Murine Fetal Development Enhanced by Dietary Vitamin A, Corn Oil, and Inositol (43627)

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Abstract. **Previous work on the effects of dietary vitamin A on craniofacial anomalies in mice revealed that 18-day-old fetuses from dams given 200 IU of vitamin A in corn oil daily in their diet weighed approximately 10% more than fetuses from mothers fed the unsupplemented standard mouse diet, Purina 5001. In the experiments reported here, it has been found that water-soluble vitamin A (200 IU/day) and *myo*-inositol (5 mg/day) added separately to the diets of pregnant mice increased the weight of 11-day gestations by approximately 25% and enhanced development of the eyes by the equivalent of 0.5–1.0 day without significantly affecting development of the liver or hind limbs. Corn oil alone (0.2 ml/day) had a similar effect on weight and eye development of 11-day fetuses and, in addition, growth of the hind limbs was enhanced modestly. The addition to the diet of vitamin A (200 IU/day) dissolved in corn oil (0.2 ml/day) resulted in a 25% increase in the weight of the 11-day fetuses and enhanced development of the eyes and hind limbs by the equivalent of about 1 day of gestation, suggesting that corn oil contains a factor(s) that interacts with vitamin A to accelerate limb development. Corn oil contains very small quantities of β-carotene or retinol (<1.0 IU vitamin A/ml); however, it is a rich source of the essential growth factors linoleic and linolenic acids and of inositol esters. The data suggest that the growth-promoting actions of dietary corn oil are due in part to the inositol esters.** [P.S.E.B.M. 1993, Vol 203]

A review of previous work (1–4) on the effects of dietary vitamin A on cleft palate and other craniofacial anomalies in mice has revealed that 18-day-old fetuses from dams given 200 IU of vitamin A in corn oil daily in their diets weighed approximately 10% more than fetuses from mothers fed a standard mouse diet, Purina 5001 (Table I). In an effort to determine how early in development dietary vitamin A expresses its effects on growth and organogenesis, 11-day-old embryos from dams fed the standard diet or the same diet supplemented with 200 IU of vitamin A in corn oil were compared as to overall growth (weight) and development of the eyes and hind limbs. Because early results revealed that fetuses from a control group fed a diet supplemented only with corn oil containing no significant quantity of vitamin A showed enhanced growth and development comparable to that observed in the group supplemented with vitamin A in corn oil, the effects of supplemental water-soluble vitamin A and of inositol, a constituent of corn oil, also were assessed.

Materials and Methods

The congenic strains B10.BR and B10.A(18R) were maintained in this laboratory by brother × sister matings. In the experiments, one male and two virgin 10- to 12-week old female mice were placed in each cage between the hours of 1800 and 0700 the next day. The day a vaginal plug was detected was considered to be Day 0 of pregnancy. On the 11th day of gestation, the dams were sacrificed, the uterus was removed, and individual implantations were fixed in 10% neutral formalin for 24 hr. The fetuses were dissected free, weighed, and graded for development of liver, hind limb, and eyes according to the criteria outlined in Figure 1. Fetuses that scored 0 in all categories were considered to have died before Day 11. The dams were weighed on Days 0 and 11.

Limb size and shape and eye development (pigmented neural epithelium) are accepted criteria of normal development for 11- to 15-day fetuses (5, 6), and weight is a more accurate measure of overall mass than

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is crown-rump height. Enumeration of somites is not useful for estimating stage of development beyond Day 10.5 of gestation (6).

The average pregnant female mouse consumes approximately 5 g of food per day (7). The dams were started on one of the following diets on Day 0: (i) Purina 5001 (15 IU vitamin A/g) = 75 IU/day; (ii) Purina 5001 + 0.2 ml corn oil/5g (<1.0 IU vitamin A/ml) = 75 IU/day; (iii) Purina 5001 + 200 IU of vitamin A in 0.2 ml corn oil/5 g = 275 IU/day; (iv) Purina 5001 + 200 IU water-soluble vitamin A/5 g = 275 IU/day; (v) Purina 5001 + 500 IU water-soluble vitamin A/5 g = 575 IU/day; and (vi) Purina 5001 + 0.1% *myo*-inositol (w/w, 5 mg/5 g) = 75 IU/day. The retinol palmitate in vegetable oil (1.6×10^6 USP units/g), water-soluble vitamin A, and *myo*-inositol were purchased from Sigma Chemical Co., St. Louis, MO. The corn oil (Mazola) was obtained locally. The agents were added to the 5001 bisquits by soaking them in the appropriate volume of solution, e.g., 100 g of 5001 plus 4.0 ml of corn oil = 0.2 ml corn oil/5 g, and 100 g of 5001 plus 100 mg *myo*-inositol in 3 ml of 0.9% NaCl = 5001 plus 0.1% *myo*-inositol. The dose selected for *myo*-inositol was calculated from its approximate content in corn oil so that the daily intake would represent the median amount in the diet with corn oil alone (inositol esters, 1–2% [8, 9]). Purina 5001 is not considered a deficient diet, certainly not with regard to total fat or vitamin A content (7, 10, 11), and the effects on growth and development of corn oil and especially vitamin A at doses that are demonstrably teratogenic (1–3) probably should be considered pharmacologic rather than nutritional.

