Effect of Maternal Alcohol Consumption on the Fetal Thyroid in the Rat (42937)

MASAKO YAMAMOTO, MASANORI TOGUCHI, KAZUYOSHI ARISHIMA, YASUNOBU EGUCHI, JOSEPH LEICHTER,* AND MELVIN LEE*

Department of Anatomy II, Azabu University School of Veterinary Medicine, Sagamihara, Kanagawa 229, Japan and Division of Human Nutrition, University of British Columbia, Vancouver, B.C., V6T 1W5, Canada*

Abstract. **To investigate the effect of maternal alcohol consumption on the development of the fetal thyroid gland, Sprague-Dawley rats were given 20% ethanol for 4 weeks prior to mating and 30% ethanol throughout gestation. Pair-fed controls received an isocaloric amount of corn starch and chow, with water *ad libitum*, and *ad libitum* controls received rat chow and water. On Days 17, 18, 19, and 20 of gestation, the fetuses were weighed and the fetal thyroids were removed for histometric observation. On Days 19 and 20, the fetal thyroids of alcohol-exposed fetuses weighed significantly less than those of the two control groups, but more than the control thyroids 1 day earlier. Maternal alcohol consumption caused a significant decrease in both the follicular cell height and the follicle diameter of the fetal thyroid on all days examined. In the alcohol group on Days 19 and 20 of gestation, the cell height was less than, and the follicle diameter was approximately equal to those in the two controls 2 days earlier. These results indicate that, as a consequence of maternal alcohol consumption, growth of the fetal thyroid gland is retarded, and there are indications of fetal hypothyroidism, as seen from the histometric data. This latter is suggestive of a retarded thyrotropic activity of the fetal pituitary gland.** [P.S.E.B.M. 1989, Vol 191]

It has been well established that, in both humans and laboratory animals, maternal alcohol consumption during gestation results in retarded fetal growth which persists after birth (1–5). Some investigators have shown a reduced transfer of nutrients to fetuses of rats given alcohol during gestation (6–8). Jones *et al.* (9) demonstrated that chronic alcohol consumption leads to a redistribution of blood, with a diminished blood supply to the placenta.

In relation to this, it should be noted that restriction of pregnant rats to 50% of their normal food intake results in small fetuses and placentas on Day 21, with a marked reduction in placental blood flow (10). Furthermore, in 50% calorie-restricted pregnant rats, the fetal thyroid glands were retarded in growth in proportion to the reduction in fetal weight compared with the normal-fed condition (11).

In the case of maternal protein deprivation throughout gestation, the thyroid tissues of the fetal and newborn rats also showed retarded morphogenesis and function (12, 13). Hence, it is of interest to see whether a similar retardation of thyroid morphogenesis occurs in fetuses as a consequence of maternal alcohol consumption prior to and throughout gestation.

Accordingly, this study was undertaken to determine the influence of alcohol consumption before and throughout gestation by rats on the histologic development of the fetal thyroid gland.

Materials and Methods

Virgin female Sprague-Dawley rats (Japan CLEA Co. Ltd.) weighing approximately 170 g were individually housed in stainless steel screen-bottom cages at 23 ± 2°C with a 12-hr on- and 12-hr off-light cycle. After a 1-week adjustment period, they were randomly assigned to one of three dietary regimens. Group 1 (alcohol) received 10% ethanol (v/v) as the sole source of liquid and rat chow (Labo MR Breeder) *ad libitum*. After 1 week the alcohol was increased to 20%. Group 2 (pair-fed controls) was given water *ad libitum* and the same amount of rat chow as had been consumed by the alcohol group during the previous 24 hr, plus an amount of corn starch (Wako Chemical Industries, Tokyo, Japan) calorically equal to the amount of alcohol consumed by the alcohol-fed rats. Thus, the pair-fed rats had caloric intakes equal to those of the alcohol-

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fed rats, but did not receive alcohol. A previous study showed that pregnant rats given ethanol in drinking water exhibited a moderate degree of dehydration (4), but that this did not contribute significantly to retarded fetal growth. For that reason, no attempt was made in the present study to match the amount of liquid consumed by the alcohol-fed and pair-fed controls. Group 3 (*ad libitum* controls) received rat chow and water *ad libitum*.

After approximately 4 weeks on this regimen all animals were bred overnight to males of the same strain. The appearance of sperm in the vaginal smear on the following morning established Day 0 of gestation. At that time the Group 1 rats were changed to 30% ethanol and the pair-fed group was adjusted accordingly.

On Day 17, 18, 19, or 20 of gestation, pregnant rats were sacrificed, and the fetuses were removed quickly, selected at random, and weighed. Among the weighed fetuses, some were further selected at random for histologic observation of fetal thyroids. The removed fetal thyroids were weighed and fixed in Bouin's fluid.

