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Histopathology of the pituitary gland in neonatal little *(lit)* mutant mice

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Summary. The pituitary gland in the little *(lit)* mutant mouse was analyzed with respect to the cytoarchitecture of the pars distalis and the volumetric density of immunoreactive growth hormone (GH) cell granules in neonatal *lit/lit* and normal C57BL mice. At 8 days postnatally the volume of GH granules/total tissue was significantly less in the *lit/lit* pars distalis, and the cells were loosely arranged, as compared with the normal pars distalis. In newborn mice a statistically significant difference could not be detected between normal and *lit/lit* mice with respect to the volumetric density of GH granules; however, differences occurred in the cytoarchitectural organization of the pars distalis. These differences included prominent vascular channels and well-defined cords and clusters of cells in the normal newborn mice, in contrast to indistinct vascular elements and a more diffuse arrangement of cells in *lit/lit.*

Key words: Pituitary, Neonatal mice, *Lit* mutant

Introduction

The little (*lit*) mutant mouse is characterized by a deficiency of growth hormone (GH) resulting in decreased growth that is detectable postnatally, even though the GH gene is present in these mice (Eicher and Beamer, 1976; Phillips et al., 1982; Cheng et al., 1983). A lack of responsiveness to growth hormone releasing factor has also been demonstrated (Jansson et al., 1986). Although the pituitary of *lit/lit* mice shows typical GH cells (Christensen and Wilson, 1981; Cheng et al., 1983; Wilson and Wyatt, 1986), ultrastructural morphometry of the immature pars distalis at 16 - 24 days postnatally and in adults has demonstrated a volumetric deficiency in the population of GH secretory granules relative to normal values (Wilson et al., 1988). An important question concerning this mutant is whether deficiencies are already present in the neonatal *lit/lit* pituitary at birth and during the first week postnatally, i.e., prior to the manifestation of retarded growth. The current immunocytochemical and morphometric study thus analyzes the population of GH secretory granules as well as the overall organization of cells in the pars distalis in newborn and 8 - day *lit/lit* mice.

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Materials and methods

Normal C57BL/6J mice and C57BL/6J *lit/lit* mutant mice were maintained on a 14 hour light and 10 hour dark cycle. Homozygous *lit/lit* and homozygous C57 matings were established and pregnant females were checked daily for the birth of litters, with date of birth counted as day zero (newborn). For 8-day mice, if the female was not producing milk, pups were fostered to a lactating female. Three *lit/lit* males and 3 normal C57 control males were used for the newborn stage and for the 8-day stage.

Pituitaries were removed at approximately 9 AM, rinsed in normal saline and fixed in 3% glutaraldehyde in 0.1 M phosphate buffer, pH 7.3, at room temperature for 1 1/2 hours. Tissue was rinsed in phosphate buffer, routinely dehydrated and embedded in araldite 502. Trimmed blocks were thick sectioned with glass knives, and sections were stained with methylene-azure blue to verify that samples were obtained from the lateral wings of the pituitary. Light micrographs of thick sections were taken with Kodak Panatomic-X film. A diamond knife was used to collect five sets of thin sections, 20 μm apart for each 8-day pituitary, and 8 μm apart for each newborn pituitary. Sections were then mounted on bare nickel grids for immunocytochemical processing as follows.

After a 2 min. rinse in glass distilled water, grids were etched in H_2O_2 for 2 minutes, rinsed again in glass distilled water and preincubated for 10 - 30 minutes in either 0.3 - 2% BSA (Bovine Serum albumin fraction V,

J.T. Baker or Sigma) in TBS (0.05 M Tris, 0.15 M NaCl, pH 7.6) or a 1:5 dilution of FBS (fetal bovine serum, Gibco) in BSA-TBS. Grids were then incubated for 3 hours at room temperature in rabbit anti-human growth hormone (Dako, diluted from 1:200 to 1:500 with BSA-TBS) or a 1:50 dilution of non-immune rabbit serum (ICN) which served as a control. Radioimmunoassay by the supplier indicates that the anti-GH reacts at 100% to GH and has a cross reactivity of 1% for prolactin and less than 1% for HPL, FSH, TSH and LH. After rinsing in BSA-TBS, grids were incubated for 1 hour at room temperature in secondary antibody-gold complex (Janssen goat anti-rabbit IgG fraction conjugated to 15 nm colloidal gold diluted from 1:45 to 1:100 with BSA-TBS). Grids were rinsed in BSA-TBS, TBS and glass distilled water before post fixing for 2 minutes in 1% OsO4 and routine staining with uranyl acetate and lead citrate.

