surface of the kidney as it enters the loops of Henle; it reappears 10–15 sec later in the distal segments which appear filled with dye 25–120 sec after the initial green blush in the renal vessels. An 'early' distal tubule was taken as one which filled with dye within the first 15 sec of the appearance of dye in any distal tubule. Most of our 'early distal' samples would therefore correspond to the cortical part of the diluting segment after the tubule had met its own glomerulus (Burg, 1976). Some 'late' distal tubules (where the dye appeared 30–60 sec after the beginning of the distal transit) were punctured; but on examination after Microfil injection (see below), it became apparent that in this group a number of cortical collecting ducts had been sampled. Since it is impossible to determine how many distal tubules supply each cortical collecting duct, collections from different animals are not necessarily comparable and so the attempt to collect late distal samples in this series of experiments was abandoned. There were nineteen, eighteen, nineteen and seventeen punctures in virgin, 6, 12 and 19 day pregnant animals respectively.

Once an early distal tubule was identified, 2–3 min were allowed for the total clearance of the dye from the nephron and the tubule was then punctured with a micropipette of tip diameter 10 μm. Because the flow of fluid was so much slower than in proximal tubules, great care was taken when inserting the bolus of castor oil to ensure that it only moved distally. Pressure adjustments were also made to ensure there was no movement of the oil block and that the tubular diameter remained constant. Fluid was collected for 8–10 min, as described for proximal tubules, and the punctured tubules were then drawn for future reference.

At the end of the experiments, punctured proximal and distal tubules were re-identified and filled with Microfil (silicone rubber; Canton Biomedical Products Inc. Boulder, CO, U.SA.) using a pipette with a tip diameter of 12–15 μm inserted through the original puncture site. The kidney was removed, stored overnight in de-ionized water at 4 °C and, next day, partially digested in 20% NaOH for 15–20 min. The silicone rubber casts of the tubules were dissected out. Proximal tubules, accepted as such if they had an attached glomerulus, were drawn using a camera lucida attachment for a stereo-microscope. The length of the drawing was measured from the glomerulus to the puncture site and compared with an object micrometer drawn at the same magnification.

Distal tubules were confirmed as such if they had (*a*) no attached glomerulus, (*b*) few surface convolutions, (*c*) distally placed branches where Microfil had retrogradely entered other distal nephrons through the cortical collecting ducts. Measurement of length was not attempted, however, since the beginning of the distal tubule is impossible to define in this preparation. (Technically, of course, the 'distal tubule' begins where the ascending limb of the loop of Henle meets the afferent and efferent arterioles of the corresponding glomerulus, but injection of Microfil in this experiment could not define the position of the corresponding glomerulus). Accordingly, all 'early' distal tubules were grouped together for statistical purposes and no attempt was made to correlate any variable with the distance of the puncture site along the distal tubule.

Analyses

After measuring the volume of the fluid collected in a constant bore capillary (approximately 0·3 mm internal diameter) aliquots of proximal and distal fluid were delivered into vials for measuring [^{3}H]inulin concentrations in a liquid scintillation counter with PCS scintillant (Radiochemical Centre, Amersham). Appropriate aliquots of urine and plasma were counted at the same time. Distal tubular samples and plasma had their sodium and potassium concentrations measured on a Helium Glow Photometer (Aminco Inc., Silver Springs, MD, U.S.A.), their chloride concentration measured using the ultramicro method of Ramsay, Brown & Croghan (1955), and their osmolality measured on a Nanolitre Osmometer (Clifton Technical Physics, Hartford, NY, U.S.A.)

Calculations

Whole kidney glomerular filtration rate (G.F.R.)	$C_{in} = U_{in} V/P_{in}$
Single nephron filtration rate (S.N.G.F.R.)	$v(TF_{in}/P_{in})$
Nephron filtered load	S.N.G.F.R. $\times P_A$
Percentage of fluid remaining in tubule,	$100\ P_{in}/TF_{in}$
Percentage of filtered load remaining in the tubule	$100\ (TF_A/P_A)/(TF_{in}/P_{in})$
Percentage reabsorption of a substace	100 − percentage of load remaining in the tubule
Net reabsorptive flux of a solute, Φ_A	(S.N.G.F.R. $\times P_A$) − (vTF_A)
Net reabsorptive flux of water, Φ_W	$v((TF_{in}/P_{in})-1)$
'Osmotic deficit'	osmolality − 1·86 (Na + K).

Where C_{in}, U_{in}, TF_{in} and P_{in} are the clearance, urine, tubular fluid and plasma concentrations of inulin; TF_A and P_A are the concentrations of any substance, A, in tubular fluid and plasma; V is the urine flow rate and v is the tubular fluid collection rate.

(*B*) *Stationary microperfusion studies*

In this series, an initial intravenous injection of 1 ml. 0·9% saline was given followed by an infusion of 0·9% saline at 1 ml. hr^{-1}. Microperfusion was commenced 1–1½ hr after the start of the infusion.

Double barrelled micropipettes, tip diameter 13–15 μm, were used to puncture straight segments of randomly selected superficial proximal convoluted tubules. Castor oil, stained with Sudan Black, was injected from one barrel and subsequently split with a Ringer solution (containing NaCl, 145; $NaHCO_3$, 5; KCl, 5; $CaCl_2$, 1·5 m-mole $l.^{-1}$ bubbled with 95% O_2 5% CO_2 for one hour before use) from the second barrel (see Garland, Brunt, Taylor & Green, 1979). The split drops were photographed on Ektachrome commercial (7252) film using a Beaulieu 16 mm movie camera attached to a Zeiss operating microscope at 2 frames sec^{-1}. Processed films were replayed on a motion analysis projector and the rate of shrinkage of the injected ringer droplet was measured using a computerized image analysis system ('Magiscan', Joyce Loebl Ltd., Team Valley, Gateshead) as described previously (Garland *et al.* 1979). For each sequence, frames were analysed for droplet length (l) measured as the shortest distance between the oil menisci, and mean tubule diameter (d). Reabsorptive half time ($t_{\frac{1}{2}}$), i.e. the time taken for half the droplet to be reabsorbed, was calculated from the linear regression line relating the logarithm of the corrected length ($l+d$; Gyory, 1971) and time. Reabsorptive rates (nl. mm^{-1} min^{-1}) were calculated using the formula:

$$\text{reabsorptive rate} = 0{\cdot}693\ \pi r^2/t_{\frac{1}{2}}$$

where r is the mean radius of the droplet (Gertz, 1963). There were a total of eighty-seven observations (ten animals) in virgins, seventy-five observations (eight animals) in 6 day, sixty-eight observations (eight animals) in 12 day and seventy-six observations (nine animals) in 19 day pregnant animals.

