ANIMAL MODEL OF HUMAN DISEASE

G_{M2} Gangliosidosis

Animal Model: Porcine Cerebrospinal Lipodystrophy (G_{M2} Gangliosidosis)

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Biologic Features

This genetic disease occurs naturally in purebred Yorkshire swine and appears to have an autosomal recessive mode of inheritance. Affected (homozygous) pigs are of normal size at birth but have a reduced postnatal growth rate and are noticeably smaller than their normal or heterozygous (carrier) littermates at 8 weeks of age. Incoordination, usually evident in the affected pigs by the time they are 3 months old, is manifested initially by an abnormal gait ("goose-stepping") involving both rear legs. The incoordination becomes increasingly severe until the pigs become recumbent at 4 to 6 months of age. Numerous tiny white spots diffusely scattered through the retina have been seen during ophthalmoscopic examination of living affected pigs or by stereomicroscopic examination of the excised posterior chamber of the eyes of dead pigs (including nonmacerated, stillborn affected pigs). Unusually prominent, dark blue cytoplasmic granules in many neutrophils (Figure 1) and an increased frequency of azurophilic cytoplasmic granules in lymphocytes (with an increased number of granules per lymphocyte) were seen in Romanowsky-stained blood smears from some affected pigs.

Cytoplasmic inclusions develop and accumulate within neurons distributed throughout the brain and spinal cord. The cytoplasm of affected neurons in sections stained with hematoxylin and eosin has a foamy or diffusely vacuolated appearance (Figure 2). However, in plastic-embedded, 1-μ sections stained with toluidine blue, many dark blue, round, solid

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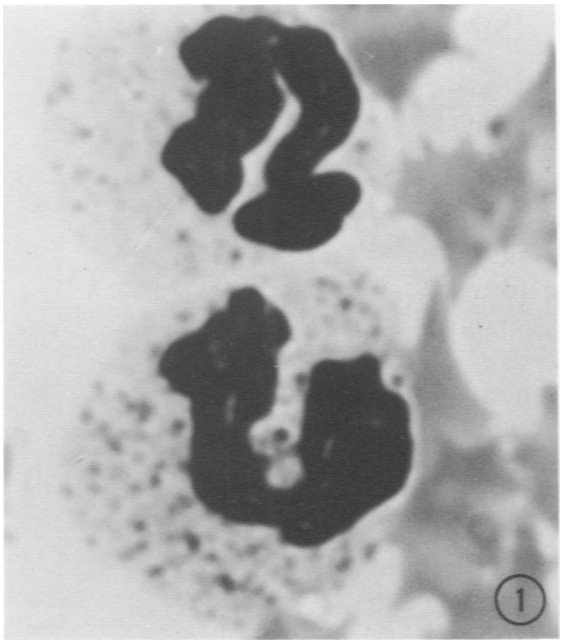

