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Histochemical Enzyme Changes in Epidermis of Manganese-Deficient Fetal Mice[1] (38005)

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A dietary deficiency of manganese during pregnancy in a number of species results in offspring which exhibit ataxia, skeletal abnormalities, and high neonatal mortality (1–3). The ataxia was found to result from a congenital absence of otoliths in the inner ear (4). A coat-color mutant in mice, *pallid,* also causes impaired otolith development. Abnormal otolith development in both manganese-deficient and pallid young can be alleviated by supplementation with manganese during specific periods of gestation (5). This finding began a series of studies aimed at characterizing the genetic and nutritional interactions of the pallid gene and the trace element manganese. Because of the inner ear abnormalities in manganese-deficient and pallid mutant animals, and also because of the influence of the pallid gene on epidermal structures (6), it was of interest to examine enzyme activities in cephalic tissues. The present report describes such a histochemical investigation in manganese-deficient and pallid fetal mice.

Materials and Methods. Hybrid mice (derived from a four-way cross of C57Bl/6J, C3H/J, AKR/J, and DBA/2J[2]) and mice of the C57Bl/10J strain carrying the pallid gene (*pa*) were maintained in this laboratory. At birth, litters from hybrid females were given purified diets containing either 1 ppm manganese (deficient) or 45 ppm manganese (control).[3] The C57Bl/10J-pa litters were given a stock pelleted diet[4] throughout the experiment.

Hybrid females who had received the purified diets since birth were the experimental dams. They were mated with stock-fed males. Pallid females (*pa/pa*) were mated with C57Bl/10J ± *pa* (black) males to obtain litters consisting of the beige-colored pallid mutant mice and their black littermate controls. The day of finding vaginal plugs was considered Day 0 of gestation. The mice were mated in such a way that females from every experimental group became pregnant on each of three different days, thus ensuring treatment of specimens from each group to occur together for three different analyses.

On Day 17 of pregnancy, the females were anesthetized with Diabutol and when available two fetuses from each dam were removed and frozen in liquid-nitrogen-cooled isopentane. Specimens were taken from 4 dams fed the 1-ppm manganese diet, 4 dams fed the 45-ppm manganese diet, and 3-pallid dams with pallid and nonpallid (heterozygous) fetuses.

Fetuses from the four experimental groups were sectioned in a cryostat. Trans-

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[2] Stocks graciously provided by G. E. Bradford, Department of Animal Science, University of California—Davis.

[3] Composition of purified diet, in percent: cerelose, 54.5; casein, 30.0; corn oil, 8; salt mix, 6.0; vitamin mix, 1.5 (see Ref. 4 for composition of vitamin mix). Composition of salt mix in percent: $CaCO_3$, 30.000; K_2HPO_4, 32.100; NaCl, 16.800; $MgSO_4 \cdot 7H_2O$, 12.500; $CaHPO_4$, 6.000; $FeSO_4 \cdot 7H_2O$, 2.500; KI, 0.080; $ZnCO_3$, 0.025; $CuSO_4 \cdot 5H_2O$, 0.0300; $MnSO_4 \cdot H_2O$, for deficient (1 ppm) 0.00515, for control (45 ppm) 0.23175.

[4] Purina Laboratory Chow, Ralston Purina Co., St. Louis, MO.