All data are presented as means ± S.E. of the mean and differences assessed for significance using Student's t test, unless otherwise stated.

RESULTS

Animals gained weight as expected during pregnancy and there was a significant increase in weight at each stage of pregnancy studied (see Table 1); increases in weight are comparable to those recorded previously (Garland, Green & Moriarty, 1978) although it should be noted that all animals in this study were the same age at the time of the experiment and that greater length of pregnancy did not mean older animals. Mean arterial pressure (M.A.P.) was not significantly different between series (Table 1).

The major ionic constituents of plasma are also presented in Table 2. Plasma samples were obtained hourly during free flow experiments; there was no significant

variation of any of the ionic constituents with time and so the average of the values obtained throughout the experiment for each animal was taken and the mean and standard error for the four groups was calculated. There are only small differences in plasma sodium, potassium and chloride composition at different stages of pregnancy in these animals undergoing saline diuresis; the only one that reached the level of statistical significance ($P < 0.05$) was the difference between the plasma chloride concentration of 6 and 12 day pregnant animals. Small changes in plasma

TABLE 1. Body weights, mean arterial pressure (M.A.P.) and plasma analysis at different stages of pregnancy in anaesthetized rats

	Virgin controls	Pregnant		
		6 days	12 days	19 days
Weight (g)	213·6 (18) ±4·1	232·8 (16) ±4·6	253·3 (15) ±5·9	316·9 (16) ±8·6
M.A.P. (mmHg)	108·8 (18) ±2·64	108·4 (16) ±2·40	113·8 (15) ±3·91	100·4 (16) ±4·67
Plasma Na (m-mole $l.^{-1}$)	142·1 (8) ±4·8	138·1 (8) ±2·6	136·9 (7) ±2·6	139·6 (7) ±2·5
Plasma K (m-mole $l.^{-1}$)	4·74 (8) ±0·24	4·65 (8) ±0·25	4·71 (7) ±0·21	4·77 (7) ±0·20
Plasma Cl (m-mole $l.^{-1}$)	115·7 (8) ±2·4	117·9 (8) ±2·4	111·0 (7) ±1·9	115·1 (7) ±1·8
Plasma osmolality (m-osmole kg $water^{-1}$)	287·9 (8) ±1·6	284·4 (8) ±3·9	286·9 (7) ±2·1	285·1 (7) ±4·0

Note: weights and mean arterial pressures are from all animals used, plasma analysis only from the animals used for free flow micropuncture.

chloride during pregnancy have been reported previously (Atherton & Pirie, 1981) but whether the small difference reported here is of physiological significance is not known.

Glomerular filtration rate and single nephron filtration rate

Total G.F.R. for the left kidney varied at different stages of pregnancy. Changes of G.F.R. over the three hours during which urine and plasma samples were collected are shown for each group of animals in Fig. 1. In none of the groups was there a significant variation with time; and since micropuncture samples (where S.N.G.F.R. was measured) were collected over the second and third hours of urine collection, the G.F.R. for these latter two periods was averaged for each animal and means and S.E. of the mean calculated from each group (Fig. 2*A*). At the sixth day of pregnancy, G.F.R. was significantly higher than in virgin controls ($P < 0.05$) and there was a further significant increase by 12 days of pregnancy ($P < 0.01$ compared with virgins; $P < 0.05$ compared with 6 day pregnant). In the 19 day pregnant animals, however, the G.F.R. was similar to the value at 6 days; significantly less than at 12 days ($P < 0.05$) but still significantly higher than in the virgins ($P < 0.01$). These changes are qualitatively similar to those previously reported (see Atherton & Pirie, 1981).

Single nephron filtration rate (S.N.G.F.R.) was measured in both proximal and distal

tubules. At later stages of pregnancy the S.N.G.F.R. calculated from proximal tubule punctures (hereafter called proximal S.N.G.F.R.) was significant higher than distal S.N.G.F.R. although in virgin and 6 day pregnant animals the small differences were not statistically significant (Fig. 2*B*). Distal S.N.G.F.R. was not significantly different from controls at 6, 12 or 19 days of pregnancy although it was significantly higher at 19 days than at 6 days ($P < 0.05$). Proximal S.N.G.F.R. was considerably raised later

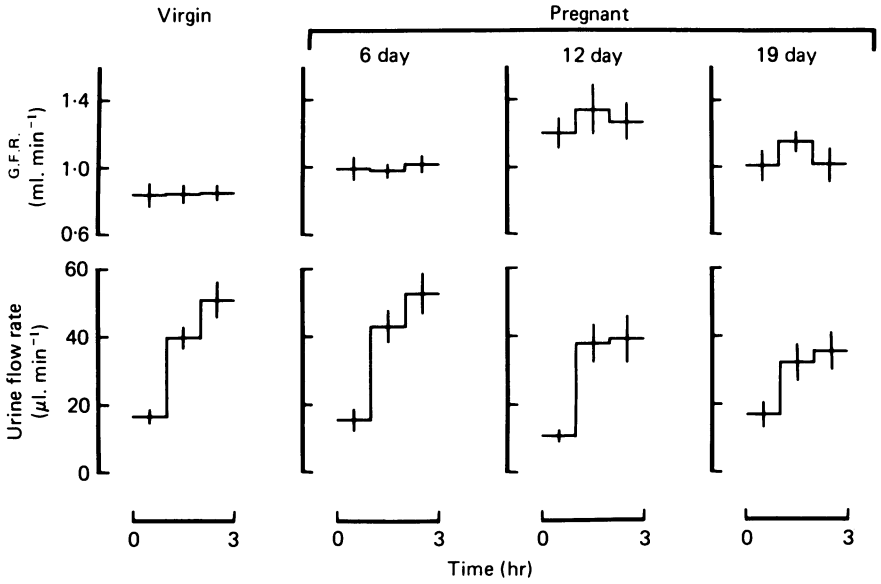

Fig. 1. Glomerular filtration rate and urine flow rates over the three hours of collection for virgin and pregnant rats. Bars = ±S.E. of the mean.

in pregnancy ($P < 0.001$ compared with either virgin or 6 days pregnant) but these later proximal S.N.G.F.R.s may be artificially raised (see Discussion).

