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Changes in blood urea nitrogen (BUN) concentration during pregnancy in the rat with or without obstructive uremia

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With one figure

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Summary

Bilateral ureteral ligation changes the blood urea nitrogen (BUN) concentration in pregnant and non-pregnant rats. Marked increases of the BUN concentration occurred in both pregnant and non-pregnant specimens after ligation. The BUN concentration in sham-ligated rats did not change from day 16 through 19 of pregnancy, but increased on day 20 of pregnancy and thereafter. There was no significant difference in BUN between ligated pregnant rats on days 16 and 17 of pregnancy and ligated non-pregnant rats. As pregnancy progressed from day 18 onward, the BUN in ligated pregnant rats decreased when compared with that of ligated non-pregnant rats, but did not reach the level in sham-ligated pregnant rats. The BUN of mothers and fetuses both in the ligated and the sham-ligated groups were similar. Amniotic fluid urea nitrogen levels were higher than in blood on late fetal days. In ligated mothers, a significant relationship between maternal BUN and the number of fetuses was noted on day 22 of pregnancy.

These results suggest that the fetal kidney becomes functional for excreting urea in late fetal life.

Introduction

Kidney function in pregnant rats has been the subject of several investigations (3, 7, 10). Glomerular filtration rate and renal reabsorption are higher in pregnant animals than in virgin ones (4), indicating that changed renal function is associated with pregnancy. Also, it has been suggested that the rat can serve as a useful model for the renal changes occuring in human pregnancy since rats demonstrate similarities with human pregnancy at the whole kidney level (7). With the increasing use of ovulation inducers in human obstetrics more women are becoming polytocous. Despite the fact that rats are polytocous animals, there are few reports which consider litter size and maternal renal function during pregnancy.

It is commonly believed that throughout pregnancy the organ of excretion in the fetus is the placenta, not the fetal kidney, and that fetal waste products are processed exclusively by the placenta and transfered into the maternal circulation to be voided into the maternal urine (13). Thus one might expect some relationship between maternal renal function during pregnancy and litter size.

The present study was designed to explore kidney function during pregnancy using blood urea nitrogen (BUN) levels as an indicator. Pregnant rats with and without uremia caused by bilateral ureteral obstruction were the chief focus of this study. A further aim was to clarify the relationship between maternal BUN levels and the number of fetuses at the different stages of pregnancy when obstructive uremia was induced.

Materials and Methods

One hundred and fifty-five female adult Wistar rats were allowed free access to water and a commercial diet (Oriental Pellets NMF). The animals were divided into two groups, "Pregnant" and "Non-pregnant". "Pregnant" rats were bred in the evening in our laboratory, with the following morning considered day one of pregnancy.

All surgery was performed under ether anesthesia and consisted of either bilateral ureteral ligation or a "sham" operation. In both cases animals were subjected to midventral laparotomy and the apex of the urinary bladder was grasped with a hemostat to expose the ureters. Each ureter was doubly ligated near the bladder neck with cotton thread, or, in the sham operations, the thread was passed around the ureters and then removed. After the operation, the bladder was returned to the pelvic cavity and the incision was closed with sutures. In the "Non-pregnant" group, blood samples were drawn at either one or two days post-surgery from the abdominal aorta while the rats were under ether anesthesia. BUN determinations were made on these samples with a Rapid Blood Analyzer (Chugai Pharmac. Tokyo, Japan). In the "Pregnant" group, operations were performed and blood was drawn from animals representing each day from day 15 through day 21 of pregnancy, and second blood collections for BUN determination were obtained at either one or two days after the operation. Blood of fetuses on days 21 and 22 of gestation was collected by decapitation and pooled from each litter to determine BUN concentration. Blood collected from sham-operated groups served as controls.

To ascertain whether there might be some relationship between maternal BUN level and litter size, bilateral ureteral ligation was performed on pregnant rats which had small numbers of fetuses. Since the rats we normally keep in our laboratory always have about 10—13 fetuses per litter, some of the fetuses were surgically removed on day 12 or 13 of pregnancy. In these animals the ureteral ligation was performed on days 15, 18, and 21 of pregnancy respectively, and blood was collected to determine BUN concentration one day after each operation.

Further, to determine urea nitrogen (UN) in amniotic fluid, the fluid on days 20, 21 and 22 of gestation was collected from each amniotic cavity using a syringe and a hypodermic needle and pooled from two litters for UN determination. Urine from fetuses from ligated mothers was also used for UN determination. The urines were collected through the wall of the bladder using a syringe and a hypodermic needle.

