# Nucleic Acids in Fetal Kidney after Maternal Nephrectomy* (35600)

RONALD A. MALT AND DENISE A. LEMAITRE
(Introduced by Paul S. Russell)

*Surgical Services, Massachusetts General Hospital, Boston, Massachusetts 02114, and Department of Surgery, Harvard Medical School, Boston, Massachusetts*

Reports are conflicting on whether uninephrectomy in the pregnant rat stimulates growth of the fetal kidney. Rollason (1) inferred from both the elevated mitotic index in the nephrogenic zones of the fetal kidneys on the first postoperative day and from histochemical data that differentiation of the fetal proximal-tubule cells became more rapid after bilateral maternal nephrectomy on day 18 of a 21-day gestation. Goss (2) could find no increase in mitotic index in the cortical areas of fetal kidneys delivered from mothers uninephrectomized on day 19 of gestation.

The fetal response might, however, be hypertrophy rather than hyperplasia; the responses might be more subtle than those studied; and the renoprival state in the mother might require more than 2 or 3 days to cause an observable effect. To help clarify these points, we have examined the relative content of RNA and DNA in fetal kidneys derived from mice uninephrectomized early in the last trimester of pregnancy.

* Supported by grants from the NIH (HD-01988), American Heart Association, and Shriners Burns Institute.

*Materials and Methods. Animals.* Normal young adult mice (Charles River strain) were received at 42–45 days of age. Pregnant young adult female mice were received 10 days after breeding and an animal chosen for nephrectomy randomly was caged with one chosen for sham operation. They were permitted to have food and water except at the time of operation. On day 14 of pregnancy (dated from breeding), left nephrectomy or sham operation was performed. Mice were then caged individually. Three to 4 randomly chosen experimental and control mice were decapitated between 8:00 and 10:00 a.m. on day 17 of pregnancy, and the remainder, provided that they had delivered, on day 19. Uteri were dissected into cold Hanks' solution and all fetal kidneys were rapidly removed (3). Only fetuses with milk in their intestinal tract were used. Kidneys from a

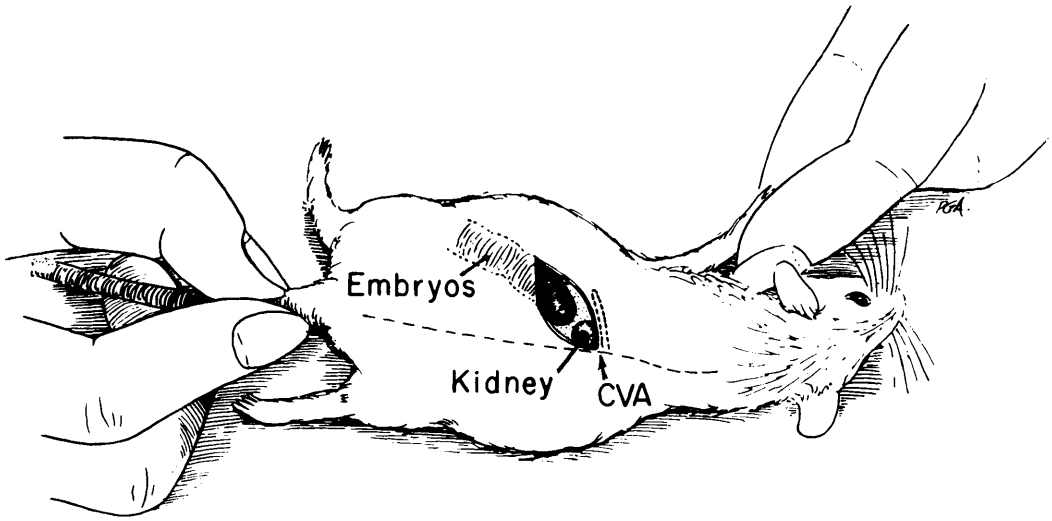


FIG. 1. Position of pregnant mouse and incision for exposure of kidney.

single litter and the corresponding maternal kidney were separately collected in cold Hanks' solution, weighed, and frozen at —70°.