The ratio G.F.R./S.N.G.F.R. has been used as a crude index of relative distribution of filtrate between superficial cortical and juxtamedullary nephrons, or as a measure of the total number of functional glomeruli (Solomon, 1977). Since it has been demonstrated that there are no significant changes in the number of glomeruli during pregnancy (Balmer, Garland, Green, Moriarty & Richardson, 1977) an increase in the G.F.R./S.N.G.F.R. ratio in the present experiments would indicate a greater *proportion* of filtrate from juxtamedullary nephrons, and a decrease would indicate a greater proportion from the superficial cortical nephrons. Because proximal S.N.G.F.R.s may not give a true picture of changes (see Discussion) only the G.F.R./distal S.N.G.F.R. ratio is presented (Fig. 2*C*). This shows a significant increase in 6 day pregnant animals when compared with virgins ($P < 0.05$). Ratios in 12 and 19 day pregnant animals were lower than at 6 days but were not statistically significantly different from control virgin values.

Urine flow

Urine flow rate increased significantly in all groups after the first hour of urine collection (i.e. after the second hour of infusion), but although there were further small increases after the second hour, in no group did they reach the level of statistical

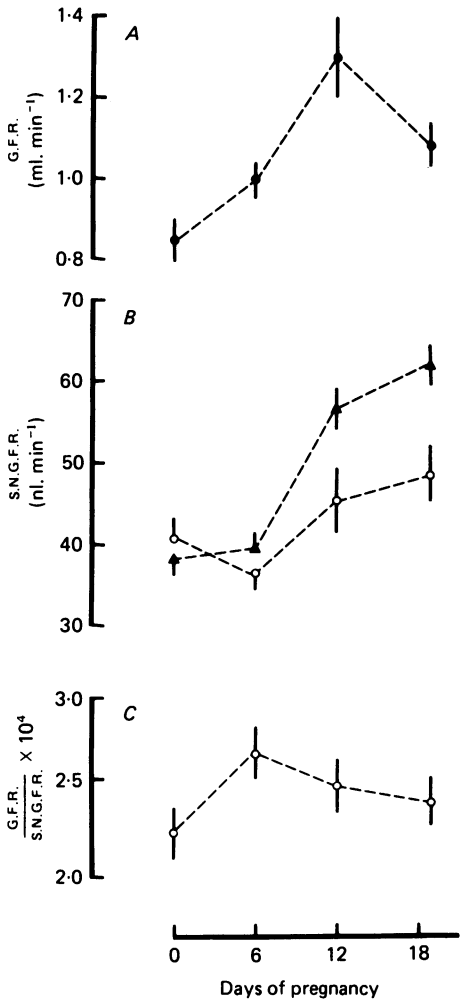

Fig. 2. Changes in glomerular filtration rate (*A*), single nephron glomerular filtration rate (*B*) calculated from proximal (▲) and distal (○) data, and glomerular filtration rate: distal S.N.G.F.R. ratio (*C*) during pregnancy. Bars = ±S.E. of the mean.

significance (Fig. 1). When comparing flow rates in virgin and 6 day pregnant animals, it can be seen that at comparable times there were no significant differences. Although the flow rate in 12 and 19 day pregnant animals was similar to that in virgins and 6 day pregnant animals during the first hour of collection, thereafter, the flow rate was less in the 12 and 19 day pregnant animals; this achieved statistical significance

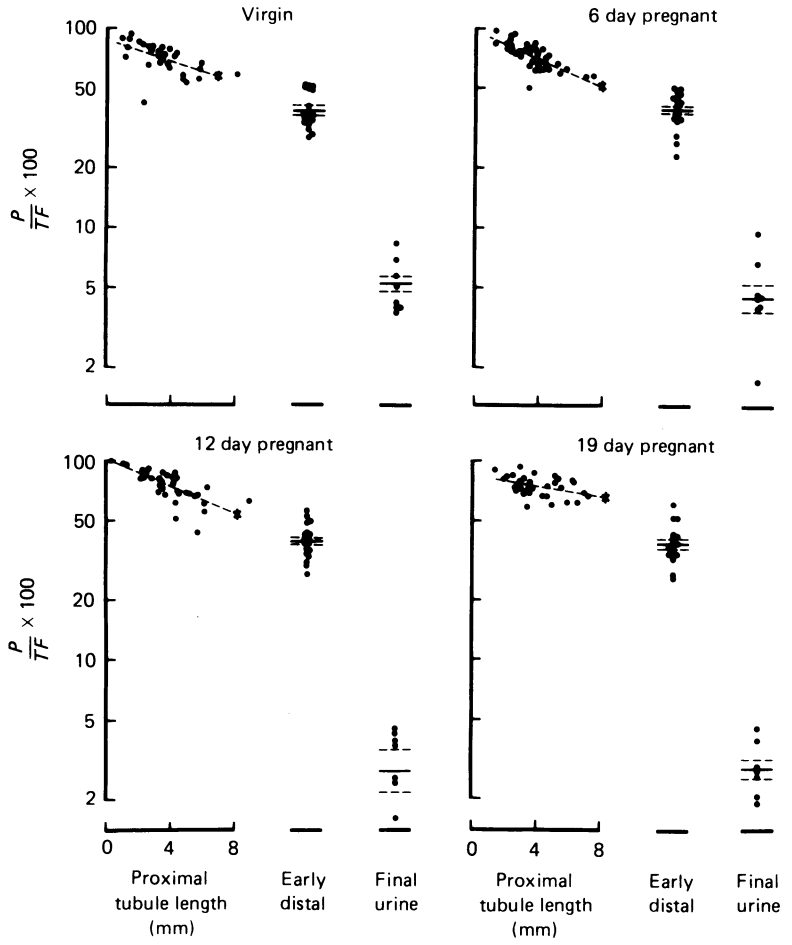

Fig. 3. Percentage reabsorption along the proximal tubule, in early sistal tubules and in the final urine during pregnancy. For proximal tubular data the dashed line is the regression line calculated from least squares regression; the symbol (X) represents the mean lengths of proximal tubule (data from Garland *et al.* 1978) and the bar represents ±S.E. of the mean of this point. For early distal tubule and final urine the mean is indicated by the continuous line and the dashed lines indicate ±S.E. of the mean.

when 19 day pregnant animals were compared with virgins or 6 day pregnant animals ($P < 0{\cdot}05$).

Fractional fluid reabsorption

The percentage of the filtered fluid which is not reabsorbed is presented in Fig. 3 for proximal tubule (where it is correlated with distance of the puncture site from the glomerulus), early distal tubule, and for final urine, calculated as the mean of the second and third hour of collection. The percentage of fluid excreted was similar in virgin and 6 day pregnant rats but significantly less than either of these earlier values

in later stages of pregnancy ($P < 0.01$ in all cases). Thus for the whole kidney percentage reabsorption of fluid was greater in later stages of pregnancy.