Table I. Effect of Added Dietary Vitamin A (200 IU/day) on Weights of 18-Day-Old Fetuses from Mothers Injected with Dexamethasone (80 mg/kg) on the 12th Day of Gestation

	Vitamin A added	No. of fetuses	Mean wt (g ± SD)
B10.BR	–	120	0.77 ± 0.10
	+	115	0.86 ± 0.10[a]
B10.A(18R)	–	108	0.79 ± 0.13
	+	105	0.87 ± 0.11[a]

[a] $P < 0.001$, Mann-Whitney test.

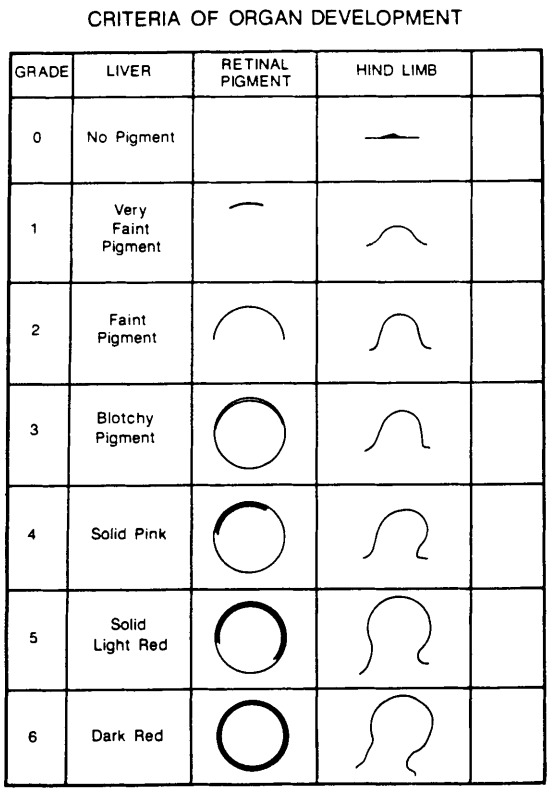

Figure 1. Criteria of organ development. See text for details.

On the 11th day of gestation, the liver was excised from one B10.A(18R) dam from the groups given the control diet and the diets supplemented with water-soluble vitamin A (200 IU), corn oil only, and vitamin A (200 IU) in corn oil; the retinoids were extracted and assayed spectrophotometrically (12–14).

The data sets are presented as means and standard deviations based on variations between litters; however, because certain data sets did not appear to have normal distribution, comparisons with the control groups (5001 alone) were made using the unpaired *t* test, the Mann-Whitney two-sample test, and multiple regression analysis (NCSS statistical program; NCSS, Kayville, UT). The most conservative *P*-value is reported without correction for multiple determinations.

Results

In studies with B10.BR mice, it was found that the addition to the diet of corn oil alone or vitamin A in corn oil resulted in 11-day-old fetuses that were 21–26% heavier and had more advanced development of their hind limbs and eyes than did fetuses from mothers fed the control diet (Table II). Fetuses from mothers fed the diet with 200 IU of water-soluble vitamin A were 17% heavier than the controls and eye, but not hind limb, development was significantly advanced. Statistically, the group given 500 IU of water-soluble vitamin A daily did not differ from the control group in any respect; however, regression analysis (data not shown) suggests that at this dose of vitamin A, eye development was depressed in the higher weight fetuses and limb growth was retarded in those of lower weight. None of the experimental diets appeared to influence liver myeloid development in this strain.