A Zeiss 9 electron microscope was used to photograph sections at a direct magnification of 4,800 x, which was enlarged in the final micrographs to 14,400 x. Four micrographs were obtained from each of the 5 sections for each animal by photographing the center of a grid square in each of four quadrants. All photographs were morphometrically analyzed by means of the square grid point counting method of Weibel (1969). For normals and mutants at 8 days and newborn, a comparison of mean values (± standard error of mean) for volume densities of GH secretory granules/total tissue were subjected to statistical analyses by means of the Man-Whitney U test (Sokal and Rohlf, 1981).

Results

In the following account, «normal» refers to C57BL mice, and «abnormal» refers to *lit/lit* mice.

Cytoarchitecture

At 8 days postnatally the pars distalis of the adenohypophysis in normal mice is typically elongated, with the long axis lying mediolaterally. Cross sections show a compact arrangement of cells with tortuous vascular channels permeating throughout the tissue (Fig. 1A). The abnormal pituitary is likewise elongated and contains tortuous vascular channels, but the gland is

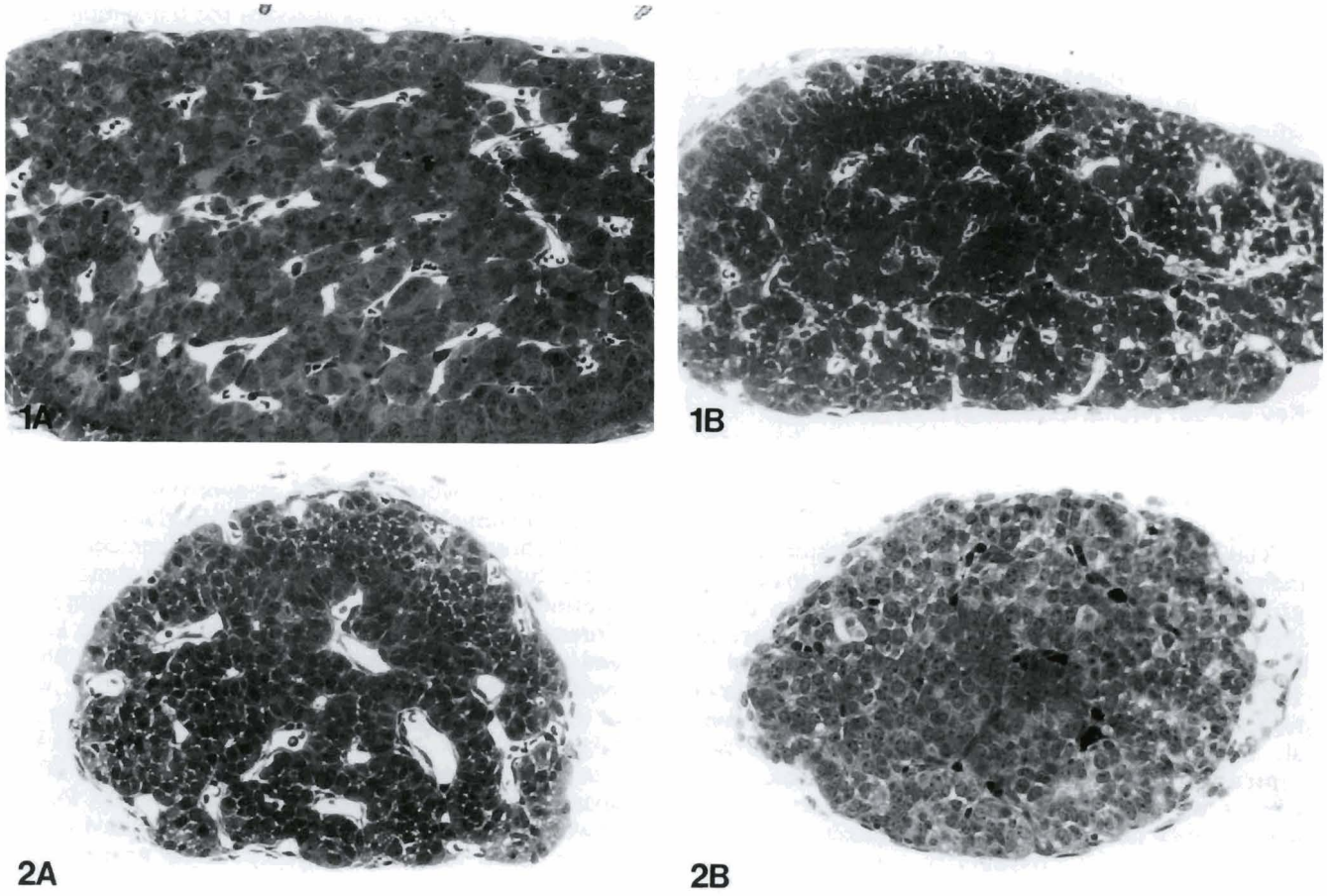

Fig. 1. Cross sections of the postnatal pars distalis at 8 days. x 250. A, Normal. B, Abnormal, with loosely arranged cells.

