J. Physiol. (1980) **301**, *pp.* 129–136
Printed in Great Britain

CHANGES IN RENAL HAEMODYNAMICS AND KIDNEY WEIGHT DURING PREGNANCY IN THE UNANAESTHETIZED RAT

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(*Received 5 July 1979*)

SUMMARY

1. Glomerular filtration rate ([^{3}H]inulin clearance) and renal plasma flow (PAH clearance) were measured in unanaesthetized Sprague-Dawley rats early in pregnancy (8–10 days) and near term (18–20 days); the results were compared with studies in virgin litter-mates. Evidence of kidney growth was sought by analysing renal dry weights as well as [^{14}C]choline incorporation into membrane phospholipids of renal cortical slices.

2. Inulin clearances were similar early in pregnancy (pregnant $2{\cdot}36 \pm 0{\cdot}12$ (S.E. of mean) *vs.* non-pregnant $2{\cdot}33 \pm 0{\cdot}10$ ml./min) but near term values were significantly increased (pregnant $2{\cdot}80 \pm 0{\cdot}05$ *vs.* non-pregnant $2{\cdot}39 \pm 0{\cdot}05$ ml./min, $P < 0{\cdot}001$). PAH clearances were similar in pregnant and litter-mate control animals at each stage of the study.

3. Total renal weight increased significantly both early and late in pregnancy, but renal dry weights as well as [^{14}C]choline incorporation were similar in pregnant and non-pregnant animals both at 8–10 days gestation and near term.

4. Data confirm our previous findings on renal haemodynamics in anaesthetized rats. Observations that renal dry weight and [^{14}C]choline incorporation into phospholipids are similar in litter-mate pregnant and control animals suggests that renal enlargement in this species is due to increments in water content and not to accelerated growth.

INTRODUCTION

Physiological alterations which accompany normal human pregnancy include marked increases in glomerular filtration rate (g.f.r.) and renal plasma flow, and perhaps increments in kidney size (Lindheimer & Katz, 1977). The increases in renal haemodynamics occur soon after conception, are maximal in the first trimester and are sustained till near term (Davison, 1974; Davison & Hytten, 1974).

Data concerning kidney function and size in pregnant rats are sparse and confusing. Early observers either reported that no changes occurred in g.f.r. throughout gestation or that increments were present in mid-pregnancy which returned to normal near term (Lichton, 1963; Sims, 1963; Matthews & Taylor, 1960; Lichton

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0022-3751/80/7610-0355 $07.50

& Hugh, 1968; Herrera, Kropp & Freinkel, 1969). In 1971, Lindheimer & Katz, using constant infusion techniques showed that inulin and *p*-aminohippurate (PAH) clearances were similar in anaesthetized 14 day pregnant Sprague-Dawley rats and their virgin litter-mates, but near term (gestational day 20) g.f.r. was significantly greater in the pregnant animals. More recently investigators studying age-matched, pregnant and non-gravid Sprague-Dawley animals observed that g.f.r. increases much earlier during pregnancy (Atherton & Pirie, 1977). In their studies [^{3}H]inulin clearance was elevated as early as 5 days post-conception. The same changes were observed in pseudopregnant rats, suggesting that development of placenta and fetus were not essential factors (Atherton & Pirie, 1979). Furthermore, workers from the same laboratory reported that renal dry weight increased 13% and proximal tubular length 20% by the seventh day of gestation (Balmer, Garland, Green, Moriarty & Richardson, 1977; Garland, Green & Moriarty, 1978). Such findings resemble those seen in rats undergoing compensatory renal hypertrophy after unilateral nephrectomy (Hayslett, Kashgarian & Epstein, 1968; Nowinski & Goss, 1969) and prompted the present protocols designed to measure renal haemodynamics in unanaesthetised pregnant rats and compare them with virgin littermates studied under identical conditions. In addition, evidence of change in renal size was sought both by analysing renal wet and dry weights as well as by the incorporation of [^{14}C]choline into membrane phospholipids, a sensitive marker of renal growth (Toback, Smith & Lowenstein, 1974).

METHODS

Litter-mate Sprague-Dawley rats (Sprague-Dawley Farms, Madison, Wisconsin) paired and caged together after weaning, were fed standard rat chow. One animal from each pair was bred when 8 weeks old. Vaginal smears were examined and pregnancy dated from the day on which spermatozoa were observed. Tests were performed on gravid rats during the 8–10th or 18–20th gestational day and in each experiment the pregnant animal was studied simultaneously with its similarly handled virgin litter-mate.