For proximal tubular punctures, it can be seen that there is a relationship between the inulin ratio and the site at which the nephron was punctured. The regression line had a significantly smaller slope in 19 day pregnant animals than in the other groups

TABLE 2. Fluid reabsorbed by parts of the nephron

		Virgin controls	Pregnant		
			6 days	12 days	19 days
Reabsorption by whole kidney (μl. min^{-1})		802·5 ± 40·8	953·6 ± 48·4	1265·2 ± 64·0	1103·4 ± 55·3
Reabsorbed up to early distal tubule (nl. min^{-1})		25·4 ± 1·5	22·6 ± 1·8	28·2 ± 2·8	30·4 ± 2·9
Reabsorbed by* proximal tubule (nl. min^{-1})	(*a*)	15·9 ± 2·0	16·9 ± 2·65	26·0 ± 3·6	21·1 ± 3·7
	(*b*)	17·1 ± 2·3	15·8 ± 2·9	20·8 ± 3·9	16·5 ± 3·7
Rate of reabsorption in proximal tubule, from stationary microperfusion experiments (nl. min^{-1} mm^{-1})		1·77 ± 0·06	1·83 ± 0·06	1·71 ± 0·06	1·69 ± 0·05

* Reabsorption up to the end of the proximal tubule is calculated in two ways: (*a*) fractional reabsorption at end of proximal (from Fig. 3) × prox. S.N.G.F.R.; (*b*) fractional reabsorption at end of proximal (from Fig. 3) × distal S.N.G.F.R. The latter calculation assumes that for each stage of pregnancy fractional reabsorption along the proximal tubule is independent of proximal S.N.G.F.R. and so any errors in proximal S.N.G.F.R. (see discussion) will not alter fractional reabsorption.

($P < 0.05$) which did not differ significantly among themselves: i.e. for the 19 day pregnant animals the fractional fluid reabsorption per unit length of proximal tubule was significantly less than in controls and earlier stages of pregnancy. However, because the proximal tubule becomes longer during pregnancy (Garland *et al.* 1978) the over-all fractional fluid reabsorption of the proximal tubule would be better described by assessment of the fraction of the filtered fluid remaining at the end of this segment in each group. Accordingly in Fig. 3 the fractional reabsorption is shown (as mean ± S.E.M.) for the total length of the proximal tubule in each series (values from Garland *et al.* 1978). This has been calculated from the regression equation of the proximal tubular data. While there are no significant differences between the amount remaining at the end of the proximal tubule in virgin, 6 and 12 day pregnant animals (58·4, 52·4 and 54·3 % respectively), in the 19 day pregnant animals 66·1 % still remained, significantly more than in any other group.

In contrast, by the early distal tubule the percentages of fluid still remaining in the tubule were essentially similar in each series (approximately 40 %): i.e. the percentage reabsorption was the same in spite of apparent differences at the end of the proximal tubule. Thus by the early distal tubule there is reabsorption of some

60 % of the filtrate in virgins and at all stages of pregnancy. Since only 3–5 % of filtered fluid is excreted in the final urine this means that a further large fraction (35–37 % of the fluid filtered) must be reabsorbed beyond the early distal tubule.

Absolute fluid reabsorption

From the G.F.R. and fractional reabsorption data the values for fluid reabsorption can be calculated at different sites in the kidney (Table 2). For the whole kidney the absolute fluid reabsorption followed the pattern of changes of G.F.R. with a significant rise by 6 days of pregnancy ($P < 0{\cdot}05$), a further significant rise at 12 days ($P < 0{\cdot}005$ compared with 6 days) and a fall at 19 days (when compared to 12 day $P < 0{\cdot}05$) although remaining higher than in virgin animals ($P < 0{\cdot}001$).

Reabsorption by the loop of Henle and the proximal tubule (i.e. in samples collected from early distal tubules) is remarkably similar in all four series, the only statistically significant difference being between the 6 and 19 day pregnant animals. It is more difficult to quantify proximal reabsorption by this technique because of problems with proximal S.N.G.F.R. (see Discussion); however, making the assumption that glomerulotubular balance occurs in the proximal tubule of rats at each stage of pregnancy one can calculate the fluid reabsorption from the calculated reabsorption at the end of the proximal tubule (Fig. 3) and either proximal or distal S.N.G.F.R. (Fig. 2); it can be seen in Table 2 that there are significant differences only in 12 day pregnant animals when proximal S.N.G.F.R. is used in the calculation ($P < 0{\cdot}01$ compared with virgins and 6 days), and that when distal S.N.G.F.R.s are used there are no significant differences.

In an attempt to estimate reabsorption from the proximal tubule by an independent means, fluid reabsorption per unit length was measured using stationary microperfusion methods. In these experiments there was no flow of fluid along the nephron and so changes in S.N.G.F.R. could have no effect on reabsorption. Reabasorption rates were not significantly different at the different stages of pregnancy (see Table 2). Taken together with the altered lengths of the proximal tubules in pregnancy (Garland *et al.* 1978) this would imply (if reabsorption is constant along the nephron) that in pregnancy more fluid was reabsorbed by the proximal tubule because of its greater length and not because of any alteration of intrinsic reabsorptive capacity.

Solute handling

Proximal tubular handling of solutes was not investigated in these experiments but the composition of the fluid in the early distal tubules was. Since, at least for sodium, reabsorption by the proximal tubule is at a similar concentration to plasma, marked differences between the concentration of a solute in tubular fluid and in plasma indicate activity in the loop of Henle. Early distal tubular fluid concentrations are presented (part *A* of each graph) for each series (because plasma concentrations were so similar in the four series the changes in $TF:P$ ratios followed the same pattern as the changes in absolute concentrations and are not presented). The amount of solute filtered at the glomerulus calculated from distal S.N.G.F.R. and plasma concentration (part *B*) and the amount reabsorbed (part *C*) are depicted, and then the percentage of solute not reabsorbed at the site of puncture (part *D*) expressed logarithmically because it is not normally distributed.

