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Serum Prolactin Levels in Fetal and Neonatal Hamsters and the Relationship to Maternal Levels (39056)

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(Introduced by George C. Kent)

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Prolactin has been identified in fetal rats by disc electrophoresis of pituitary homogenates (1) and has been measured using radioimmunoassay (RIA) in the pituitaries and/or plasma of fetal and neonatal rats (2, 3) and in the plasma of neonatal rats only (4). In addition, prolactin cells were localized in fetal rat adenohypophyses using the peroxidase-labeled antibody technique (5), and the pituitary of mouse embryos has been shown to contain as well as secrete this hormone (6). Since iodinated prolactin (^{125}I-PRL) has been shown to cross the placenta in monkeys and enter amniotic fluid and fetal circulation (7), the question arises as to whether fetal serum prolactin levels are, in part, a contribution from a maternal source.

The present study was undertaken to determine whether fetal and neonatal hamster serum contains immunoassayable amounts of prolactin, and to determine its relationship, if any, to maternal levels. The possibility of a maternal source for some of this hormone in fetal serum was also investigated.

Materials and methods. Animals. Multiparous cyclic female hamsters were maintained on a 12L:12D lighting schedule, 2400 hr being the midpoint of the dark period. Each cyclic female hamster was mated on the night of estrus and at 0900 hr the following morning, fetuses were considered to be half a day of gestation. Pups were born during the dark hours at the end of 16 days of gestation, and these animals were considered to be one-half day old the morning following parturition. All animals were sacrificed and sera collections made at 0900–1100 hr.

Maternal blood was collected in tubes after the animals had been sacrificed by decapitation. Serum was harvested from the clots after a period of 2 hr and centrifuged to remove any residual blood cells.

For collection of fetal blood, the maternal uterus was opened by a single longitudinal incision and each fetus was carefully lifted from the uterus and blotted dry of amniotic fluid and maternal blood. Whenever possible, the umbilical cord was left intact and placental association with the uterus was maintained while each fetus was decapitated and blood collected in capillary tubes. Immediately after blood collection the samples were centrifuged for 15 min in a hematocrit centrifuge, after which sera from the fetuses of each litter were pooled. Each sample represented pooled sera from a single litter of animals, except at 13.5 day gestation, when sera of two litters were pooled to obtain a large enough sample.

All sera collections and centrifugations were carried out at room temperature and the samples were immediately frozen and stored at $-12°$ until assayed.

Iodination of prolactin. Prolactin was iodinated by modification of the procedure provided by the Rat Pituitary Hormone Distribution Program, National Institute of Arthritis and Metabolic Diseases (NIAMD), National Institutes of Health (NIH). NIAMD Rat PRL-I-1 (30 IU/mg) was radiolabeled with ^{125}I, New England Nuclear Co., NEZ 033. One mCi ^{125}I in 25 μl of 0.1 *N* NaOH was neutralized with an equal volume of 0.1 *N* HCl in a reaction vessel containing 25 μl of 0.1 *M* NaH_2PO_4 buffer, pH 7.6. Twenty microliter (2 μg) of NIAMD

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Rat PRL-I-1, previously stored at −20° in 0.01 *M* NaH_2PO_4 buffer containing 0.15 *M* NaCl with 0.1% NaN_3 (PBS), pH 7.6, was added to the reaction vessel and immediately followed by the addition of 10 μl of chloramine-T (25 mg/ml PBS without NaN_3) that had been prepared immediately prior to use. The reaction vessel was gently agitated for 60 sec after which 25 μl of freshly prepared sodium metabisulfite (25 mg/ml PBS without NaN_3) was added, and the entire reaction mixture was applied to a Bio Gel P-60 gel filtration column, Bio Rad Labs., 0.7 × 20 cm glass column, 100–200 mesh. The gel column was prepared for the reaction mixture by equilibrating with PBS. Then, 2 ml of 2% BSA in PBS was added to the column, after which 5 ml of PBS was added. The reaction mixture was eluted from the column with PBS. Twenty 0.5 ml fractions were collected singly in culture tubes containing 50 μl of 2% BSA in PBS. Ten microliter of each fraction was then placed in a liquid scintillation vial containing 10 ml of Aquasol, New England Nuclear Co. Each vial was gently agitated, then counted at 2% error in the full tritium window of a Beckman LS-100C liquid scintillation counter. Radioactivities of each vial were recorded as counts per minute (cpm).