*Nephrectomy in pregnancy* (Fig. 1). In the pregnant mouse the anatomic approach described for the normal adult (4) will uncover only the intrauterine fetuses. Exposure of the maternal kidney demands that the animal be rolled to a semiprone position so that the vertebral column is well off the operating table and that the incision be made exactly in the angle between last rib and the spine. Even so, the kidney will be much further posterior than in the nonpregnant mouse; the method of nephrectomy is otherwise the same (4). Operating time is 1–2 min.

*Nephrectomy of fetus* (Fig. 2). Stripped of the membranes, the fetus is impaled by 2 pins onto a disc cut from a No. 10 rubber stopper. A flank-to-flank incision is made below the umbilicus without cutting the intestines. The peritoneal contents are swept upwards with a cotton swab to reveal the translucent, whitish kidneys in a position much more caudal than might be supposed. The kidneys are easily avulsed by pulling the pedicle with an iris forceps (Lawton Surgical Instruments). Operative time is less than a minute. As the adrenal is also removed by the maneuver, it is necessary to harvest the kidneys into a bath of cold Hanks' solution and then to pluck the adrenals under a dissecting microscope. A length of ureter often attached must also be removed.

*Analysis.* Total RNA and DNA content were estimated (5) by Munro and Fleck's (6) modification of the Schmidt-Thannhauser scheme. Results from 3 such experiments were pooled for evaluation of significance by Student's $t$ test.

*Results.* Tables I and II show that RNA/DNA in the nephrectomized mothers increased about 22% but that RNA/DNA in the fetuses was unchanged ($p>0.5$). The total RNA content of the kidney increased about 264% between days 17 and 19 of ges-

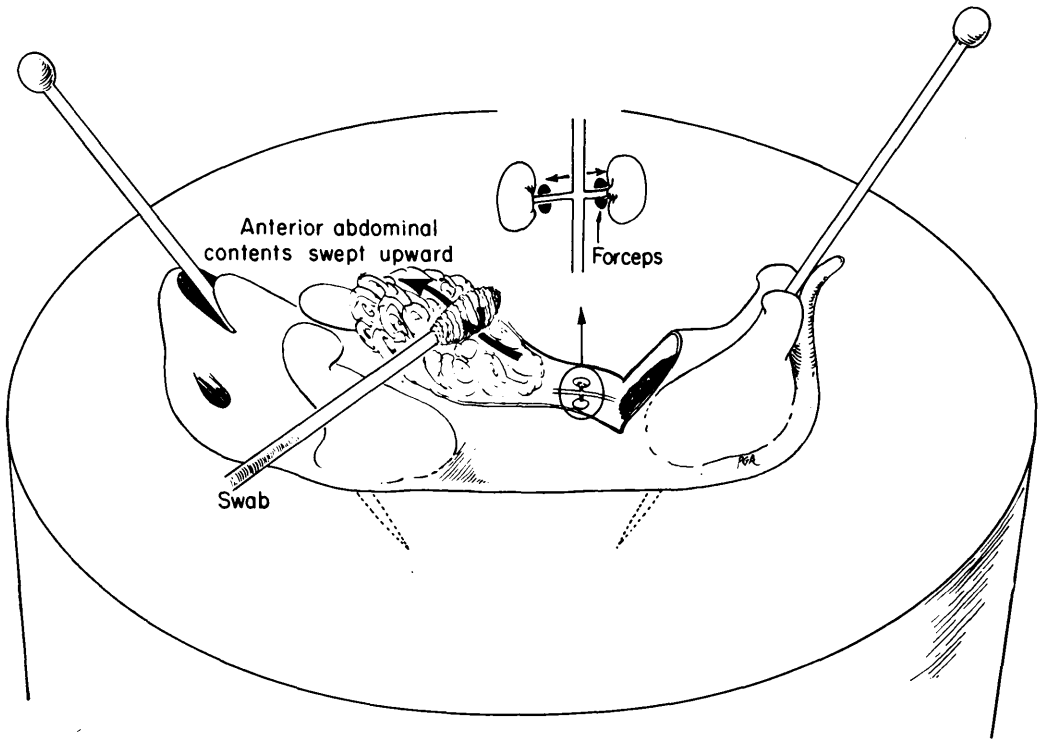


FIG. 2. Method of rapid nephrectomy in fetus.