Analyses of data were made with Student's t-test. A P value of less than 0.05 was considered significant statistically.

Results

Effects of ureteral ligation on BUN concentration

The results of bilateral ureteral ligation are shown in tables 1 and 2.

Table 1. BUN concentrations in non-pregnant rats

Group	Number of animals	Numer of days of treatment	BUN (mg/dl) mean ± SEM	P value
NP-N	6	0	21.4 ± 1.0	$0.2 < P < 0.5$
NP-C	6	2	23.5 ± 2.2	$P < 0.001$
NP-E	5	1	168.8 ± 3.7	

Abbreviations: NP-N, no-treatment; NP-C, sham-ligation; NP-E, bilateral ureteral ligation.

Table 2. BUN concentrations in pregnant rats

Group		Number of animals	Duration of pregnancy in days at		Number of days of treatment	BUN(mg/dl) mean ± SEM	P value
			Ligation	Autopsy			
P-C	mother	5	20	22	2	21.4 ± 2.2	$0.2 < P < 0.5$
	fetus	58 (5)				24.0 ± 1.7	
P-E-20,	mother	7	20	22	2	194.1 ± 14.1	$0.5 < P < 0.8$
	fetus	80 (7)				200.2 ± 11.3	
P-E-21,	mother	5	21	22	1	122.2 ± 5.2	$0.8 < P$
	fetus	54 (5)				120.1 ± 6.5	

Abbreviations: P-C, sham-ligation; P-E, bilateral ureteral ligation. Blood samples of fetuses were pooled from each litter. The numbers in parentheses stand for the number of litters pooled.

Table 3. BUN concentrations in sham-ligated and bilaterally ligated rats at different days of pregnancy

Group		Number of animals	Duration of pregnancy in days at		BUN (mg/dl) mean ± SEM	P value
			Ligation	Autopsy		
P-C-15		5	15	16	22.8 ± 1.7	$P^1 < 0.001$
P-E-15		5	15	16	162.7 ± 7.6	0.7 < P^2 < 0.8
P-C-16		5	16	17	21.4 ± 1.3	0.9 < P^3
						$P^1 < 0.001$
P-E-16		5	16	17	163.6 ± 8.1	0.8 < P^2 < 0.9
P-C-17		5	17	18	21.4 ± 1.3	0.1 < P^3 < 0.2
						$P^1 < 0.001$
P-E-17		5	17	18	145.2 ± 6.6	0.4 < P^2 < 0.5
P-C-18		5	18	19	20.1 ± 1.1	0.4 < P^3 < 0.5
						$P^1 < 0.001$
P-E-18		6	18	19	140.5 ± 1.9	0.001 < P^2 < 0.01
P-C-19		5	19	20	27.1 ± 1.2	0.1 < P^3 < 0.2
						$P^1 < 0.001$
P-E-19		5	19	20	129.7 ± 7.4	0.6 < P^2 < 0.7
P-C-20,	mother	5	20	21	25.8 ± 1.9	0.5 < P^3 < 0.6
						0.5 < P^4 < 0.8
	fetus	55 (5)			25.2 ± 1.6	$P^7 < 0.001$
						$P^8 < 0.001$
P-E-20,	mother	5	20	21	124.1 ± 3.1	0.4 < P^5 < 0.5
						0.5 < P^4 < 0.8
	fetus	53 (5)			121.6 ± 5.9	
						0.7 < P^3 < 0.8
P-C-21,	mother	5	21	22	28.7 ± 3.0	0.7 < P^6 < 0.8
						0.8 < P^4
	fetus	64 (5)			28.9 ± 2.2	$P^7 < 0.001$
						$P^8 < 0.001$
P-E-21,	mother*	5	21	22	122.2 ± 5.2	
						0.8 < P^4
	fetus*	54 (5)			120.1 ± 6.5	

Abbreviations: P-C, sham-ligation; P-E, bilateral ureteral ligation. Blood samples of fetuses were pooled from each litter. The numbers in parentheses designate the number of litters. *, Data repeated from table 2. P^1, Significance of the difference of the mean between P-C and P-E group on each day of pregnancy. P_2, Significance of the difference of the mean between days in P-C group. P^3, Significance of the difference of the mean between days in P-E group. P^4, Significance of the difference of the mean between mother and fetus in each group. P^5, Significance of the difference of the mean of the mother between days in P-C group. P^6, Significance of the difference of the mean of the mother between days in P-E group. P^7 and P^8, Significance of the difference of the mean of the mother (P^7) or fetus (P^8) between P-C and P-E group on each day of pregnancy.