Immunoassay of prolactin. Immunoassay procedures were carried out in accordance with methods provided by NIAMD. In preparing a standard curve, NIAMD-Rat PRL-RP-1 (11 IU/mg) was employed as a reference preparation. Prolactin antiserum utilized in the assay was NIAMD-anti-rat PRL S-2. The minimum and maximum volume of serum used in the assay was 50 μl and 200 μl, and duplicate hormone determinations from each sample were employed using the same or different volumes of serum. The slope of the dose-response curve for pooled sera from pregnant hamsters was not significantly different from the slope of the standard curve. Samples were counted as described for the iodination curve samples.

Injection of ^{125}I-PRL and recovery from mother and fetus. Immediately after purification of the iodinated prolactin on Bio Gel columns, 13.5, 14.5, and 15.5 day pregnant hamsters were administered 0.1 ml ^{125}I-PRL (620,000 cpm/0.1 ml PBS) at 0900–1100 hr by a single intracardiac injection. Exactly 10 min after the ^{125}I-PRL injection the pregnant animals were sacrificed by rapid decapitation for collection of maternal and fetal blood. Serum was harvested as described previously. All liquid scintillation vials containing 10 ml Aquasol were precounted for background radioactivity. One hundred μl of serum from each pregnant hamster was then placed in a liquid scintillation vial, gently agitated, and counted at 3% error. Radioactivities were recorded as cpm. One hundred μl of each fetal serum sample was counted as described for maternal serum.

To determine whether radioactivity measured in serum was still bound to prolactin, 200 μl of 15.5 day maternal serum was then subjected to gel filtration using the method previously described for separation of ^{125}I-PRL from other iodination products, except that 0.5 ml fractions were collected directly in scintillation vials.

One hundred to 150 μl of each fetal serum sample was also subjected to gel filtration. Twenty 0.5 ml fractions were collected in scintillation vials from the gel filtration of the 15.5 day fetal samples, and six 0.5 ml fractions were collected for the remaining fetal serum samples. Each vial was counted at 3% error and cpm recorded.

Statistics. An analysis of variance with a completely randomized design was conducted on hormonal data from maternal and fetal hamsters to determine whether the two hormonal patterns were similar.

Results. Fetal prolactin concentrations were found to be relatively high (approximately 4.0 ng) at 13.5 days and 15.5 days of gestation, whereas a drop in levels occurred on day 14.5 (Fig. 1). Little or no prolactin was detected in the serum of newborn animals on the morning after parturition. Two of the 5 litters contained no measurable prolactin whatever at that time. Of particular interest is the consistent manner in which the fetal prolactin levels reflect the rising and falling of maternal serum levels of the hormone before birth (Fig. 1).

In order to determine whether prolactin

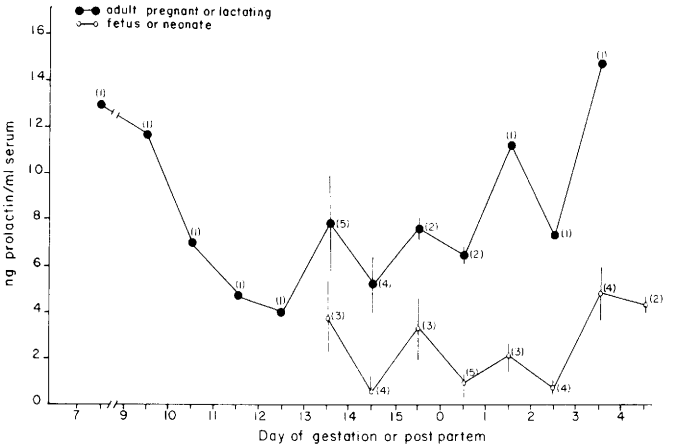

FIG. 1. Serum prolactin (PRL) levels (ng/ml serum) of pregnant or lactating hamsters and fetal or neonatal hamsters. The number of samples assayed each day is in parentheses.

in fetal serum could be attributed to placental transfer from maternal sources, ^{125}I-PRL was injected into pregnant females. Ten min later a measurable quantity of radioactivity was detected in fetal serum (Table I) and calculated to be an average of 3.9% of the radioactivity (cpm/ml) recorded in the maternal serum.