TABLE I. RNA and DNA Contents (± SE) of Fetal Mouse Kidneys on Day 17 of Gestation and at Birth (day 19).[a]

| | Gestation (days) | N | RNA (μg/kidney) | DNA (μg/kidney) |
|---|---|---|---|---|
| Nephrectomy | 17 | 12 | 15.6 ± 1.4 | 20.3 ± 2.2 |
| | 19 | 12 | 41.2 ± 2.4 | 59.0 ± 4.6 |
| Sham operation | 17 | 11 | 15.2 ± 1.3 | 21.0 ± 1.8 |
| | 19 | 11 | 38.4 ± 2.8 | 56.8 ± 6.3 |

[a] Unilateral nephrectomy or sham operation was performed on the mother on day 14 of gestation.

TABLE II. RNA/DNA of Uninephrectomized Mothers and Their Fetuses.[a]

| | Gestation | | | |
|---|---|---|---|---|
| | N | 17 Days (ratio) | N | 19 Days (ratio) |
| Nephrectomy | | | | |
| Mothers | 10 | 0.98 ± 0.04 | 7 | 0.96 ± 0.03 |
| Fetuses | 12 | 0.79 ± 0.03 | 12 | 0.72 ± 0.04 |
| Sham operation | | | | |
| Mothers | 9 | 0.81 ± 0.04 | 8 | 0.78 ± 0.02 |
| Fetuses | 11 | 0.76 ± 0.02 | 11 | 0.74 ± 0.05 |

[a] A maternal kidney and the entire harvest of fetal kidneys from a litter each constitutes one sample. Maternal kidneys from only the first 2 experiments were analyzed (see text). Since these results were calculated directly from raw data, they differ slightly from ratios calculated from the secondary data in Table I.

tation in fetuses of nephrectomized mothers compared with 253% in fetuses of sham-operated mothers. The comparable increases for total DNA content were 291 and 270%.

*Discussion.* These data show that although RNA/DNA in the kidneys of pregnant females was slightly lower than the ratio for nonpregnant females (5), the increase of 22% after uninephrectomy in the last trimester of pregnancy was the same. The RNA/DNA in the fetal kidney after maternal nephrectomy, however, was unchanged compared with RNA/DNA in fetal kidneys from sham-operated mothers. RNA/DNA and the decrease in ratio with advancing age in both these groups of fetal kidneys was the same as that in normal mouse fetuses of the same age (3), allowing for small differences because the onset of pregnancy was timed by different conventions in this and in the previous work.

Interpretation of the results rests on the premise that the ratio of RNA to DNA is a measure of cytoplasmic hypertrophy because the nuclear DNA and RNA content is nearly constant (7). Since this assumption is probably valid when applied to the essentially diploid cells of fetal kidneys (maximal mitotic index about 5/1000 cells (2), the experiments show that uninephrectomy of the mother during the last trimester of pregnancy has no effect on hypertrophic activity of the fetal mouse kidney. They do not, however, eliminate the possibility that there may be differential changes in classes of RNA too small to be detected by measurement of total RNA alone (8). There is no evidence that maternal nephrectomy interferes with nucleic acid synthesis in the fetal kidney.

Most explanations proposed by Goss (2) to account for the absence of hyperplastic activity during a 2-day period after maternal nephrectomy in the rat would hold for these experiments as well. Kidney growth may already be maximal and incapable of being stimulated further during late fetal life, fetal kidneys may be unresponsive to the action of influences that would otherwise stimulate growth, and maternal stimuli may be excluded from the fetus. Only the possibility that the stimuli may be physical rather than chemical can now be less well considered, as it is known that the denervated, transplanted kidney undergoes compensatory growth (9).