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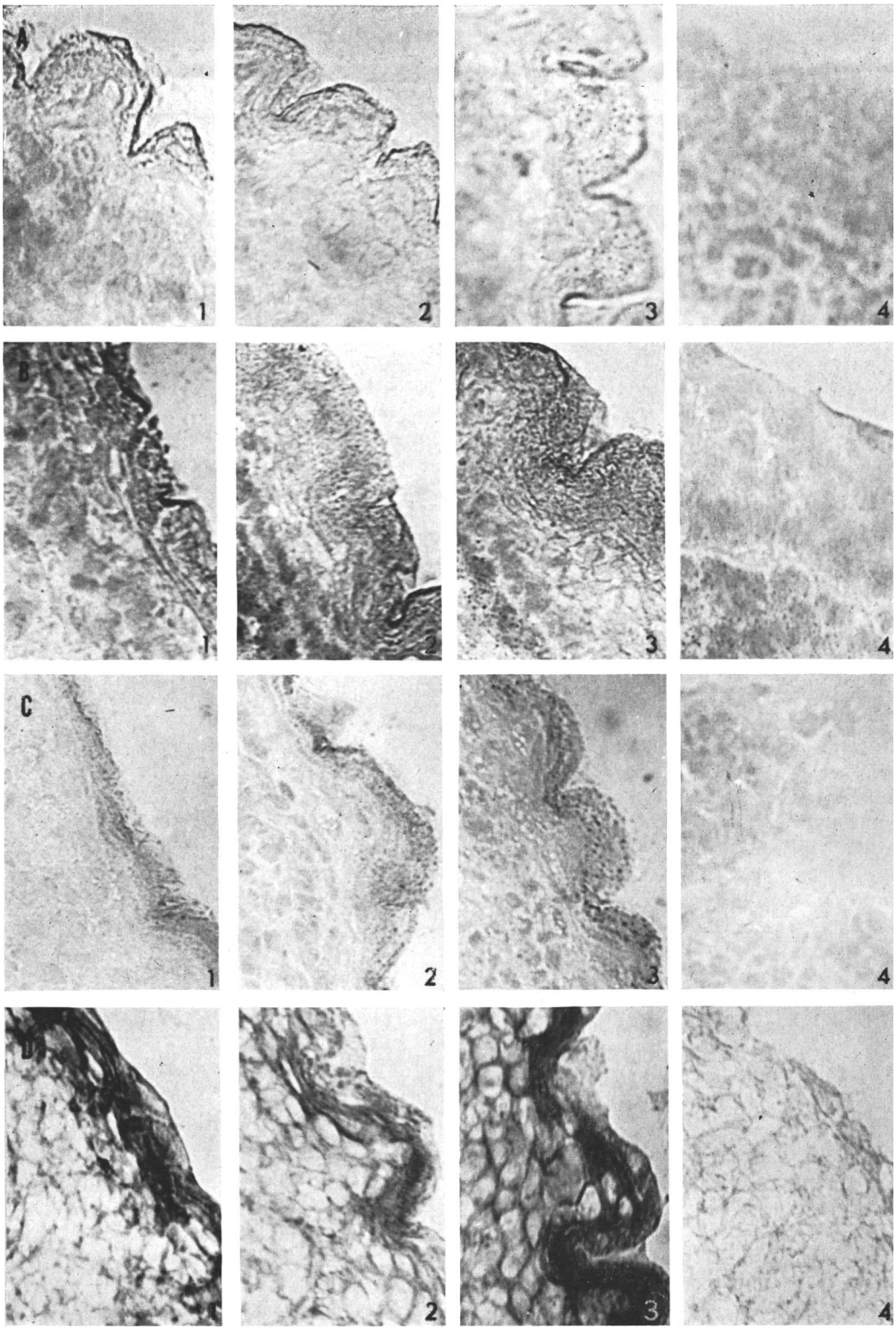

FIG. 1. Cryostat sections of epidermis from 17-day mouse fetuses stained histochemically. Enzyme reactions: A = cytochrome oxidase; B = alkaline phosphatase; C = choline esterase;

verse head sections (10 μm thick) were placed on cover slips and put in coplin jars for various histochemical analyses. Testing each particular reaction for the manganese deficient, manganese control, pallid and nonpallid (black) groups was done simultaneously in one jar. All enzyme analyses were completed on the day the specimens were taken. Reactivity of cytochrome oxidase (7), lactic dehydrogenase (8), glucose-6-phosphate dehydrogenase (8), succinic dehydrogenase (8), malic dehydrogenase (8), acid phosphatase (9), alkaline phosphatase (8), and choline esterase (8) and the concentration of choline lipids (10) were examined histochemically. Enzyme reactions were viewed and photographed immediately after incubation.

Results. All enzymes found in the inner ear were localized in all samples in the sensory epithelium. Alkaline phosphatase was also localized in the matrix surrounding the otoliths. Choline lipids were similarly localized in the neural epithelium of the inner ear and also in the otolithic matrix. Choline lipids appeared to be slightly reduced in intensity in the manganese-deficient tissue.