Sodium. (Fig. 4). By the time tubular fluid had reached the early distal tubule sodium concentration was much less than in plasma; in virgin animals it was 80 m-mole $l.^{-1}$, only 57 % of the plasma concentration. In all the pregnant animals the concentration of sodium in early distal tubular fluid was significantly less than in

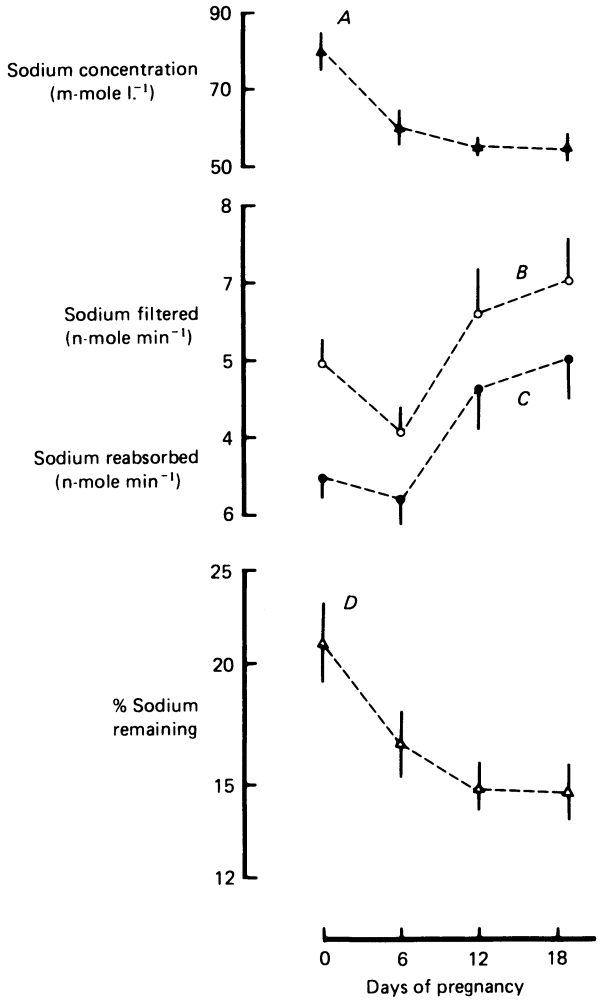

Fig. 4. Sodium concentration (*A*), amount of sodium filtered (*B*), amount of sodium reabsorbed (*C*) and the percentage of the sodium filtered which remains in early distal tubular samples (*D*) at different stages of pregnancy. Bars = ±S.E. of the mean.

virgin animals (Fig. 4*A*; $P < 0{\cdot}01$ in all cases); there were no statistically significant differences between the different stages of pregnancy.

In the 6 day pregnant group distal S.N.G.F.R. and plasma sodium concentration were both slightly less than in the virgins, consequently the filtered load of sodium (Fig. 4*B*) was considerably less and reached the level of statistical significance ($P < 0{\cdot}05$), even though the difference in its two components did not. In the 12 day pregnant group the filtered sodium was significantly higher than in the 6 day group ($P < 0{\cdot}05$),

but not statistically different from virgins, and although the mean value rose higher in the 19 day pregnant group it was still not significantly different from the virgin group (however, $P < 0.005$ when compared with 6 day pregnant value). Reabsorption of sodium prior to the puncture site (Fig. 4*C*) by the 6 day pregnant animals was not significantly different from that reabsorbed by the virgins and this means that

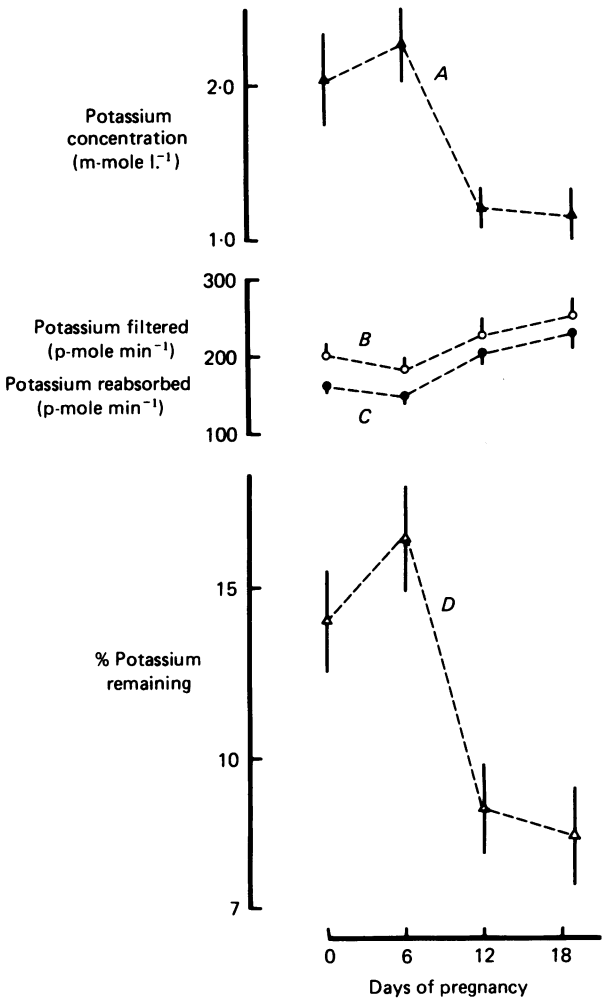

Fig. 5. Potassium concentration (*A*), amount of potassium filtered (*B*), amount of potassium reabsorbed (*C*), and the percentage of the potassium filtered which remains in early distal tubular samples (*D*) at different stages of pregnancy. Bars = ±S.E. of the mean.

a significantly smaller percentage of the filtered load (Fig. 4*D*) was left in the tubule when compared to the virgins ($P < 0.05$). The amount of sodium reabsorbed by 12 and 19 day pregnant animals was significantly higher than in either the virgins or 6 day pregnant ($P < 0.05$ for 12 day pregnant, $P < 0.01$ for 19 day pregnant when compared with either virgins or 6 day pregnant). However, since the filtered load had also increased when compared with 6 day pregnant animals the percentage of the

filtered load was not significantly different between the three different stages of pregnancy. It is instructive to note, however, that single nephrons in 12 and 19 day pregnant animals reabsorb some 25–35% more sodium than do virgin controls; the absolute amount remaining for reabsorption in more distal parts is less in pregnant animals than in the virgin controls (e.g. 19 day pregnant animals 1·0 n-mole min^{-1}, virgins 1·5 n-mole min^{-1}).