*Summary.* The effect of the renoprival state in the mother on renal growth of the

fetus was studied in the progeny of mice uninephrectomized on day 14 of pregnancy. Fetal kidneys removed on day 17 and at birth (day 19) and the comparable solitary maternal kidney were analyzed for content of RNA and DNA. The ratio RNA/DNA (RNA/av cell) increased about 22% in the maternal kidney compared with sham-operated controls, but no change was found in fetal RNA/DNA. Therefore, either no humoral stimulus is produced as a consequence of maternal nephrectomy, or the fetal kidney cannot respond to it.

1. Rollason, H. D., Anat. Record **141**, 183 (1961).
2. Goss, R. J., Nature **198**, 1108 (1963).
3. Priestley, G. C. and Malt, R. A., J. Cell Biol. **37**, 703 (1968).
4. Malt, R. A., *in* "Compensatory Hypertrophy of the Kidney," (W. W. Nowinski and R. J. Goss, eds.) Academic Press, New York.
5. Malt, R. A. and Lemaitre, D. A., Am. J. Physiol. **214**, 1041 (1968).
6. Munro, H. N. and Fleck, A., *in* "Methods of Biochemical Analysis" (D. Glick, ed.), Vol. 14, p. 113. (Interscience), Wiley, New York (1966).
7. Halliburton, I. W. and Thomson, R. Y., Cancer Res. **25**, 1882 (1965).
8. Malt, R. A. and Miller, W. L., J. Exptl. Med. **126**, 1 (1967).
9. Ogden, D. A., Porter, K. A., Terasaki, P. I., Marchioro, T. L., Holmes, J. H., and Starzl, T. E., Am. J. Med. **43**, 837 (1967).

Received Sept. 4, 1968. P.S.E.B.M., 1969, Vol. 130.

## Sexual Receptivity in Weanling Rats Treated with PMS and Gonadal Hormones* (35601)

ZAHIR U. KHAN AND ROLAND K. MEYER
*Department of Zoology, University of Wisconsin, Madison, Wisconsin 53706*

Many attempts have been made to induce artificial precocious sexual maturity in immature female animals by gonadotropins. It has been reported that pregnant mare's serum gonadotropin produces ovulation in the immature rat (1–3). It was observed (1) that immature rats could become pregnant if treated with pregnant mare's serum (PMS). Ovulation was induced in 26–31-day-old rats with a single injection of PMS (4). Also mating was produced in immature animals by treating them with low doses, 3 RU, of PMS at 22 days of age. These observations were extended and it was concluded that the pituitary and the central nervous system were involved in the induction of ovulation in immature female rats (5). It was further observed that a single injection of 20 IU of PMS on day 30 and 1.2 IU of HCG given intravenously on day 32 caused superovulation in rats and if these rats were mated on the evening of day 32, an average of 22.8 implantation sites was counted on days 10 to 13 of pregnancy (6). It has also been shown that ovulation can be induced consistently in 24-day-old rats (7) and that a dose of 3 IU of PMS in 22-day-old rats produces ovulation consistently.

The work reported here is a part of a program, the purpose of which is to investigate the hormonal factors involved in the attainment of sexual maturity in the female rat. The report describes specifically the data concerned with the induction of sexual receptivity, ovulation, embryo implantation and maintenance of pregnancy in the 22-day-old rat treated with PMS.

*Materials and Methods.* Twenty-one-day-old immature rats, weighing 50–55 g, were received from the Holtzman Company, Madison, Wisconsin. They were kept 8–10 per cage under conditions of 14 hr light and 10 hr dark daily. The lights were turned on at 5:00 a.m. Central Standard Time (CST)

* Supported by Public Health Service Training Grant No. 1 T1 HD 104-03, The National Institute of Child Health and Human Development and by The Ford Foundation, Grant No. 63-505, and by The Population Council through a two year Fellowship for Mr. Khan.