Fig. 2. Cross sections of newborn pars distalis. x 250. A, Normal. The cells are grouped in cores and clusters around well defined vessels. B, Abnormal. Note diffuse arrangement of cells and lack of well developed vascular elements..

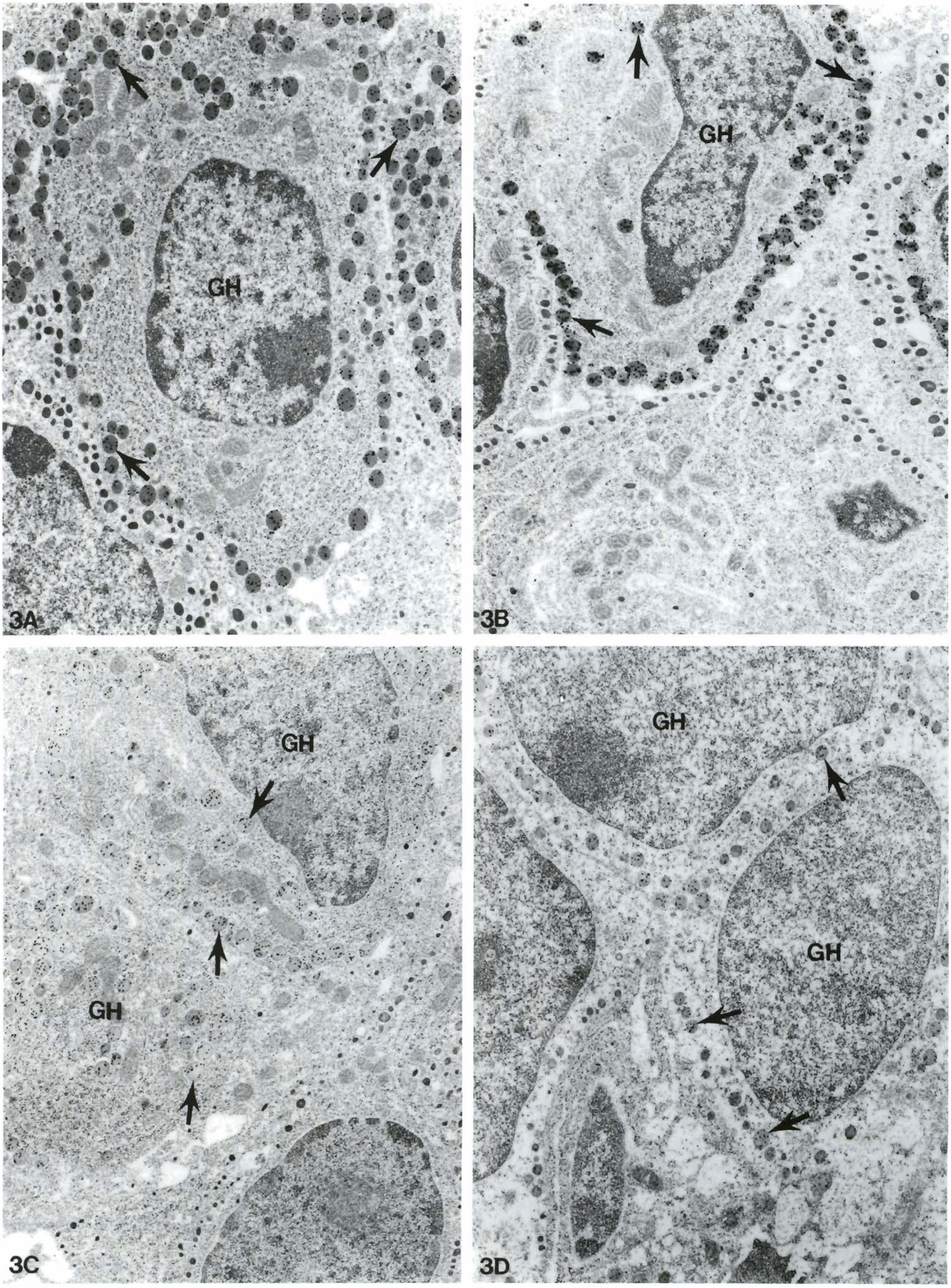
GH
3A
GH
3B
GH
GH
3C
GH
GH
3D

Fig. 3. Electron micrographs of the postnatal pars distalis at 8 days (A,B) and in the newborn (C,D), showing secretory granules (arrows) in growth hormone (GH) cells. Note positive reaction to anti-growth hormone (black particles) overlying GH secretory granules, x 14,400. A, C. Normal. B, D. Abnormal.

smaller than the normal, and the cells are more loosely arranged (Fig. 1B).