Table 4. UN concentrations in amniotic fluid and fetal urine

Group	Day of pregnancy	Number of animals	Average number of fetuses per litter	UN (mg/dl)	
				Amniotic fluid	Fetal urine
P-N-20	20	2	11.0	32.8	*
P-N-21	21	2	11.0	32.2	*
P-N-22	22	2	11.0	91.3	*
P-C-21	22	2	13.0	62.5	*
P-E-21	22	8	10.8	175.0	346.0

Abbreviations: P-N, no-treatment; P-C, sham-ligation; P-E, bilateral ureteral ligation in which the ureters were ligated bilaterally on day 21 of pregnancy. Amniotic fluid and fetal urine were pooled from two litters for UN determination. *, Since we failed to collect sufficient quantities of urine from the fetuses of non-treated mothers, no UN determination was made.

Table 1 shows the BUN concentrations in non-pregnant rats with or without ureteral ligation. The BUN concentration in ligated rats showed about an eight fold increase when compared with that in either sham-ligated or non-treated controls. There was no statistically significant difference in the BUN concentration between the two control groups; non-treated and sham-ligated.

Table 2 shows the BUN concentration in near term pregnant rats with and without bilateral ureteral ligation. Bilateral ureteral ligation induced marked increase in the BUN concentration both one and two days after treatment. BUN concentrations in the fetal rats were similar to those of their mothers in both the sham-ligated and the ligated groups.

The effects of ureteral ligation at different days of pregnancy are shown in table 3. The BUN concentration in sham-ligated rats did not change from day 16 through 19 of pregnancy, but increased slightly from 20.1 mg/dl on day 19 of pregnancy to 27.1 mg/dl on day 20 of pregnancy ($0.001 < P < 0.01$), and remained at this level by day 22 of pregnancy. On the other hand, the BUN concentration in ligated mothers was always about five times that of controls starting on day 16 of pregnancy. From day 18 onward the BUN concentration decreased but did not reach the level of BUN in sham-ligated mothers by day 22 of pregnancy, the end of gestation.

The BUN values in fetal rats from both the ligated and the sham-ligated mothers on days 21 and 22 of gestation were similar to those of their mothers.

Changes in the urea nitrogen (UN) concentration in amniotic fluid and fetal urine

The urea nitrogen concentrations in amniotic fluid and fetal urine are shown in table 4. The UN concentrations in amniotic fluid from non-ligated mothers on days 20, 21 and 22 of pregnancy were higher than those of maternal and fetal blood and increased strikingly between days 21 and 22 of pregnancy (see tables 2 and 3).

Maternal bilateral ureteral ligation caused a remarkable increase in the UN concentration in amniotic fluid.

We failed to collect sufficient quantities of urine from the fetuses of non-treated mothers for UN determination since the urinary bladders of the fetuses from non-treated mothers were virtually empty. Bladders of the fetuses from ligated mothers were filled with urine, and the UN concentration in the fetal urine averaged 346.0 mg/dl.

Relationship between maternal BUN and the number of fetuses at different stages of pregnancy

The relationships between maternal BUN and the number of fetuses on days 16, 19 and 22 of pregnancy after bilateral ureteral ligation are shown in fig. 1. There was no significant correlation between maternal BUN and the number of fetuses on days 16 ($r = -0.078$, $0.8 < P < 0.9$) and 19 ($r = -0.441$, $0.1 < P < 0.2$) of pregnancy. On day 22 of pregnancy, however, a highly significant inverse relationship between maternal BUN and the number of fetuses was noted ($r = -0.774$, $0.001 < P < 0.01$).

Discussion

In our sham-ligated (control) pregnant rats, the BUN concentration showed a significant increase on day 20 of pregnancy and maintained the increased level till the end of gestation. One explanation for this increase might be that the growth spurt seen in the later days of rat gestation generates a large burden of waste products.