Renal function

Before the clearance studies, each animal was lightly anaesthetized with ether. A polyethylene catheter was introduced into the bladder and sewn securely in place. The urethra was occluded with a clip at the vulva. Polyethylene tubing (PE 50) was also placed in a femoral artery to allow blood sampling. Isotonic saline equal to 1% of body weight was infused through a PE50 catheter into a jugular vein to replace estimated surgical fluid loss and this tubing which was tunnelled sub-cutaneously around the back of the neck was used for the primary dose and constant infusion of [^{3}H]inulin and PAH.

After cannulation of blood vessels and bladder, animals were placed in restraining cages, secured in part with black silk sutures in the skin of the back, and allowed to recover from anaesthesia. After priming doses, the sustaining infusion was commenced. The infusate, containing 5 μc [methoxy-^{3}H]inulin and 4 mg PAH/ml. hypotonic saline, was delivered with a constant infusion pump (Harvard Apparatus Co., Inc. Model 975, Millis, Mass.) at a rate of 0·04 ml./min in both pregnant and control animals. After a 1 hr equilibration period urine was collected in pre-weighed test tubes and volumes determined by re-weighing the tubes shortly after completing a collection. In each experiment, collections from four consecutive periods of 30 min each were obtained, and a heparinized blood sample was taken at the mid-point of each period. Experiments in which steady-state conditions did not prevail in both gravid and control animals, were rejected.

Following sacrifice, the uterus and its contents were weighed and subtraction of this figure from the animal's total body weight determined the non-conceptus body weight of the pregnant rat. Uterine weight after removal of fetuses, placentae and amniotic fluid was on average 2–3 g heavier than that of virgin litter-mates. Correction for this small underestimation of maternal non-conceptus weight does not alter the statistical significance of any of the differences observed.

Plasma and urine concentrations of [methoxy-^{3}H]inulin and PAH were measured in duplicate samples. [^{3}H]inulin was measured in a β-liquid scintillation spectrometer (Packard Instrument Co., Chicago, Illinois) with appropriate corrections for quenching and PAH was determined by a semi-micromodification of the diazotisation method (Smith, Finkelstein & Aliminosa, 1945).

[^{14}C]choline incorporation into renal phospholipid

After completion of renal clearances, as well as in separate experiments, rats were exsanguinated by decapitation. The left kidney was then quickly removed, immediately decapsulated and weighed. Within 2 min, two or three cortical slices, each about 0·4 mm thick, were obtained with a Stadie–Riggs microtome. Each slice was incubated in 2 ml. iced Krebs–Ringer–bicarbonate with 20 μM-[methyl-^{14}C]choline chloride (specific activity 30 mc/m-mole, 83 d.p.m./p-mole, New England Nuclear, Boston Mass.). Radioactivity of the trichlororacetic acid-insoluble and soluble fractions were then determined as described by Toback *et al.* (1974). The rate of [^{14}C]choline incorporation into phospholipid was expressed as disintegrations per minute [^{14}C]-choline incorporated into the acid-insoluble fraction per milligram dry weight per 30 minutes of incubation. No [^{3}H]activity was detected in acid extracts of the kidney slices from rats infused with [methoxy-^{3}H]inulin. Values for each animal were calculated as the mean rate of incorporation in two to three slices.

In experiments designed to determine renal water content, kidneys were weighed after decapsulation, and dried to constant weight in an oven at 105 °C.

RESULTS

Results are presented as mean ± S.E. of mean. The significance of differences between pregnant and virgin litter-mate controls was assessed by non-paired Student's *t* test; values less than 0·05 are reported as statistically significant.

Renal function and kidney weight

Results of all clearance studies from 19 paired infusion studies are summarized in Table 1. There were no differences in animals pregnant for 8–10 days compared with littermate virgin controls. However, by 18–20 days gestation [^{3}H]inulin clearance was significantly greater than that measured in controls ($P < 0·001$) although PAH clearance was still similar in both groups.

Table 1 also summarises non-conceptus body weight and renal wet and dry weights. Significant increases had occurred in non-conceptus weight ($P < 0·05$) and renal wet weight ($P < 0·05$) by the second gestational week, but renal dry weight remained similar in pregnant and non-pregnant animals. Near term the increment in non-conceptus weight in the pregnant rat was most striking ($P < 0·001$), renal wet weight remained increased ($P < 0·05$) and there were still no differences in renal dry weight when pregnant and virgin litter-mate controls were compared. Note also that kidney weight data for near term animals includes only five littermate pairs. These were the animals used in the choline incorporation experiments (below). However, in eleven additional littermate pairs the wet weight of both

TABLE 1. Non-conceptus body weight, renal haemodynamics and renal weights in pregnant and virgin litter-mate control rats (mean ± SE)