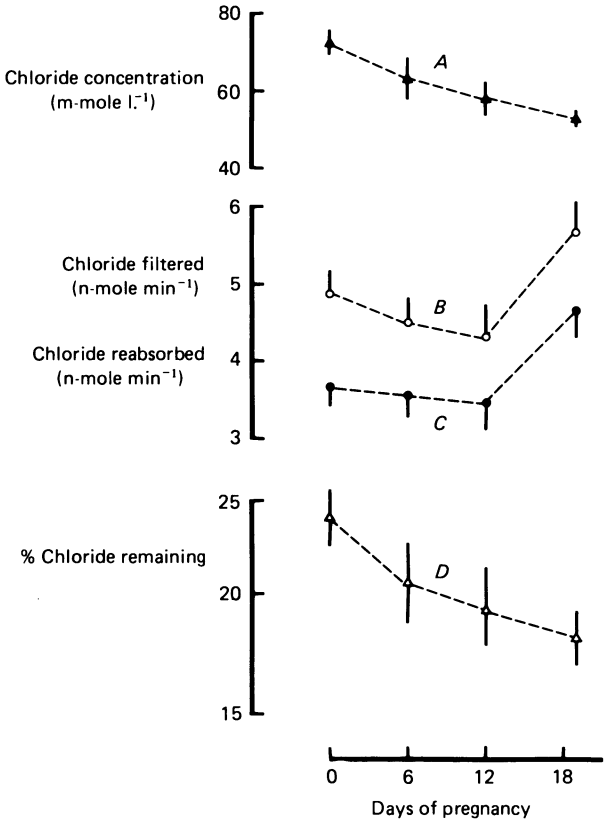

Fig. 6. Chloride concentration (*A*), amount of chloride filtered (*B*), amount of chloride reabsorbed (*C*), and the percentage of the chloride filtered which remains in early distal tubular samples (*D*) at different stages of pregnancy. Bars = ±S.E. of the mean.

Potassium. (Fig. 5). The concentration of potassium in early distal tubular fluid is significantly less than in plasma in all groups; in absolute terms in virgins the concentration was about 2 m-mole $l.^{-1}$ and this was slightly, but not significantly, higher in 6 day pregnant animals (Fig. 5*A*). Early distal potassium concentration was dramatically reduced in 12 and 19 day animals when compared with either virgins or 6 day pregnant animals ($P < 0{\cdot}01$ in both cases).

Changes in the amount of potassium filtered by the glomerulus were small and not statistically significant in 6 and 12 day pregnant animals (Fig. 5*B*). In 19 day pregnant animals, however, more potassium was filtered than in either the virgins ($P < 0{\cdot}05$) or the 6 day pregnant animals ($P < 0{\cdot}01$). The amount of potassium

reabsorbed up to the puncture site (Fig. 5*C*) was not significantly different in the 6 day pregnant group and virgins but thereafter, there was a significant increase in the 12 day pregnant group ($P < 0{\cdot}05$ compared with virgins and 6 day pregnant) and a still higher level in the 19 day pregnant group ($P < 0{\cdot}01$ compared with virgins; $P < 0{\cdot}005$ compared with 6 day pregnant). The percentage of the filtered potassium

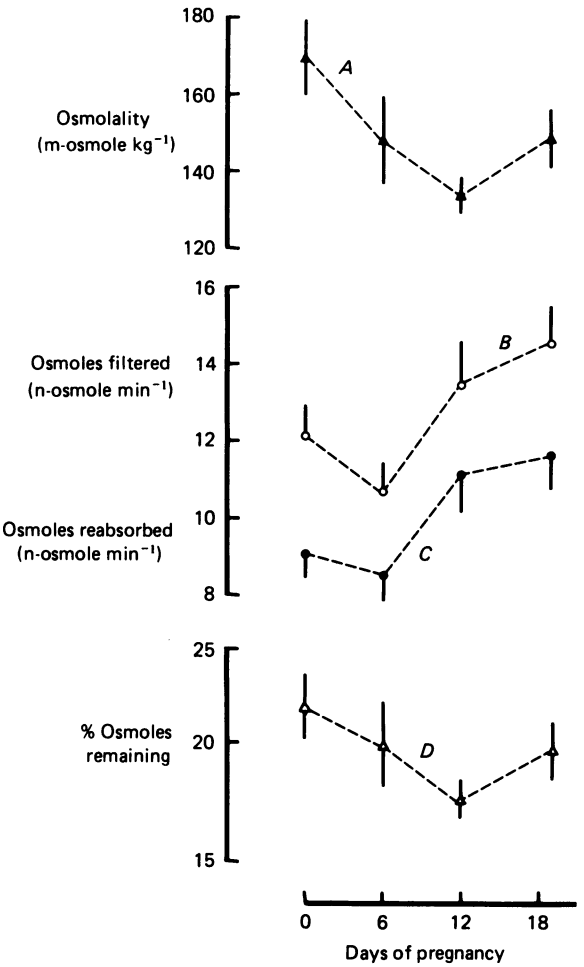

Fig. 7. Osmolality (*A*), filtered osmolal load (*B*), osmolal reabsorption (*C*), and percentage osmolal excretion in early distal tubular samples (*D*) at different stages of pregnancy. Bars = ±S.E. of the mean.

remaining in the tubule (Fig. 5*D*), and hence the percentage reabsorption, was not significantly different in the virgin and 6 day pregnant animals. Subsequently there was a marked reduction in the percentage of filtered potassium remaining in the tubule in both 12 and 19 day pregnant animals ($P < 0{\cdot}01$ compared with controls; $P < 0{\cdot}001$ compared with 6 day pregnant, in both cases).

Chloride. (Fig. 6). In general, changes in chloride handling followed changes in sodium handling, as might be expected; there were, however, some points of

difference. Chloride concentration in early distal tubular fluid in virgins was 63% of the plasma concentration, being some 73 m-mole $l.^{-1}$ (Fig. 6*A*). This was lower at each succeeding stage of pregnancy and the fall reached statistically significant levels in the 12 day pregnant group ($P < 0.05$ compared with virgins) and the 19 day pregnant group ($P < 0.001$ compared with virgins); there were no statistically significant differences between the three groups of pregnant animals.

The amount of chloride filtered was not statistically different in the virgin, 6 and 12 day pregnant groups, but was significantly higher in the 19 day pregnant group than any of the others (Fig. 6*B*; $P < 0.005$ in all cases). Changes in absolute reabsorption of chloride were similar (Fig. 6*C*); again only in the 19 day group was there a significantly higher value ($P < 0.05$) compared with all other groups. The percentage of the filtered chloride remaining in the tubule decreased steadily throughout pregnancy (Fig. 6*D*) but only reached statistically significant levels, when compared to virgins, in the 12 day pregnant group ($P < 0.05$) and the 19 day pregnant group ($P < 0.01$).

Osmoles. (Fig. 7). It might be expected that changes in osmolality of tubular fluid and in osmolal reabsorption would parallel the changes in sodium but this is not the case. The osmolality of early distal tubular fluid (Fig. 7*A*) was about 55% of that in plasma with a mean value of 170 m-osmole kg^{-1} in virgin animals. Osmolality of early distal tubular fluid in 12 day pregnant animals was significantly less than in virgins ($P < 0.005$), but in the 6 and 19 day pregnant groups the osmolality was not significantly different from either virgins or 12 day pregnant animals.