Bouin-fixed thyroids were dehydrated in a graded series of ethanol, embedded in Paraplast (Sherwood Medical Industries), and sectioned serially at 5 μm. The sections were stained with periodic acid-Schiff reagent and hematoxylin. The average diameter of follicles and the average height of the follicular cells were determined as described by Eguchi and Morikawa (14).

Data were analyzed by Duncan's new multiple range test (15). An overall F value and a corrected significance level between groups was calculated. A probability value less than 0.05 was considered to be statistically significant.

Results

The average daily food and alcohol consumption and weekly body weights of rats given ethanol, pair-fed, or fed *ad libitum* before and during pregnancy have been reported previously (3). Briefly, during pregnancy alcohol contributed about 28% of the calories ingested. Total calorie intakes of pair-fed and alcohol-fed animals were approximately 60% of that of the *ad libitum* controls. This was reflected in the significantly lower weight gain of alcohol and pair-fed animals. The mean maternal plasma alcohol concentration in the alcohol-treated group on Days 15–17 of pregnancy was about 76 mg/100 ml. There was no maternal or fetal motality observed in any of the three groups of rats.

Fetuses of alcohol-fed rats weighed significantly less than those of pair-fed or *ad libitum*-fed controls on all gestational days examined (Table I). The fetal thyroid weight of alcohol-exposed fetuses on Days 17 and 18 was not significantly different from that of the two control groups. However, on Days 19 and 20 fetal thyroid weight was significantly less in alcohol-exposed fetuses, compared with controls of the same age (Table I).

Histologically, control fetal thyroids on Day 17 were already inlaid with small colloid-storing follicles in the parenchyma of irregularly arranged cords. In alcohol-exposed fetuses, the thyroids consisted mainly of radially arranged cell groups, with very few colloid-storing follicles. On Day 18 of gestation, fetal thyroids of alcohol-exposed rats had developed follicles (Fig. 1A), but they were fewer in number than those of fetuses of pair-fed or *ad libitum*-fed controls (Fig. 1, B and C). On Day 20 of gestation, the thyroids of fetuses of pair-fed and *ad libitum*-fed controls had well developed follicles whose cell height and follicle diameter were increased (Fig. 1, E and F). In contrast, the thyroids of alcohol-exposed fetuses had smaller follicles containing a large amount of colloid and the follicle cell height was less (Fig. 1D) than that of the two control groups (Fig. 1, E and F).

Histometrically, the follicular cell heights in the two control groups steadily increased during the period of observation (Table II). The follicle cell height in alcohol-exposed fetuses also increased until Day 20, but the rate of the increase seems to be less than that of the two control groups on all days examined. On Days 19 and 20, the follicle cell height of alcohol-exposed fetuses had not attained a value equal to that of either control group on the same day of gestation. In fact, follicle cell height on Days 19 and 20 was less than that of the two control groups 2 days earlier. Follicle diameters in fetuses of pair-fed and *ad libitum*-fed animals increased linearly from Day 17 to Day 20 of gestation (Table II). In the fetuses of ethanol-fed rats, the follicle diameter was significantly less than in the two control groups on all days examined. On Days 19 and 20 follicle size was approximately equal to that of the controls 2 days earlier (Table II).

Table I. Body Weights and Thyroid Weights of Fetuses from Alcohol-Fed, Pair-Fed, and *Ad libitum*-Fed Rats (mean ± SE)

Group	Fetal age (days)	No. of observation[a]	Body weight (g)	Thyroid weight (mg)
Alcohol-fed	17	12 (3)	0.97 ± 0.02a[b]	0.27 ± 0.03a
Pair-fed		14 (4)	1.05 ± 0.02b	0.32 ± 0.02a
Ad libitum-fed		20 (5)	1.04 ± 0.02b	0.30 ± 0.01a
Alcohol-fed	18	18 (5)	1.57 ± 0.05a	0.54 ± 0.04a
Pair-fed		21 (5)	1.74 ± 0.02b	0.59 ± 0.02a
Ad libitum-fed		17 (4)	1.68 ± 0.03b	0.59 ± 0.04a
Alcohol-fed	19	19 (5)	2.17 ± 0.07a	0.62 ± 0.05a
Pair-fed		19 (5)	2.66 ± 0.05b	0.79 ± 0.02b
Ad libitum-fed		16 (4)	2.56 ± 0.03b	0.73 ± 0.02b
Alcohol-fed	20	16 (4)	3.18 ± 0.12a	0.85 ± 0.04a
Pair-fed		21 (6)	3.95 ± 0.08b	0.98 ± 0.03b
Ad libitum-fed		13 (3)	4.35 ± 0.07c	1.02 ± 0.05b

[a] Number of litters in parentheses.
[b] a, b, c, Figures in the same column for a single given age not sharing the same letters are significantly different at $P < 0.05$.