The amount of ^{125}I-PRL still bound to protein was determined by columning aliquots from each serum sample from mother or fetus. In each case, the higher level of radioactivity was found in fraction 4 (Fig. 2).

Discussion. We have established by RIA that there are measurable quantities of prolactin hormone in the serum of fetal and neonatal hamsters. The fact that exogenous ^{125}I-PRL crossed hamster placentae and entered fetal circulation indicates the possibility that at least some of the endogenous hormone may have a maternal source in the unborn animal. Josimovich *et al.* (7) reported a similar observation when injecting pregnant monkeys with ^{125}I-PRL. The hormone was determined to cross the placenta in minute amounts and enter fetal circulation (0.5% of maternal serum levels) and amniotic fluid (1.0% of maternal serum levels). They were also able to show that the fetal pituitary released prolactin following an injection of exogenous TRF.

Immunohistochemical techniques have demonstrated the presence of a small number of pituitary prolactin cells in the fetal hamster as early as 13.5 days gestation, and at the same age, electron micrographs first showed cells with some of the morphological characteristics of prolactin cells (Thompson and Trimble, unpublished). Thus, a fetal contribution of prolactin would be possible at 13.5 days of gestation but not earlier. Because of the scarcity of immunoreactive cells and the small number of cytoplasmic granules in such cells, it does not seem likely that there are enough competent prolactin cells to account for the relatively high amount of circulating prolactin at this age. At 14.5 days there were considerably more granulated cells, yet the concentration of prolactin in the fetal serum drops.

An average of 3.9% of circulating ma-

TABLE I. RADIOACTIVITY RECOVERED FROM FETAL AND MATERNAL SERUM 10 MINUTES POST-INTRACARDIAC INJECTION WITH ^{125}I-PRL[a]

Day of gestation	Cpm/ml serum (corrected for bkg)		Percentage of Maternal levels (embryo/adult)
	Adult	Embryo	
13.5	1574.9[b]	58.9[c]	3.73
14.5	1180.1	34.4	2.91
14.5	681.3	36.5	5.35
15.5	1081.2	53.6	4.95
15.5	1938.5	50.4	2.59

[a] 620,000 cpm/.1 ml PBS.
[b] Average cpm of two adult serum samples.
[c] cpm of pooled sera from 2 litters.

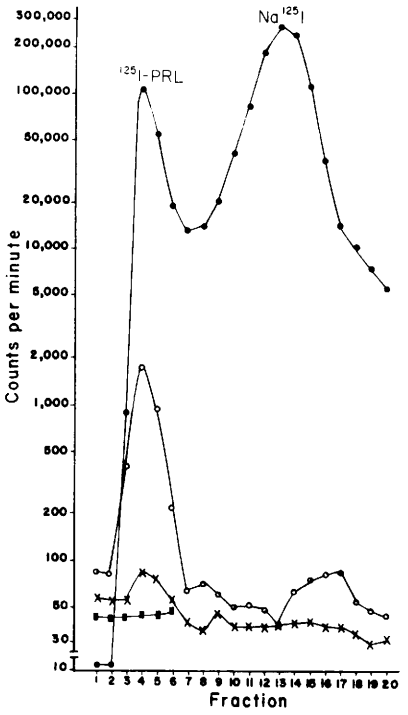

FIG. 2. Gel filtration patterns. (●—●) products from the iodination of NIAMD-Rat PRL-I-1 in which a 1 μl aliquot of 0.5 ml fractions were counted. (○—○) maternal or (×—×) fetal serum containing ^{125}I-PRL (mean of 4 samples, 6 litters, counted for fractions 1–6); 0.5 ml fractions were collected and counted. (■—■) nonradioactive hamster serum; 0.5 ml fractions were collected and counted. The shape of the purification curve for freshly iodinated prolactin is similar to the filtration curves determined for radioactivity recovered in fetal and maternal serum except that there is little or no Na^{125}I in fractions 9–17 recovered from serum samples.

ternal serum levels of ^{125}I-PRL was measured in pooled fetal serum ten minutes after a single injection of ^{125}I-PRL into pregnant hamsters on days 13.5–15.5, whereas on the same days endogenous fetal serum prolactin levels were, on the average, 37% of maternal levels as revealed by RIA. The difference between measured endogenous fetal-maternal ratio and the percentage of maternal ^{125}I-PRL recovered in fetal serum, could be due to the limitations of the injection procedure and rapid serum hormone clearance rates (8). However, some of the difference may be an indication of the relative amount of prolactin contributed by fetal or maternal pituitary secretion.