The osmolal load delivered to the tubule was greater in 12 and 19 day pregnant animals than in 6 day pregnant animals ($P < 0.05$ and $P < 0.005$ respectively) but none of them were significantly different from the load delivered in virgins (Fig. 7*B*). Changes occurred in net osmolal reabsorption (Fig. 7*C*); there was no difference in osmolal reabsorption in virgins and 6 day pregnant animals but reabsorption in the 12 day pregnant group was significantly higher than in the 6 day pregnant group ($P < 0.05$) and reabsorption in the 19 day pregnant group was higher than that in both the virgins ($P < 0.05$) and the 6 day pregnant group ($P < 0.01$). The percentage of the osmolal load remaining in the tubule (Fig. 7*D*) was significantly less than in virgins only in the 12 day pregnant group ($P < 0.05$).

'Osmotic deficit'. The fact that there were differences between osmolal handling and sodium handling suggests that some other substance (or substances) must contribute to changes in osmolality (Bishop, Green & Thomas, 1981). The concentration of such substances can be calculated as the 'osmotic deficit' (see Methods). Whatever constitutes this 'deficit' it is greater during pregnancy than in virgin animals accounting for 17.3 ± 3.5 m-osmole $l.^{-1}$ in virgins and 32.4 ± 6.3, 29.4 ± 3.7 and 44.6 ± 6.6 m-osmole $l.^{-1}$ in 6, 12 and 19 day pregnant animals respectively ($P < 0.05$ in all cases); it is not certain whether the 'deficit' concentration changes throughout pregnancy but it is higher in 19 day pregnant animals than in 12 day pregnant ones ($P < 0.05$).

However since the substance(s) responsible for the osmotic deficit is not known (Bishop *et al.* 1981), or cannot be demonstrated with certainty, in these experiments its plasma concentration cannot be measured and so net tubular secretion cannot be assessed.

DISCUSSION

The major findings in the present experiments which require discussion are firstly the changes in whole kidney G.F.R. and S.N.G.F.R. at different stages of pregnancy; secondly the reabsorption of fluid by different nephron segments during pregnancy; thirdly the changes in tubular handling of various solutes resulting in changes in distal tubular solute concentrations throughout pregnancy.

Glomerular filtration rate

Changes in whole kidney G.F.R. through pregnancy in the rat are similar to those described by others (see Atherton & Pirie, 1981). While at least some of these changes may be due to changes in renal plasma flow (Baylis, 1980), to changes in extracellular fluid volume (Atherton & Pirie, 1981) or to changes in hormonal levels (El Karib & Green, 1981), the relative contribution of these factors and whether they have similar effects on different populations of nephrons is not known. Changes in S.N.G.F.R. have not previously been measured throughout pregnancy.

Differences between S.N.G.F.R. measured in proximal and distal tubules are well known and are a consequence of the operation of the tubuloglomerular feed-back system (Navar, Ploth & Bell, 1980). Tubuloglomerular feed-back is inhibited, or has its sensitivity decreased, by a high salt diet (Kaufman, Hamburger & Flamenbaum, 1976), by infusion of large volumes of saline (Schnermann, Davis, Wunderlich, Levine & Horster, 1971) and by the relative level of the renal arterial pressure with respect to the autoregulatory range (Ploth, Schnermann, Dahlheim, Hermle & Schmidmeier, 1977); in the current study all these inhibiting factors were present in all the groups. What is surprising, therefore, is the enhancement of tubuloglomerular feed-back, with a consequent increased difference between proximal and distal S.N.G.F.R. at 12 and 19 days of pregnancy, especially since later stages of pregnancy are accompanied by an increased salt intake in the rat (Churchill *et al.* 1980; Atherton, Dark, Garland, Morgan, Pidgeon & Soni, 1982) and an expansion of extracellular and blood volume Atherton & Pirie, 1981; Atherton *et al.* 1982). Moreover, there is no detectable increase in blood pressure (Table 1). It may be that there is increased sensitivity of the tubuloglomerular feed-back and that this is related to the increased plasma renin activity in the later stages of pregnancy (Schneider & Mulrow, 1973; Whipp, Coghlan, Shulkes, Skinner & Wintour, 1978) but a causal relationship has not yet been demonstrated. On the other hand, from the present data it is not possible to rule out the effect of a small (non-significant) rise in the amount of fluid delivered to the macula densa area of the distal tubule. Nevertheless there is enhancement of tubuloglomerular feed-back in later stages of pregnancy and for that reason, in later stages of pregnancy distal S.N.G.F.R. (where flow past the macula densa is normal) is likely to be a more 'physiological' value than proximal S.N.G.F.R. Data derived from total proximal collections must, therefore, be treated with considerable reserve and we prefer to think that distal S.N.G.F.R.s are the 'true' values.

It must be emphasized that what has been discussed so far are values from superficial cortical nephrons, and there is no *direct* information in this study about the S.N.G.F.R. in the deep or juxtamedullary nephrons. Examination of the results (Fig. 2), however, shows that large changes in the juxtamedullary nephron filtration

rate must also occur. The increased G.F.R./distal S.N.G.F.R. ratio in the 6 day pregnant group indicates a redistribution of G.F.R. but gives no indication of the magnitude. That this is a real change is confirmed by similar changes occurring at 6 days of pregnancy in the G.F.R./proximal S.N.G.F.R., even though this latter value must be treated with reservation because of uncertainties over proximal S.N.G.F.R. It is impossible with these techniques to predict the juxtamedullary S.N.G.F.R. accurately since there is insufficient information about the total number of nephrons and the proportion of juxtamedullary nephrons in the animals used, but if we assume that 22·5% of the nephrons are juxtamedullary in type (Horster & Thurau, 1968) out of a total of 25,000 nephrons (this is slightly lower than the mean of 27,500 predicted for the left kidney of mature female Sprague-Dawley rats by Solomon (1977)), then the juxtamedullary S.N.G.F.R. would be about 10 nl. min^{-1}. Although under hydropenic conditions many studies have shown that S.N.G.F.R. is higher in juxtamedullary than cortical nephrons (Jamison, 1970; Bonvalet, Bencsáth & de Rouffignac, 1972; Horster & Thurau, 1968) it has also been shown that under conditions of increased dietary intake of salt and with a saline infusion, juxtamedullary S.N.G.F.R. falls to levels comparable with the 10 nl. min^{-1} calculated above (Horster & Thurau, 1968). The juxtamedullary S.N.G.F.R. obviously increases dramatically in the early stages of pregnancy. Using the same estimate for total nephron population as above, by the sixth day of pregnancy the juxtamedullary S.N.G.F.R. would be 50 nl. min^{-1}, and although the *relative* proportion did not alter significantly at 12 days the juxtamedullary S.N.G.F.R. would have risen to 75 nl. min^{-1}, falling to 25 nl. min^{-1} in the 19 day pregnant group. Presumably the increased juxtamedullary S.N.G.F.R. is associated with an increased blood flow in the vasa recta.