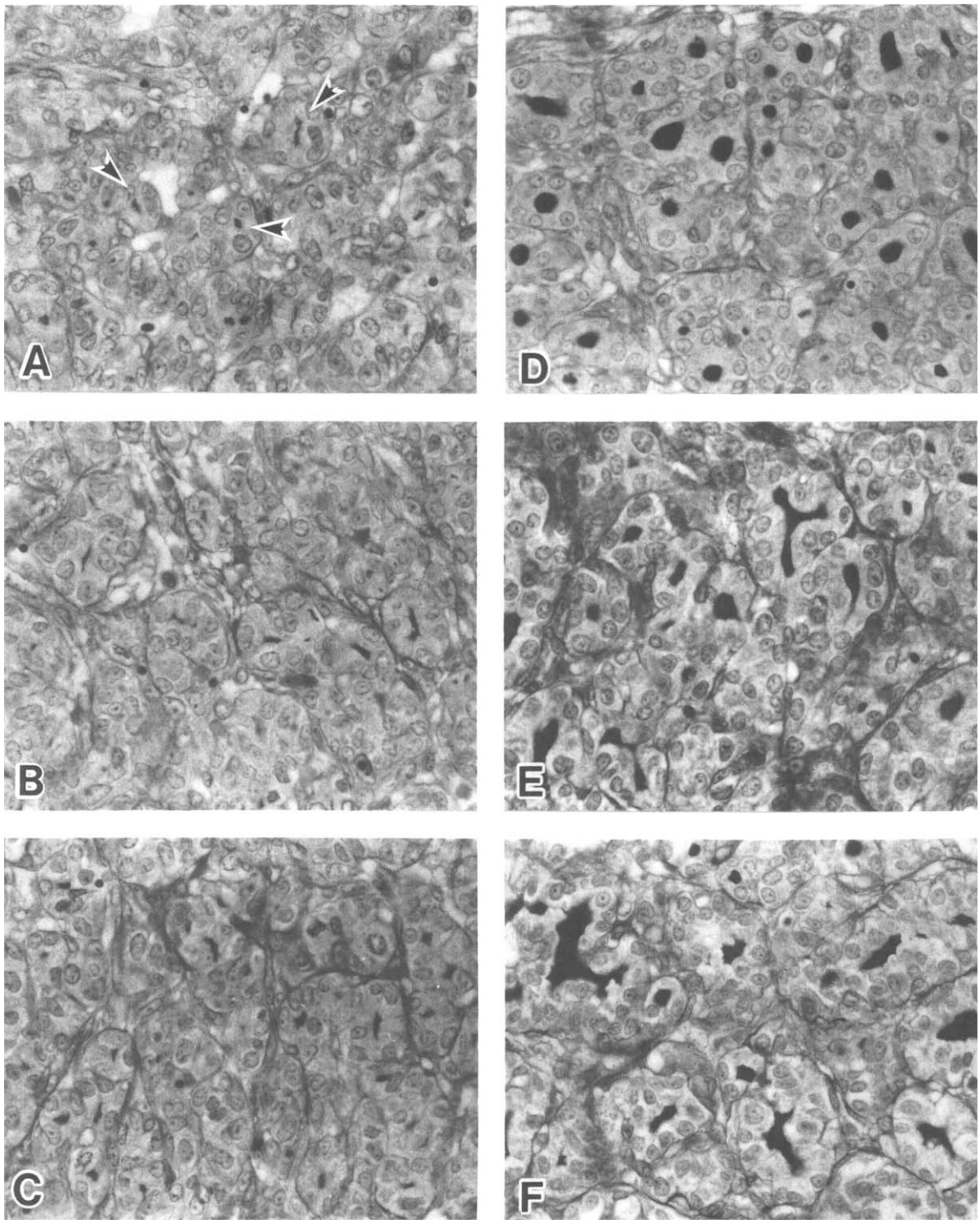

Figure 1. Figures represent thyroids on Day 18 (A–C) and Day 20 (D–F) of gestation. All sections were stained with periodic acid-Schiff and hematoxylin. (A) The thyroid of an alcohol-exposed fetus showing follicles containing periodic acid-Schiff-positive colloids (arrowheads). Both the follicular cell height and the follicular diameter are less than those of pair-fed control (B) and *ad libitum*-fed control (C) (original magnification ×600). (B) The thyroid of a pair-fed control showing developing follicles (original magnification ×600). (C) The thyroid of an *ad libitum*-fed control showing developing follicles (original magnification ×600). (D) The thyroid of an alcohol-exposed fetus showing numerous follicles filled with colloid in their lumina. The cell height of follicles is less than those of the pair-fed (E) and *ad libitum*-fed controls (F) (original magnification ×600). (E) The thyroid of a pair-fed control showing an increased height of follicle cells compared with that of a similar control 2 days earlier (B) (original magnification ×600). (F) The thyroid of an *ad libitum*-fed control. The follicle cell height is comparable to that of the pair-fed control (E) (original magnification ×600).