Eleven-day-old fetuses from B10.A(18R) dams fed the control diet generally were smaller and had less well-developed livers than did B10.BR fetuses (P = 0.097 and 0.004, respectively). The addition of corn oil or vitamin A to the diet had the same effects on weight

Table II. Effects of Vitamin A and Corn Oil on Growth and Development of Fetuses from Two Mouse Strains

Purina 5001 plus	B10.BR					B10.A(18R)				
	No. of litters (fetuses)	Fetal wt (mg)	Stage of development			No. of litters (fetuses)	Fetal wt (mg)	Stage of development		
			Liver	Hind limb	Eyes			Liver	Hind limb	Eyes
	12 (60)	24.0 ± 6.1	1.7 ± 0.7	2.0 ± 0.5	1.2 ± 0.9	10 (64)	21.6 ± 7.5	1.3 ± 0.5	2.0 ± 0.4	1.1 ± 0.5
Corn oil, 0.2 ml	10 (53)	29.1 ± 3.5[a]	1.9 ± 0.2	2.4 ± 0.4[b]	2.0 ± 0.2[a]	6 (23)	24.9 ± 4.6[d]	2.0 ± 0.6[a]	2.3 ± 0.5[c]	2.1 ± 0.2[a]
Vitamin A, 200 IU in corn oil, 0.2 ml	11 (63)	30.4 ± 4.0[a]	1.9 ± 0.4	2.7 ± 0.4[a]	2.2 ± 0.2[a]	8 (38)	28.2 ± 7.3[a]	1.9 ± 0.4[a]	2.7 ± 0.8[a]	2.0 ± 0.7[a]
Vitamin A, 200 IU, water soluble	8 (43)	28.1 ± 3.9[b]	1.6 ± 0.4	2.2 ± 0.5	1.9 ± 0.5[a]	7 (33)	28.4 ± 4.4[a]	1.9 ± 0.7[b]	2.1 ± 0.3	2.1 ± 0.4[a]
Vitamin A, 500 IU, water soluble	7 (42)	20.8 ± 3.4	1.6 ± 0.7	2.2 ± 0.3	1.3 ± 0.6	ND	ND	ND	ND	ND
Inositol, 5 mg/d	ND	ND	ND	ND	ND	10 (43)	29.7 ± 8.8[a]	1.6 ± 0.5	2.2 ± 0.4	1.9 ± 0.7[a]

Note. Values are expressed as mean ± SD. Comparison with control group: [a] $P < 0.0000$; [b] $P < 0.0001$; [c] $P < 0.001$; [d] $P < 0.01$, Mann-Whitney two-sample test.

and development of the hind limbs and eyes as they did on B10.BR fetuses. In contrast to the results with B10.BR, however, liver myeloid development was enhanced in this strain by corn oil and by vitamin A.

In both strains, eye development was enhanced equally by corn oil alone and by water-soluble vitamin A (200 IU). With the exception of the B10.A(18R) dams given the diet supplemented only with corn oil, the experimental diets induced comparable weight gains in the fetuses of both strains (i.e., 15% vs 25–31% weight gains). Mean hind limb development was not affected by water-soluble vitamin A at either dose level, but was enhanced by corn oil (15–20%) and by vitamin A in corn oil (35%). These results suggest that at the doses given, dietary vitamin A alone has little effect on hind limb development but that it may act additively when given with corn oil.

In the final experiment, *myo*-inositol was added to Purina 5001 at a concentration of 0.1% (the average pregnant dam would consume approximately 5 mg of added inositol per day). On the 11th day of gestation, the weights of the B10.A(18R) fetuses from mothers fed the supplemented diet on average were 36% greater than those from dams fed the control diet, and eye development, but not liver or hind limb development, was enhanced as noted in the group given the diet supplemented with 200 IU of vitamin A per day.

Maternal weight gain did not differ significantly among the several diet groups (data not shown). On the 11th day of gestation, maternal liver retinol values (μg/g) were: 5001 diet only, 259; 5001 plus corn oil only, 245; 5001 plus 200 IU of water-soluble vitamin A, 401; 5001 plus 200 IU of vitamin A in corn oil, 385.