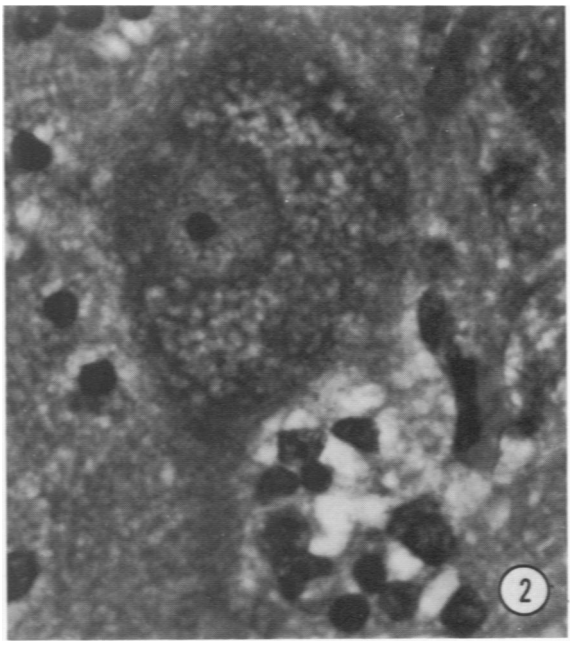

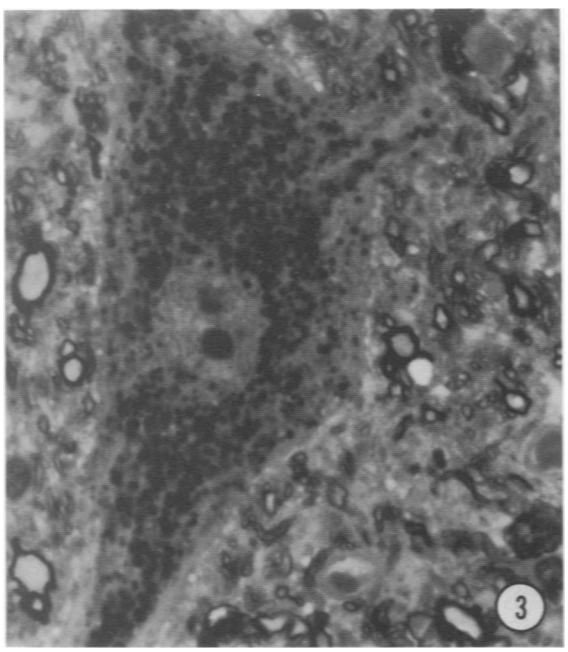

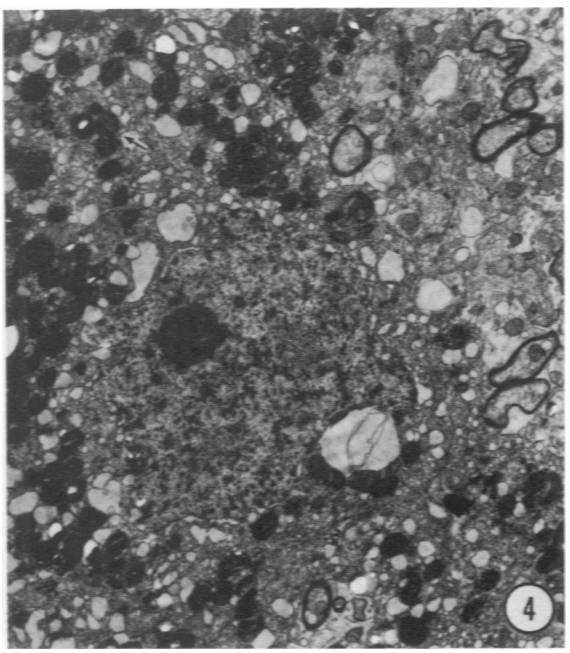

Figure 1—Two neutrophils in the blood of a lipodystrophic pig. Prominent cytoplasmic granulation (lower neutrophil) was observed only in affected pigs (Wright stain, × 2800). **Figure 2**—Neuronal cytoplasmic vacuolation in a section of paraffin-embedded lumbar spinal cord from a newborn affected pig (H&E, × 740). **Figure 3**—Granular appearance of the cytoplasm of a large motor neuron in a 1-μ section of plastic-embedded lumbar spinal cord from a 69-day-old affected pig (Toluidine blue, × 740). **Figure 4**—Neuron in the cerebral cortex from an 18-day-old, affected pig; the cytoplasm contains a moderate number of membranous inclusions (*arrow*) (Uranyl acetate and lead citrate, × 5150).

inclusions are visible in the cytoplasm of affected neurons (Figure 3). Electron microscopic examination of affected neurons has revealed these cytoplasmic inclusions to be membrane-bounded, 0.6 to 1.0 μ in diameter and partially or completely filled with membranes arrayed in a laminar pattern (Figure 4). The predominant inclusion in older pigs is completely filled with a single, concentrically whorled array of membrane and is considered to be the fully developed membranous cytoplasmic body (MCB).[1] Various forms of membranous cytoplasmic inclusions have been observed also in various types of cells with established phagocytic capability, such as microglial cells, reticuloendothelial cells, and neutrophils. Visceral histiocytosis has not been demonstrated, although ganglion cells in the submucous and myenteric plexuses appear to be affected.

The total ganglioside content of the cerebral cortex is 50 to 100% higher in affected pigs than in control (normal or carrier) pigs. This increase in total gangliosides is due to a marked increase in the amount of one monosialoganglioside, G_{M2} ganglioside, in the brain of the affected pig.

This familial disease of swine apparently is due to insufficient intracellular activity of *N*-acetyl-β-D-hexosaminidase. The level of activity of this enzyme in homogenates of buffy coat (blood), brain, and liver is distinctly lower in affected than in normal or carrier pigs. The level of hexosaminidase activity in buffy coat homogenates has provided a reliable basis for *in vitro* identification of affected pigs but is somewhat less reliable for separating carrier from normal swine. However, pigs can be reliably classified as *affected, carrier,* or *normal* on the basis of the level of hexosaminidase activity in their serum. Affected swine have the highest, normals have the lowest, and carrier pigs have intermediate levels of serum hexosaminidase activity. This serum enzyme assay provides a reliable test for use in detecting affected or carrier pigs in the general population of Yorkshire swine. The isoenzyme studies completed to date in affected swine are inconclusive, but no evidence of a complete deficiency of one isoenzyme has been found.

Comparison With Human G_{M2} Gangliosidoses

G_{M2} gangliosidosis in Yorkshire swine has many features in common with juvenile (Type 3) human G_{M2} gangliosidosis [later age of onset and death, no cherry red spot in the retina, no macroencephaly, late onset of blindness, moderate (two to three times) increase in total (and G_{M2}) ganglioside content of the brain, and, possibly, a partial rather than complete deficiency of hexosaminidase isoenzyme A].[2-6] However, the morphology of the cytoplasmic inclusions and the degree of neuronal involvement are similar to human Type 1 and 2 G_{M2} gangliosidoses and

the very low levels of hexosaminidase activity in cellular homogenates from affected pigs are similar to the low (1 to 3% of normal) levels of hexosaminidase in the brain in Sandhoff's disease (Type 2 G_{M2} gangliosidosis).[7,8] The elevated levels of serum hexosaminidase in affected pigs are not typical of any of the three types of human G_{M2} gangliosidosis.[2,3]

Usefulness of the Model

Porcine G_{M2} gangliosidosis is a genetically determined disease of potential value as an experimental model on which studies of various problems pertaining to human G_{M2} gangliosidoses (e.g., enzyme therapy in lysosomal enzyme deficiencies) can be conducted.

Availability

The frequency and distribution of this defect among Yorkshire swine is not presently known. A small group of heterozygous sows and 1 boar are currently being maintained by the Department of Veterinary Pathology, Texas A&M University. Use of the serum hexosaminidase assay to survey Yorkshire swine very likely would identify heterozygotes in sufficient number to establish a colony satisfactory for research purposes.

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