Group	Non-conceptus body weight (g)	C_{inulin} (ml./min)	C_{PAH} (ml./min)	Urine flow (μl./min)	Single kidney wet weight (mg)	Single kidney dry weight (mg)
8–10 days pregnant	209 ± 5·0 (12)	2·36 ± 0·12 (8)	6·78 ± 0·33 (8)	44·4 ± 5·3 (8)	761·0 ± 14·6 (9)	163·8 ± 2·1 (9)
Virgin litter-mates	195 ± 4·9	2·33 ± 0·10	6·56 ± 0·26	46·0 ± 6·4	714·9 ± 16·9	156·0 ± 5·2
P	< 0·05	N.S.	N.S.	NS	< 0·05	N.S.
18–20 days pregnant	244 ± 4·8 (11)	2·80 ± 0·05 (11)	6·70 ± 0·30 (11)	38·3 ± 4·6 (11)	806·8 ± 18·2 (5)*	177·5 ± 5·5 (5)*
Virgin litter-mates	203 ± 2·4	2·39 ± 0·05	6·56 ± 0·24	45·3 ± 5·4	734·6 ± 22·9	165·5 ± 3·4
P	< 0·001	< 0·001	N.S.	NS	< 0·05	N.S.

In parentheses, number of pairs of animals.

Significance of differences (*P*) between 8–10 day and 18–20 day pregnant animals and their respective controls assessed by unpaired Student's *t* test.

* In preliminary experiments similar comparisons were made in ten age-matched gravid and non-pregnant pairs, in which similar difference obtained.

kidneys was 1240 ± 20 mg in non-pregnant and 1490 ± 40 mg in 18–20 days gravid rats ($P < 0.005$). Results from infused animals were no different from non-infused animals.

TABLE 2. [^{14}C]choline incorporation into phospholipid of renal cortical slices in pregnant and virgin litter-mate control rats (mean ± S.E.)

Group	[^{14}C]choline incorporation into phospholipid* (dpm/mg dry wt. 30 min)
8–10 day pregnant	4482 ± 319
	(10)
Virgin litter-mates	4689 ± 303
18–20 day pregnant	4615 ± 353
	(5)
Virgin litter-mates	5075 ± 321

* Values for each animal were calculated as the mean rate of [^{14}C]choline incorporation in two or three slices for a single kidney.

In parentheses, number of pairs of animals.

Differences between 8–10 day and 18–20 day pregnant animals and their respective controls not significant, as assessed by unpaired Student's *t* test.

[^{14}*C*]*choline incorporation into renal phospholipids*

The rate of [^{14}C]choline incorporation into the acid-insoluble fraction of renal cortical slices from left kidneys was similar in ten pairs of virgin and 8–10 day gravid litter-mate animals (Table 2).

In preliminary experiments similar rates of incorporation were also observed when ten non-littermate 18–20 day gravid rats were compared to those of ten age-matched non-pregnant controls (non-pregnant: 5062 ± 179 *vs.* pregnant: 5350 ± 245 d.p.m./mg dry weight. 30 min, n.s.). Similar results were noted in five additional 18–20 pregnant and their virgin litter-mate controls whose results are summarised in Table 2. Incorporation results were similar whether or not clearance studies had preceded sacrifice.

DISCUSSION

Data concerning renal haemodynamics during pregnancy in the rat are conflicting. Some authors note that g.f.r. increases in the first 2 weeks of pregnancy decreasing to non-pregnant values before delivery (Sims, 1963; Matthews & Taylor, 1960; Lichton & Hugh, 1968; Atherton & Pirie, 1978), while others report increments occurring near term (Lichton, 1963; Lindheimer & Katz, 1971; Lindheimer, Koppen & Katz, 1976). Explanations for these conflicting results may be found in the different, and sometimes imprecise, methods used and have been discussed in a previous publication (Lindheimer & Katz, 1971). For example, in some studies single injection techniques were used, animals were volume expanded to increase urine flow and clearances were calculated from a single collection period.

In the present experiments each pregnant animal was studied together with a virgin littermate under identical test conditions, and results calculated from four

successive 30 min clearance periods. The results extend previous observations that g.f.r. increases only near term in pregnant Sprague-Dawley rats (Lindheimer & Katz, 1971) by demonstrating that this occurs in unanaesthetized animals. The results, also confirm previous reports that while kidney wet weights are increased during pregnancy dry weights are similar in pregnant and non-pregnant animals (Poo, Lew & Addis, 1939; Peters, Krijnen & Boyd, 1967). These observations do not, however, confirm recent reports (Atherton & Pirie, 1977; Balmer *et al.* 1977; Garland *et al.* 1978) that g.f.r., renal dry weights and proximal tubular length are increased by the second gestational week in Sprague-Dawley rats, i.e. changes in the gravid animal which resemble the compensatory renal hypertrophy that occurs after uninephrectomy. We performed additional experiments analysing [^{14}C]choline incorporation into renal cortical phospholipids, and could demonstrate no evidence of new renal growth. Thus our data are consistent with the hypothesis that alterations in renal haemodynamics occurring during late pregnancy in rats are functional rather than structural in nature.