Discussion

Vitamin A and inositol are dietary factors that are critical for normal growth and development of the vertebrate embryo (8, 10, 15). In the absence of either one of these factors from the diet of the pregnant animal, fetal growth is retarded, organogenesis is impaired, and death may result. In these experiments, it has been shown that the addition of vitamin A (200 IU/day) or inositol (5 mg/day) to a diet assumed to be nutritionally adequate (Purina 5001) resulted in a significant increase in mean fetal weight and enhanced eye, but not hind limb, development in 11-day-old fetuses. At a higher dose of vitamin A (500 IU/day), overall growth and eye development were inhibited in a subset of fetuses. Corn oil alone produced effects on growth and eye development very similar to those observed in the groups fed vitamin A (200 IU/day) or inositol; in addition, hind limb development appeared to be enhanced. This latter effect was even more apparent when the diet was supplemented with both corn oil and vitamin A (200 IU/day). This suggests that corn oil may contain a factor other than inositol (perhaps fatty acids), which interacts with vitamin A to accelerate hind limb development.

Vitamin A is known to be essential for normal fetal growth (10, 15) and to be involved in limb (16, 17) and eye development (2, 18). Using weight as an index of development, fetal growth has been found to correlate well with cord blood levels of insulin-like growth factor (IGF)-1 (19–21). After birth, growth is mediated by IGF's under the control of pituitary growth hormone (GH): *in vitro*, the release of GH from pituitary cells can be modulated by retinoic acid (22). However, GH secreted by the fetal hypophysis does not increase the synthesis or release of IGF-1 in fetal liver because of immaturity of the GH receptors (21). Fetal blood levels of IGF-1 correlate well with those of placental lactogen (PL) that has 90% homology with GH, suggesting that PL may be involved in the regulation of IGF-1 (23, 24). It is not known what effect, if any, retinoids have on the production or release of PL; however, modulation of PL levels by vitamin A could explain the increase in fetal weight noted in these studies.

Retinoids also have significant effects on differentiation and organogenesis (25). Observations on chick limb development have suggested that retinoids act as

morphogens (16) or through modulation of expression of *Hox* genes and/or growth factors (17). The eye abnormalities microphthalmia and anophthalmia can be produced by deficiencies and excesses of dietary retinoids (2, 26).

Corn oil contains very small quantities of β-carotene or retinol (<1.0 IU vitamin A/ml); however, it is a rich source of the essential fatty acids linoleic and linolenic and of inositol. Linoleic and linolenic acids have been shown to be essential for the maintenance of fertility, fecundity, and neonatal growth (27–29). Since Burr and Burr (30) demonstrated the requirement for linoleic acid in reproduction and growth of animals, many physiologic functions have been shown to be affected by deficiencies of these essential fatty acids: in pregnant rats, early and late fetal deaths are increased (31, 32), the period of gestation is often prolonged, the litter size is reduced, and postnatal mortality is increased (33, 34). The observation that corn oil but not inositol enhances hind limb development suggests that the role of these fatty acids in early fetal development should be explored.

Inositol is a six-carbon sugar alcohol present in biologic systems primarily as *myo*-inositol. In mammalian cells, inositol exists in its free form, as phosphorylated derivatives, and as various phosphoinositides (35, and references therein). Many of its physiologic and biochemical functions have been attributed to membrane phosphoinositides. The activation of the polyphosphoinositide cycle by calcium-mobilizing hormones or growth factors leads to hydrolysis of the plasma membrane phosphatidylinositol 4,5-biphosphate, generating the second messengers inositol 1,4,5-triphosphate (which triggers release of Ca^{2+} from the endoplasmic reticulum into the cytosol) and diacylglycerol, the endogenous activator of protein kinase C and associated protein phosphorylations (36). Inositol phosphates through the polyphosphoinositide cycle have been demonstrated to be critically involved in mesoderm induction (36), embryonic pattern formation (37), the formation of actin nucleation sites (38), the normal maturation of the fetal lung and eyes (35), intercellular communication (39), and in the release of placental lactogen from the trophoblastic giant cells of the placenta (40, 41). In addition, it has been shown *in vitro* that *myo*-inositol can reverse the growth inhibitory effects of hyperglycemia on early rat embryos (42).

Inositol as a component of the polyphosphoinositide cycle appears to play a critical role in many phases of fetal development, from blastocyst to neonate, and dietary deficiencies have been associated with retarded growth and development in rodent fetuses and neonates and in human premature infants. The results of the studies presented here suggest that inositol and vitamin A play roles in the regulation of maternal/fetal growth factors and gene activation and that, at low pharmacologic doses, they may enhance growth and development.

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