On the other hand, bilateral ureteral ligation caused obstructive uremia and induced high elevations of the BUN concentration in both non-pregnant and pregnant rats. There were no significant differences between the BUN concentrations one day after the ligation in non-pregnant rats, and in 16- and 17-day pregnant rats. However, the high levels of BUN noted in the 16- and 17-day ligated pregnant animals decreased in day 18 and later samples. Eventually the BUN levels were significantly lower than those in the ligated non-pregnant

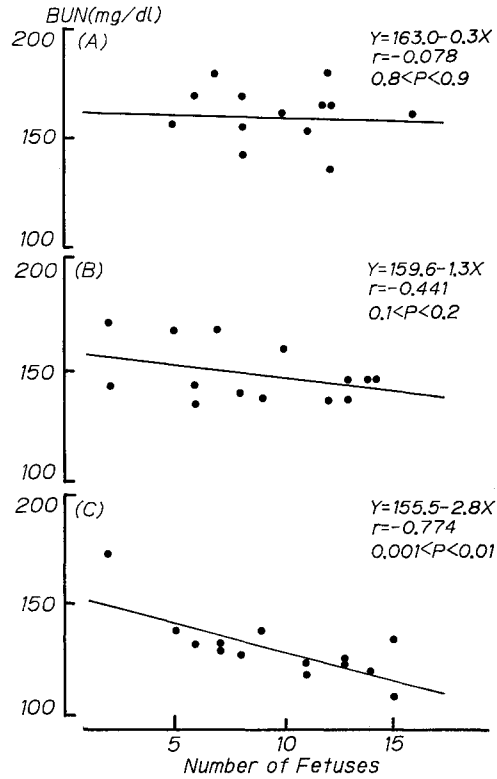

Fig. 1. Relationship between maternal BUN level and the number of fetuses at various days of pregnancy after bilateral ureteral ligation. Abbreviations: (A), Ureteral ligation was performed on day 15 of pregnancy and BUN concentration was determined on day 16 of pregnancy. (B), Ureteral ligation was performed on day 18 of pregnancy and BUN concentration was determined on day 19 of pregnancy. (C), Ureteral ligation was performed on day 21 of pregnancy and BUN concentration was determined on day 22 of pregnancy.

rats, though still much higher than those in sham-ligated control pregnant rats. This suggests that the fetal kidney becomes active on day 18 of pregnancy and thereafter, when maternal BUN levels were elevated. This idea is further supported by several lines of work. ROLLASON (15) showed that when the maternal kidneys were removed, the development of the fetal rat kidney was accelerated, and WELLS (17) demonstrated that when maternal kidneys were ligated fetal urine production rose. Also, in the present study we noted that the urinary bladders of the fetuses from ligated mothers on day 22 of gestation were filled with urine, suggesting fetal kidney function occurs.

It is generally accepted that except for the brain the liver is the only organ which produces urea in adult animals (9). In the liver of the fetal rat, however, the enzyme levels involved in urea synthesis have been reported to be relatively low (2, 14, 16). Thus, our finding that there was no significant difference in the BUN concentration between the mothers and the fetuses, in both the ligated and the sham-ligated groups, indicates that the elevated urea nitrogen in the maternal circulation following ureteral ligation crossed the placenta into the fetal circulation. This produced the same level in both the mother and the fetuses.

In our present study, the UN concentration in amniotic fluid in normal development was higher than that of maternal and fetal blood, and increased remarkably between days 21 and 22 of gestation. Further, maternal bilateral ureteral ligation induced highly elevated UN concentrations in amniotic fluid. These results indicate that the fetal kidney function

occurs on late fetal days and that elevated BUN in the fetal circulation acts as a stimulus for the function of the fetal kidney to secrete urea into the amniotic cavity through fetal urine: In our present study high UN concentration in fetal urine and amniotic fluid was observed following maternal bilateral ureteral ligation. This notion is further supported by following reports. Unlike many other animals, in rodents and man there is no large fluid-filled allantoic sac (5, 8) and the urine always passes into the amniotic cavity (12). Further, in renal agenesis little or no amniotic fluid is found (6). In normal circumstances, the fetal kidney secretes urine during the last two days of gestation in the rat (18), near term in the sheep (1) and in the mature human fetus (13). Unilateral nephrectomy causes significant hypertrophy of the remaining kidney two or three days after operation, though less than that seen in unilaterally nephrectomized newborn rats (11).

Furthermore, in our present study a highly significant inverse relationship between maternal BUN and the number of fetuses was observed on day 22 of pregnancy when the maternal ureters were ligated on day 21 of pregnancy to cause uremia. This finding further supports the view that the function of the fetal kidney is stimulated by the elevated BUN level on late fetal days, and also suggests that the fetal kidney may substitute for the maternal kidney when the maternal kidney becomes dysfunctional.

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