Lindheimer & Katz (1971) measured inulin and PAH clearances in 14 day and 20 day anaesthetized gravid animals comparing results to virgin litter-mate controls and observed that inulin clearance only increased shortly before delivery. They also noted that absolute inulin spaces increased both in the second and last gestational weeks. However, while inulin space per gram non-conceptus weight was similar in 14 day gravid and litter-mate controls, this value did increase significantly near term. The authors cautiously suggested that this latter change might influence the observed increment in g.f.r.

Alterations in volume may underly the difference between our findings and others, especially in studies of animals prepared as for micropuncture where increases in inulin clearance were noted as early as 5 days after conception (Atherton & Pirie, 1977). This might reflect the fact that gravid rats resist the dehydration that occurs during preparation for micropuncture (Gottschalk & Lassiter, 1973) better than non-pregnant animals and thus appear to have higher filtration rates. This would be consistent with the observations that renal water content increases very early after conception and that the absolute glomerular filtration rates in both the pregnant and non gravid animals studied by Atherton & Pirie (1977) are substantially lower than either those reported in anaesthetized rats (Lindheimer & Katz, 1971) or awake animals in the present investigation.

Discrepancies between renal weights reported in other studies and our own, are harder to explain. Garland *et al.* (1978) who noted increases in kidney dry weight early in gestation also observed increments in proximal tubule lengths but only the latter persisted until term and into the post-partum state (Atherton & Pirie, 1978). However, any recent increase in renal growth might be expected to be reflected in increments in [^{14}C]choline incorporation in kidney cortical slices, since renal growth relies upon formation of new cellular membranes and choline is the major precursor in their synthesis (Kennedy & Weiss, 1956; Plagemann, 1968). In a series of elegant experiments Toback and his colleagues (1974) demonstrated that when renal cortical slices are incubated with radioactive choline, over 99% of the label is incorporated in phosphatidylcholine, the major phospholipid of cellular membranes. More importantly, they demonstrated that significant increments in

incorporation are present in the contralateral kidney as early as 5 min following uninephrectomy in mice and rats, long before increases in renal function or weight occur. They have further demonstrated that this altered phospholipid metabolism or synthesis in the rat kidney persists for many days after a variety of stimuli (Toback, Ordonez, Bortz & Spargo, 1976; Toback, Havener, Dodd & Spargo, 1977). Such data imply that [^{14}C]choline incorporation is a sensitive and reliable marker of accelerated renal growth and it is of interest that we failed to demonstrate any changes in this index when either 8–10 or 18–20 day pregnant animals were compared with virgin litter-mates. These observations coupled with the absence of any changes in renal dry weight are strong arguments against the presence of morphological alterations akin to compensatory renal hypertrophy in pregnancy, but do not preclude ultrastructural changes within the cells of the proximal tubules or even medullary hypertrophy during pregnancy (Chang, Pike & Clagett, 1978; Schiebler & Danner, 1978).

Finally, the observation that PAH clearances remained unchanged while g.f.r. increased at term is of interest (these findings too confirm our previous data in anaesthetized animals, in which PAH extraction ratios were also similar in gravid and non-pregnant rats) since filtration rate is said to be plasma flow dependent in the rat (Brenner, Troy & Daugharty, 1971; Brenner, Troy, Daugharty, Deen & Robertson, 1972). Such discrepancies may be explained by alterations in intrarenal blood perfusion, but we have recently demonstrated that the distribution of radioactive microspheres is unchanged at term in pregnant Sprague-Dawley rats (Lindheimer *et al.* 1976). Contrary to previous claims filtration pressure equilibrium may not be attained by the end of the glomerulus in the rat (Arendshorst, 1979). However, there is evidence that g.f.r. is elevated by the twelfth day of pregnancy in the Munich-Wistar rat and this is accompanied by a proportional increase in renal plasma flow (Baylis, 1979). Further experiments are necessary to explore these discrepancies.

This work was supported in part by grants from the National Institutes of Health (HD-5572), the Mothers Aid Research Fund (Chicago Lying-In Hospital) and the Medical Research Council.

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