Endocrine Function of the Placenta in Thiamine-Deficient Rats[1] (34714)

WARREN G. KINZEY
(Introduced by H. H. Srebnik)
Department of Anthropology, University of California, Davis, California 95616

Thiamine deficiency in female rats leads to cessation of estrous cycles and reduction in fertility (1). Using purified diets, Nelson and Evans (2) demonstrated that reproductive failure in pregnant rats was due to a combination of inanition and B_1 deficiency. They also demonstrated that pregnancy could be maintained until day 13 in thiamine-deficient rats by daily injections of estrone and progesterone. Their results suggested that reproductive failure in thiamine deficiency might be due to inadequate secretion of pituitary hormones, ovarian steroids, and/or placental gonadotropin. The present investigation reports the effect of a thiamine-deficient diet, given in combination with oxythiamine, a thiamine antimetabolite, on reproductive performance and upon gonadotropic activity of the placenta.

Materials and Methods. Adult virgin Long-Evans rats, 175–225 g body weight, were bred with normal males and fed purified thiamine-deficient diet[2] *ad libitum* beginning the day of finding sperm in vaginal smear (day zero). Distilled water was available *ad libitum.* Animals were kept in individual cages, on screens, until sacrificed on day 19 of gestation[3], or on day 11 to obtain placentas for assay. Groups of thiamine-deficient rats were given daily ip injections of 600 μg of oxythiamine. Daily sc injections of 1 μg of estrone and 4 mg of progesterone in 0.3 ml of sesame oil were given from day 3 through day 10 of pregnancy. An additional group of rats of comparable age and weight was fed purified complete (standard) diet and sacrificed on day 11 to obtain placentas for bioassay. Vaginal smears were examined daily during gestation, and animals were weighed regularly. At autopsy, uteri were removed and examined for implantation sites and living young. Placentas were removed from animals sacrificed on day 11 and immediately frozen on Dry Ice for bioassay.

Luteotropic and mammotropic activities of the placenta were assayed simultaneously in immature hypophysectomized female rats prepared according to previously reported procedure.[4] At autopsy, the previously traumatized uterine cornua of assay rats were examined for decidual reaction and the mammary glands for lobulo-alveolar growth. The minimal effective dose (MED) for placental mammotropic activity was that daily amount of placental homogenate given over the 7-day test period which induced in two out of three recipient rats lobulo-alveolar growth in the mammary gland at the site of injection. The MED for luteotropic activity was that daily amount of homogenate which produced a positive deciduoma cell response (DCR) in two out of three recipients. Criterion for positive DCR was a 100% increase in weight of the traumatized uterine cornu over that of the nontraumatized cornu.

Results. Table I summarizes the reproductive performance of pregnant rats fed thiamine-deficient diet, with and without daily injections of oxythiamine. Without the antimetabolite, 11 of 12 rats had implantation sites and all 11 had living young at autopsy. The injections of oxythiamine increased the severity of the deficiency, for when 600 μg was injected daily 18 of 20 rats had implantation

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[2] The composition of the diets was reported previously (7).

[3] A previous experiment demonstrated that maternal mortality was high between day 19 and 21 of gestation.

[4] The bioassay procedure was previously reported in detail (8).

TABLE I. Maintenance of Pregnancy in Thiamine-Deficient Rats.

Anti-metabolite[a]	Ovarian hormones	No. of rats bred	Wt change during gestation (g)	Onset vaginal RBC (day)	Implantations (%)	Living litters[b] (%)	Maternal mortality (%)	No. of implantation sites/rat	
								Total	Living
None	None	12	+ 3	12.9	92	100	0	11.2	10.0
Oxythiamine	None	20	—21	12.4	90	17	60	11.0	1.4
	E + P[c]	20	—18	12.5	95	71	60	10.0	8.6

[a] All rats received thiamine-deficient diet. Oxythiamine was injected ip, 600 μg daily.

[b] Percentage of pregnant rats with living embryos at autopsy, day 19 of gestation.

[c] One μg of estrone + 4 mg of progesterone daily, days 3–10.

sites, but only one had living young at autopsy. In addition 60% of rats died between day 15 and 19 of gestation.

The injection of estrone and progesterone from day 3 through 10 markedly improved reproductive performance compared with that of the group injected with oxythiamine alone. Although 5% of rats failed to implant and there was no reduction in maternal mortality, 71% of hormone-treated animals sacrificed on day 19 had living young.

Table II presents a description of donor rats sacrificed on day 11 for bioassay. Control rats fed complete diet gained considerably in body weight. Thiamine-deficient rats gained about half that of control animals.

The luteotropic activity of placentas from thiamine-deficient and control rats is presented in Table III. The MED was 2 placentas/day irrespective of whether the placentas were obtained from thiamine-deficient or from control donors. The mammotropic activity of placentas from thiamine-deficient and control rats is presented in Table IV. The MED was 0.5 placenta/day regardless of whether the placentas were obtained from thiamine-deficient or from control donors.