The cause(s) of the redistribution of filtrate implied in the present study is not known. It may be due to anatomical or biochemical differences in the two types of nephrons; to a selective action of the hormones which are altered during pregnancy; or to complex interactions of the feed-back system and intrarenal hormones (for discussion see Baer & McGiff, 1980), but in the present state of knowledge it is impossible to prefer one alternative over the others.

Fluid reabsorption

During later stages of pregnancy in whole kidney studies there is an increased fluid reabsorption which more than compensates for the increase in G.F.R. and results in an increased fractional reabsorption of fluid (Atherton & Pirie, 1981). The current experiments during saline diuresis confirm this finding (Fig. 3). Since the proximal tubule is the major site of fluid reabsorption in the nephron it might be expected that there would be changes in reabsorption of fluid from the proximal tubule during pregnancy. Because of the uncertainty attached to total collections of proximal tubular fluid (see above) it has not been possible to obtain direct information on proximal tubular reabsorption in the free flow condition. Data from the stationary microperfusion experiments indicate that there may be no change in intrinsic reabsorptive capacity, but since there is an increase in proximal tubular length during pregnancy (Garland *et al.* 1978) the total reabsorption in the proximal tubule may well be raised in pregnancy. What is certain is that if there are changes in proximal fluid reabsorption these may be offset by altered reabsorption in the loop of Henle,

since fractional reabsorption is the same at all stages of pregnancy by the early distal tubule and there are only small changes in absolute fluid reabsorption. Until information about the ability of the loop of Henle to reabsorb fluid or about the TF/P inulin ratios at the end of the proximal tubule obtained without oil blockage becomes available, further speculation does not seem warranted.

What is clear, however, is that the final adjustment, which results in the increased fractional reabsorption seen in whole kidney studies, occurs behind the early part of the distal tubule. What is also striking is the reabsorption that occurs beyond the early distal tubule where some 60 % of the filtered fluid had been reabsorbed; by the final urine this had increased to 95 % reabsorption in virgin and 6 day pregnant animals and 97 % in 12 and 19 day pregnant animals indicating that 35–37 % of fluid is reabsorbed in the distal tubule and collecting duct. The mechanisms responsible for this massive reabsorption and the differences between virgin and 6 day pregnant on one hand and 12 and 19 day pregnant animals on the other are unclear but since in 12 and 19 day pregnant animals the osmolarity of the early distal tubular fluid is much less than in virgins the reabsorption of solute free water may play an important role. Whether the various hormonal changes that occur during pregnancy play any role is not yet known.

Solute handling

Although sodium and fluid are reabsorbed together in the proximal tubules, their transport paths are separated in the loop of Henle where fluid is reabsorbed in the descending limb while sodium *enters* the descending limb and is reabsorbed by a variety of mechanisms in the ascending limb (see Bishop *et al.* 1981). Thus increased reabsorption of sodium, relative to water, implies alteration in the function of the loop of Henle, probably the ascending limb, and results in a decreased sodium concentration in the distal tubule. It can be seen (Fig. 4) that such changes do occur and that they occur by the sixth day of and are maintained throughout the rest of pregnancy. Whether this increased reabsorption is sufficient to account for the sodium retention that occurs during pregnancy (Churchill *et al.* 1980; Atherton *et al.* 1982), or whether redistribution to deep nephrons also plays a significant part is not known. Nor is it apparent why Churchill *et al.* (1980) failed to find an effect of pregnancy on early distal sodium, although it may be, as they suggest, that their preparation of term pregnant animals impaired renal function. In the current experiments, as opposed to theirs, the total G.F.R. behaved in a similar fashion in experiments where micropuncture was not performed (compare Atherton & Pirie, 1981) or in experiments in conscious animals (Atherton, 1981).

The cause(s) of the changes in sodium handling are not known although four possibilities must be considered.

(1) If blood flow to the juxtamedullary nephrons increases and there is a wash-out of the medullary gradient in pregnancy, the sodium entry into the descending limb of the loop would be restricted as would water loss; both would combine (if other parts of the loop function normally) to produce a lower sodium concentration in early distal tubules.

(2) Similar haemodynamic changes could result in an increased amount of sodium being lost from the ascending limb.

(3) The capacity of the mechanism for transport of chloride and sodium in the thick ascending limb might be enhanced and more sodium and chloride would be reabsorbed.

(4) Changes in permeability to sodium could occur in any segment of the loop. In this respect, the effects of hormones on functions of the loop of Henle have not been studied.

The changes in sodium handling might be expected to produce similar changes in total solute reabsorption, as exemplified by osmolal reabsorption, but this was not so (compare Figs. 4 and 7). One reason for this could be because some osmotically active substance is secreted into the lumen of the tubule, and following the argument of Bishop *et al.* (1981) this is most likely to be urea. The reason for this increased entry of urea into the loop of Henle is not known since neither the gradient for urea movement nor the urea permeability have been measured during pregnancy. It might also be expected that changes in sodium would alter potassium handling, but whereas the changes in sodium and potassium are similar at the twelfth and nineteenth days of pregnancy, the changes in the two ions at 6 days are dissimilar and so could not be attributed to a common mechanism. The progressive reduction in the percentage of filtered chloride remaining in the distal tubule through pregnancy presumably reflects increase activity of the chloride transport system in the thick ascending limb of the loop of Henle and this could be responsible for increased sodium and potassium reabsorption. It is not an adequate explanation, however, for the divergent behaviour of sodium and potassium handling in the 6 day pregnant animals.

This discussion assumes that the changes in early distal tubule fluid reflect changes in the proximal tubule or loop of Henle. Schnermann, Schubert & Briggs (1981) have recently shown that the loop of Henle may reduce electrolyte concentrations much more than was originally thought and that diffusion back into the tubular lumen occurs in the post macula densa segment of the distal tubule. If this is found to be generally true then changes in the transport properties of this segment might be responsible for the differences obtained. As yet nothing is known about these transport characteristics.

In summary, divergent changes in G.F.R. and S.N.G.F.R., altered sodium reabsorption, similar fluid reabsorption up to the early distal tubule and altered osmotic deficit in the fluid collected after the loop of Henle argue that there are changes of function in many segments of the nephron, as well as redistribution of filtrate. What the precipitating factors(s) is in pregnancy which causes these changes to occur is not possible to determine from the present data, but a multifactorial cause seem most likely.

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