Changes in serum prolactin levels of fetal hamsters coincided closely with the changes in maternal serum as revealed by analysis of variance. This suggests that maternal prolactin enters fetal circulation, thereby influencing fetal prolactin concentrations. Even after birth, the newborn prolactin levels continued to fluctuate synchronously with maternal prolactin levels. Since it has been suggested that serum prolactin in lactating rats may pass into milk in amounts proportional to maternal circulating levels (9), and since large dietary proteins can be absorbed intact into the blood of neonatal rats (10), it is reasonable to postulate that the same mechanism may be responsible for this observation in the neonatal hamster.

Although many more fetal and newborn serum samples were measured for prolactin than were adult samples, the adult values closely followed relative values measured in pregnant and lactating hamsters by Bast and Greenwald (11), and in rats by Merchant (12). Prolactin serum values in this study were considerably lower than Bast and Greenwald's, which may be due to several factors. The animals were multiparous vs nulliparous, from different colonies, and there was a difference in NIAMD antibody source, which may have contributed to the differences in absolute levels of prolactin. One of the shortcomings of the RIA technique is that actual values are seldom duplicated from one lab to another, whereas rises and falls in levels are accurately reproduced (13).

Studies suggesting roles for growth hormone in rats (14) and for prolactin in mice (6) have been reported. The presence of prolactin in pre- and postnatal hamsters makes it interesting to speculate concerning the possibility of some contribution to the growth and perhaps to the reproductive differentiation of the developing animal.

Summary. Immunoreactive prolactin was measured by RIA in 13.5–15.5 day gestation fetal and 0.5–3.5 day neonatal hamster serum and found to significantly reflect rises and falls in maternal levels. On the average, fetal and neonatal levels were 37% of maternal prolactin serum levels. ^{125}I-PRL injected into 13.5–15.5 day pregnant hamsters was demonstrated to cross the placenta and enter fetal

circulation. Ten min after injection, fetal serum levels were calculated to be an average of 3.9% of the radioactivity recorded in maternal serum. There is a strong possibility that fetal prolactin serum levels may be, at least in part, attributed to a maternal source.

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1. Ingleton, P. M., Ensor, D. M., and Hancock, M. P., J. Endocrinol. **51,** 799 (1971).
2. D'Angelo, S. A., Wall, N. R., and Bowers, C. Y., Proc. Soc. Exp. Biol. Med. **148,** 227 (1975).
3. Ojeda, S. R., and McCann, S. M., Endocrinology **95,** 1499 (1974).
4. Döhler, K. D., and Wuttke, W., Endocrinology **94,** 1003 (1974).
5. Sétáló, G., and Nakane, P. K., Anat. Rec. **172,** 403 (1972).
6. Kohmoto, K., and Bern, H. A., Proc. Soc. Exp. Biol. Med. **137,** 807 (1971).
7. Josimovich, J. B., Weiss, G., and Hutchinson, D. L., Endocrinology **94,** 1364 (1974).
8. Koch, Y., Chow, Y. F., and Meites, J., Endocrinology **89,** 1303 (1971).
9. McMurty, J. P., and Malven, P. V., J. Endocrinol. **52,** 405 (1972).
10. Daniels, V. G., Hardy, R. N., Malinowska, K. W., and Nathanielsz, K. W., J. Endocrinol. **52,** 405 (1972).
11. Bast, J. D., and Greenwald, G. S., J. Endocrinol. **63,** 527 (1974).
12. Merchant, F. W., Amer. J. Anat. **139,** 245 (1974).
13. Horrobin, D. F., *in* "Prolactin: Physiology and Clinical Significance", p. 11. Medical and Technical Pub. Co. Ltd., Lancaster (1973).
14. Sara, V. R., Lazarus, L., Stuart, M. C., and King, T., Science **186,** 446 (1974).

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