Table II. Changes in Histometrics of Fetal Thyroid from Alcohol-Fed, Pair-Fed, and *Ad libitum*-Fed Rats (mean ± SE)

Group	Fetal age (days)	No. of observation[a]	Height of follicular cells (μm)	Diameter of follicles (μm)
Alcohol-fed	17	6 (3)	7.50 ± 0.34a[b]	20.0 ± 0.8a
Pair-fed		8 (3)	8.20 ± 0.20a	22.1 ± 0.5b
Ad libitum-fed		8 (3)	8.41 ± 0.38a	22.2 ± 0.7b
Alcohol-fed	18	11 (4)	7.53 ± 0.21a	20.8 ± 0.6a
Pair-fed		11 (4)	9.30 ± 0.37b	25.9 ± 1.4b
Ad libitum-fed		10 (4)	9.16 ± 0.49b	26.6 ± 1.7b
Alcohol-fed	19	12 (3)	8.03 ± 0.12a	24.1 ± 0.5a
Pair-fed		12 (3)	9.97 ± 0.14b	30.6 ± 0.6b
Ad libitum-fed		11 (3)	9.36 ± 0.21c	29.3 ± 0.7b
Alcohol-fed	20	12 (3)	8.90 ± 0.23a	26.5 ± 1.1a
Pair-fed		12 (3)	11.18 ± 0.16b	35.6 ± 0.9b
Ad libitum-fed		11 (3)	10.78 ± 0.40b	37.1 ± 0.3b

[a] Number of litters in parentheses.
[b] a, b, c, Figures in the same column for a single given age not sharing the same letter are significantly different at $P < 0.05$.

Discussion

The foregoing observations clearly show that maternal alcohol consumption results in retarded fetal growth, in agreement with previous reports under similar conditions (1–5). In addition, the thyroid weights of alcohol-exposed rat fetuses increased more slowly than did those of the controls. It has been reported that in rats consuming alcohol throughout gestation, the supply of glucose and amino acids from the mother to the fetus is reduced (6–8), along with a reduced blood flow to the placenta (9). Therefore, it may be that the decreased thyroid weight in fetuses of alcohol-fed rats in the present study is due to reduced placental nutrient transport as a consequence of altered placental blood flow.

Shrader *et al.* (12) have reported that, as a result of maternal protein deprivation, both follicular cell height and follicle diameter of fetal thyroids were significantly less than those of fetuses of normally fed control rats. In rats restricted to 50% of the calorie intake of controls, Eguchi *et al.* (11) found that the time of appearance of a reciprocal relationship between the pituitary and the thyroid was retarded by about 1 day, which was proportional to the 1-day retardation of fetal body weight gain.

In this study the histological observations show that colloid-containing primitive follicles appear in the fetal thyroids of pair-fed and *ad libitum*-fed controls on Day 17 of gestation, in agreement with other reports on normal thyroid development (17, 18). However, the thyroids of 17-day-old fetuses of alcohol-fed rats consisted mainly of radially arranged cell groups and colloid-storing follicles were very few. Maternal alcohol consumption resulted in a decrease in follicular cell height and follicle diameter in fetal thyroids on all days examined. This reduction in both follicular cell height and follicle diameter in fetuses of alcohol-fed rats is so great that on Days 19 and 20 of gestation, the cell height was less than and the follicle diameter was about equal to those of the controls 2 days earlier. Since the retarded development of the fetal thyroid due to maternal alcohol consumption differs from that due to reduced food intake, it suggests that different mechanisms may be involved.

With respect to the reciprocal relationship between the pituitary and the thyroid gland, it has been reported that in their initial stages of development thyroid follicles are independent of thyroid-stimulating hormone (TSH) but somewhat later in development the follicles become able to respond to TSH (19). In addition, removal of the fetal pituitary gland by surgical decapitation retards the growth of the fetal thyroid, an effect which can be prevented by the injection of TSH (20, 21). The height of follicular cells is considered to be an indicator of their response to TSH. Therefore, our results lead to the suggestion that the retardation of fetal thyroid development as a result of maternal alcohol consumption may be due in part to a reduction in TSH release by the fetal pituitary gland. Although we have not yet examined the fetal pituitary gland, it may also be retarded in development as a consequence of maternal alcohol consumption.

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