In the newborn there are marked differences between the normal and abnormal pars distalis with respect to the overall arrangement of cells. In the normals, the cells are grouped in cords, and rosette-like clusters often surround central cavities or vessels. The vascular elements are prominent, dilated and tortuous, and endothelial cells are easily distinguished (Fig. 2A). Invaginations frequently occur at the periphery where vessels enter and leave the gland. In contrast, abnormal pituitaries show a more diffuse arrangement of cells, with only occasional cords or rosette-like clusters (Fig. 2B). Although vascular elements pervade the tissue, the channels are narrow and indistinct, and endothelial cells are difficult to discern. Moreover, the surface of the perimeter of the gland is relatively smooth with few invaginating vessels.

Ultrastructural immunocytochemistry

Ultrastructurally, the normal 8-day pars distalis contains rounded GH cells with dense, uniformly stained secretory granules, most of which react positively to anti-GH (Fig 3A). Granules in other cell-types fail to react with anti-GH. Morphometric analysis shows that the mean volume density of GH granules/total tissue is 10.5% (±0.74). In the abnormal 8-day pars distalis, the ultrastructural appearance of GH cells is similar to that in their normal counterparts, and other granulated cell-types are also present. Although the GH cells react positively to anti-GH (Fig. 3B), the mean volume density of GH granules/total tissue is 4.9% (±0.75). This difference from the normal is statistically significant ($P < 0.05$).

In the normal and abnormal newborn pars distalis, GH cells are present, though not as well developed as at 8 days. The GH secretory granules react positively to anti-GH (Fig. 3C, D), whereas other cell-types are unreactive. Although the mean volume density of GH granules/total tissue is 9.1% (±3.42) in the normal and 5.5% (±0.67) in the abnormal, this difference is not statistically significant ($P > 0.05$).

Discussion

Quantitative morphometric studies on the pars distalis of *lit/lit* mice have demonstrated that the volume density of GH granules/total tissue is 12% that of normal adults and 22% that of normal mice at 16 days postnatally (Wilson et al., 1988). In the present study, 8-day postnatal *lit/lit* mice show a volume density that is 47% of the normal amount of GH granules/total tissue. Thus, a statistically significant quantitative difference in growth hormone synthesis is already present prior to the gross manifestation of the growth retardation that occurs at approximately 2 weeks of age (Eicher and Beamer, 1976; Wilson and Christensen, 1980a).

In the normal newborn pituitary, GH cells predominate in number over the various other cell types and progressively increase in granulation as postnatal development proceeds (Yamada et al., 1957; Yamashita, 1969; Dearden and Holmes, 1976; Wilson and Christensen, 1980b; Christensen and Wilson, 1981; Hoeffler et al., 1985). In *lit/lit* newborns the current results show that the volume density of GH granules/total tissue is 60% that of normal mice. However, this difference was not statistically significant. Since a previous nonquantitative immunofluorescent study of GH cell density suggested that in *lit/lit* the central most region of the lateral wings of the pars distalis appears to be less sparsely populated with GH cells than in normal mice (Wilson and Wyatt, 1988), it is possible that a morphometric analysis confined solely to this central area might show subtle but statistically significant differences in volume density of GH granules/total tissue not only in newborn mice but perhaps even prenatally. It is also possible that differences may occur with respect to the volumetric density of GH cells/total tissue or granules/GH cells in newborn and 8-day *lit/lit* mice. However, these parameters could not be tabulated in the current study because of the realtively imprecise cell boundaries that occur in normal as well as abnormal pituitaries processed immunocytochemically at these early stages of development.

The current results suggest that differences exist in terms of the cytoarchitecture of the pars distalis at the time of birth, as well as at 8 days postnatally. At birth, abnormal glands show less obvious cords and rosettes than in normal glands, and the vascular elements are not as prominent. Although these are non-specific features with respect to the GH cell population, it is possible that they may reflect an underlying defect in the overall pattern of organization and growth of the pars distalis. This might in turn affect GH cell differentiation and function. Since important spatial relationships and associations have been detected among various pituitary cell-types (Nakane, 1970; Watanabe, 1985), it would be useful to determine whether disruption of these relationships occurs during prenatal stages of *lit/lit* mice, thereby modifying expression of the GH gene.

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