Discussion. When thiamine deficiency was started on day of breeding, all rats which had implantation sites had living young at autopsy. This is in agreement with the data of Nelson and Evans (2). Previous investigators increased the severity of deficiency by feeding thiamine-deficient diet prior to breeding (2–4). In the present experiments, severity of deficiency was increased by injecting animals daily with the antimetabolite oxythiamine.[5] Oxythiamine was previously used as an effective competitive inhibitor of thiamine in chick embryogenesis (5).

The percentage of litters obtained in thiamine-deficient rats injected with ovarian hormones on days 3–10 was higher than that obtained in rats receiving oxythiamine alone. Thiamine deficiency, therefore, did interfere sufficiently with reproductive function to require exogenous hormones until the placenta

[5] The antimetabolite neopyrithiamine was used in a previous experiment, but at the level of 100 μg daily none of the pregnant animals survived beyond day 14 of gestation.

TABLE II. Description of Donor Rats.[a]

Experimental group	No. of rats bred	Wt day of breeding (g)	10-day wt change (g)	Implantation sites (mean ± SE)	Total no. of placentas frozen
Thiamine-deficient	44	190	+19	9.9 ± 0.2	374
Control	16	191	+41	9.3 ± 0.6	135

[a] Thiamine-deficient rats received the deficient diet, were injected daily with oxythiamine, and were injected with 1 μg of estrone + 4 mg of progesterone from day 3 through 10 of gestation. Control rats received complete diet and were uninjected. All rats were sacrificed on day 11 of gestation.

TABLE III. Luteotropic Activity of Day-11 Placenta from Thiamine-Deficient and from Control Rats.

Assay: Deciduoma reaction in immature hypophysectomized female rats.[a]

Daily dose (equivalents of placenta)	Thiamine-deficient donors (no. of assay rats)		Control donors (no. of assay rats)	
	Total	Reacting	Total	Reacting
0	12	0	12	0
0.25	8	2	8	0
0.5	12	3	10	3
1	17	6	7	4
2	4	3	2	2

[a] Assay rats were injected with 5 IU of equine gonadotropin at 26 days of age and hypophysectomized 4 days later. Injections were begun the day of operation and continued for 7 days. The uterus was traumatized on the fourth day. Autopsy was 24 hr after the last injection.

was fully established. Reproductive disturbances in thiamine-deficient rats may be attributed to reduced food intake (2, 6). It is assumed, therefore, that hormonal inadequacies seen in this study were due in part to inanition, known to cause pituitary insufficiency [*e.g.*, (7)].

Placental function in thiamine deficient rats was not severely affected by the combination of thiamine-free diet and antimetabolite, as judged by the presence of living young on day 19. Furthermore, placental luteotropic and mammotropic activities were comparable in thiamine-deficient and control groups. The greater sensitivity of the mammotropic response presumably resulted from the local nature of mammotropic response in contrast with systemic nature of luteotropic response. Similar levels of mammotropic activity of day-11 placenta were previously obtained with placentas from protein-deficient, hypophysectomized, and intact control donors (8). The MED for luteotropic activity previously reported for protein-deficient, hypophysectomized, and intact control donors, however, was 1 placenta/day rather than 2 placentas/day as reported here. No cause for this discrepancy is immediately apparent; however, comparable results were obtained in the two studies at lower dose levels. Incidence of positive DCR in recipients injected daily with 0.5 placenta in the present study (25–30%) was similar to that previously obtained in recipients injected daily with 0.5 placenta from protein-deficient donors (29%).

It was previously shown that the placenta, once established, could maintain pregnancy during dietary protein deprivation (9) and that there was no difference between protein-deficient and control rats in luteotropic and mammotropic activities of the placenta (8). Apparently neither protein deficiency nor thiamine-calorie deficiency prevented the production and secretion of gonadotropin by the rat placenta, although each of these deficiencies interfered with pituitary gonadotropin release in female rats (7).

Summary. In acute thiamine deficiency the placenta can maintain pregnancy beyond midgestation provided that estrone and progesterone are administered on days 3–10 of pregnancy. Luteotropic and mammotropic activities of the placenta on day 11 are comparable whether placentas are obtained from thiamine-deficient or from control donors. It is

TABLE IV. Mammotropic Activity of Day-11 Placenta from Thiamine-Deficient and from Control Rats.

Assay: "Local" lobulo-alveolar development in mammary glands of immature hypophysectomized female rats.[a]

Daily dose (equivalents of placenta)	Thiamine-deficient donors (no. of assay rats)		Control donors (no. of assay rats)	
	Total	Reacting	Total	Reacting
0	12	0	12	0
0.25	8	1	8	2
0.5	12	9	10	7
1	17	16	7	7
2	4	4	2	2

[a] Assay rats were injected with 5 IU of equine gonadotropin at 26 days of age and hypophysectomized 4 days later. Injections were begun the day of operation and continued for 7 days. Autopsy was 24 hr after the last injection.

suggested that endocrine function of the rat placenta is not impaired by thiamine deficiency.

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