In the sensory epithelium, activity of the enzymes studied did not differ significantly among the various groups, whether or not otoliths were present. However, where otoliths were completely absent, otolith matrix was also absent and could not of course show histochemical activity. Enzyme reactivity of other tissues in the head was also essentially the same for fetuses from dams fed 45 ppm manganese, pallid mutants, and their black controls. However, in manganese-deficient fetuses, certain enzymes were abnormal in the epidermis. Cephalic tissues of manganese-deficient fetuses except for epidermis were normal for all enzymes assayed.

In the epidermis of manganese-deficient fetuses, there appeared to be absence of cytochrome oxidase activity in the epidermal layers (Fig. 1A). The false-positive yellow reaction for alkaline phosphatase was seen in the epidermal layers in all groups except the manganese-deficient (Fig. 1B). However, the red alkaline phosphatase reaction found in the dermis was still present in this group (Fig. 1, B-4). The choline esterase (Fig. 1C) and choline lipid (Fig. 1D) reaction products were found mainly in the epidermal layers of the skin in all samples. Epidermis from manganese-deficient mice was noticeably reduced in activity of choline esterase and in choline lipid (Fig. 1, C-4 and D-4).

Morphologically, epidermis from manganese-deficient animals seemed to be retarded in infolding and in hair follicle development, but this observation could not be quantified.

Discussion. Observation of sections of the head from 17-day mouse fetuses by histochemical means did not elucidate the relationship between the pallid gene and manganese metabolism. Tissues from both groups, with and without otoliths in the inner ear, appeared to be similar. Manganese-deficient tissues were different from those of pallid mice for certain enzyme reactions, but in these cases they were also different from both control groups.

Of the cephalic tissues examined, the epidermis (particularly the soft keratin layer) of the 17-day mouse fetuses appeared to be especially affected by manganese deficiency. Reduction in activity of cytochrome oxidase, alkaline phosphatase, and choline esterase and in the choline lipid stain was found in the manganese-deficient group. Cytochrome oxidase, a mitochondrial enzyme, has previously been found histochemically to be slightly reduced in liver from manganese-deficient adult mice (11). In addition, livers from manganese-deficient mice exhibited ultrastructural mitochondrial changes and reduced oxygen uptake (12).

D = choline lipids. Experimental groups: 1 = C57B1/10J ± *pa* (black littermate control for pallid mice); 2 = C57B1/10J-*pa*/*pa* (pallid mice); 3 = manganese-control (45 ppm) hybrid mice; 4 = manganese-deficient (1 ppm Mn) hybrid mice. For each of the four histochemical reactions there is a noticeable reduction of activity in the manganese-deficient epidermis (Group 4). ×750.

The present results are thus consistent with the earlier findings. In manganese-deficient epidermis there was an absence of the false-positive yellow reaction found in the three other experimental groups stained for alkaline phosphatase. False-positive reactions have been reported in normal and pathological skin conditions but cannot be related directly to alkaline phosphatase activity (13).

The skin has been found to be an active site for fatty acid and cholesterol synthesis in humans, rats, mice, guinea pigs, and chicks (14). The reduction in nonspecific choline esterase activity and in choline lipid staining of manganese-deficient epidermis reported here may be related to this finding. There has long been known an association between manganese and choline both in lipid metabolism and in the prevention of perosis in poultry. Recently, it was suggested that liver ultrastructure resulting from a deficiency of manganese or from choline may be similar (11). Perhaps the epidermis is yet another site where these two nutrients interact.

Summary. Reactivity of cytochrome oxidase, choline esterase, lactic, succinic, malic, and glucose-6-phosphate dehydrogenases, acid and alkaline phosphatases, and concentration of choline lipids were examined histochemically in cephalic sections of fetuses from manganese-deficient and pallid mice and their controls at 17 days of gestation. In the inner ear, enzymes and choline lipids were localized in the neural epithelium, but enzyme activity did not differ among the various groups. However, in epidermis of manganese-deficient fetuses there was reduced activity of cytochrome oxidase, choline esterase, and the false-positive yellow reaction for alkaline phosphatase, and reduced concentration of choline lipids.

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