Combination of two techniques: Amniocentesis and nervous tissue explants. A pilot investigation[1]

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Summary. Addition of serum derived from amniotic fluid cells (AFS) from Tay Sachs carrier to developing mouse cerebral cortex explants results in excessive accumulation of lipid in neuronal cytoplasm.

Amniocentesis, the study of amniotic fluid cells (fibroblasts) during the 1st trimester of pregnancy, has represented a considerable advance in the prenatal diagnosis of both chromosomal and metabolic diseases. Since the co-culturing of isolated nervous tissues[3,4] with amniotic fluid cells had not previously been investigated[5], an investigation was undertaken to combine these 2 methods[6], in the hope of shedding further light on the mechanisms underlying mental retardation.

Tay-Sachs disease, a metabolic disorder[3], brings potential carriers, to the clinic for routine screening of their level of hexaminidase A. A deficiency in this enzyme results in excessive accummulation of lipid material throughout the central nervous system, in 1 out of 2600 births, resulting in amaurotic idiocy and death. It appeared relevant to begin this investigation on a well identified disease as follows:

Method. Experimental: 4 explants of cortex were removed from 16-18 day mouse fetuses and placed in a standard medium collagen coated glass coverslip assembly[3]. Feeding medium for the cultures was comprised of 70% Eagles minimal essential medium, 10% chick embryo extract ultrafiltrate and 20% horse serum, supplemented with 600 mg % glucose. 0.01 serum derived from amniotic fluid cells (AFS) were added to the cultures.

Controls: In 4 nervous system explants, treated in an identical manner, 0.01 ml of control AFS was added.

Fixation: At 4 weeks, all cultures were fixed in 2% glutaraldehyde, post-fixed in 1% O,O_4, dehydrated in Epon, and cut into 500 Å sections for electron microscopy.

Results. Light microscopic inspection gave indication of excess myelin in cerebral cortex (personal communication)[2]. Electron-microscopic examination revealed that the explants incubated with Tay-Sachs AFS in 2 cultures studied, had neuronal nuclei, whose cytoplasm was filled with lipid deposits. This resulted in a distended appearance of the cell body (figure 1). The 2 control explants did not exhibit this particular feature (figure 2).

Discussion. The findings reported combining amniocentesis and tissue culture nervous system explants, in Tay-Sachs disease relate to the previous finding by McKhann et al.[7], in which they describe that neurons isolated from a fresh Tay-Sachs biopsy, continued to accummulate lipid material (gangliosides). The specific neurohistochemical properties of the cytoplastic inclusions, and its anatomical distribution requires further investigation. Although Tay-Sachs disease is well documented, there is no available therapy. Treatment with hexaminidase A is at present not feasible, since this enzyme does not cross the blood brain barrier. In

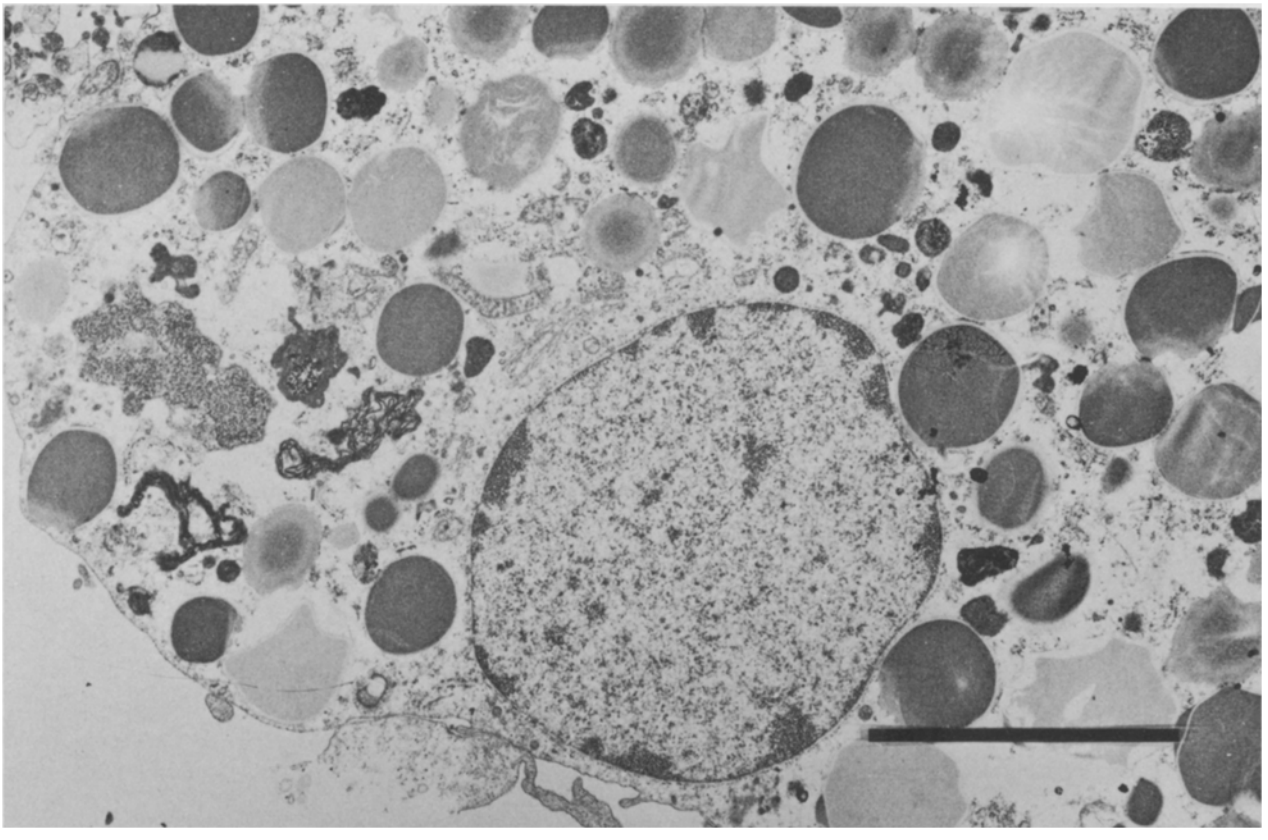

Fig.. 1. Electronmicrograph of neuronal cell, 4 weeks after gestation, in cerebral cortex of mouse. Addition of 0.01 ml of Tay-Sachs AFS results in deposition of lipid droplets in surrounding cytoplasm, giving distended appearance. Also note macrophages. × 800. Bar = 2 µm.

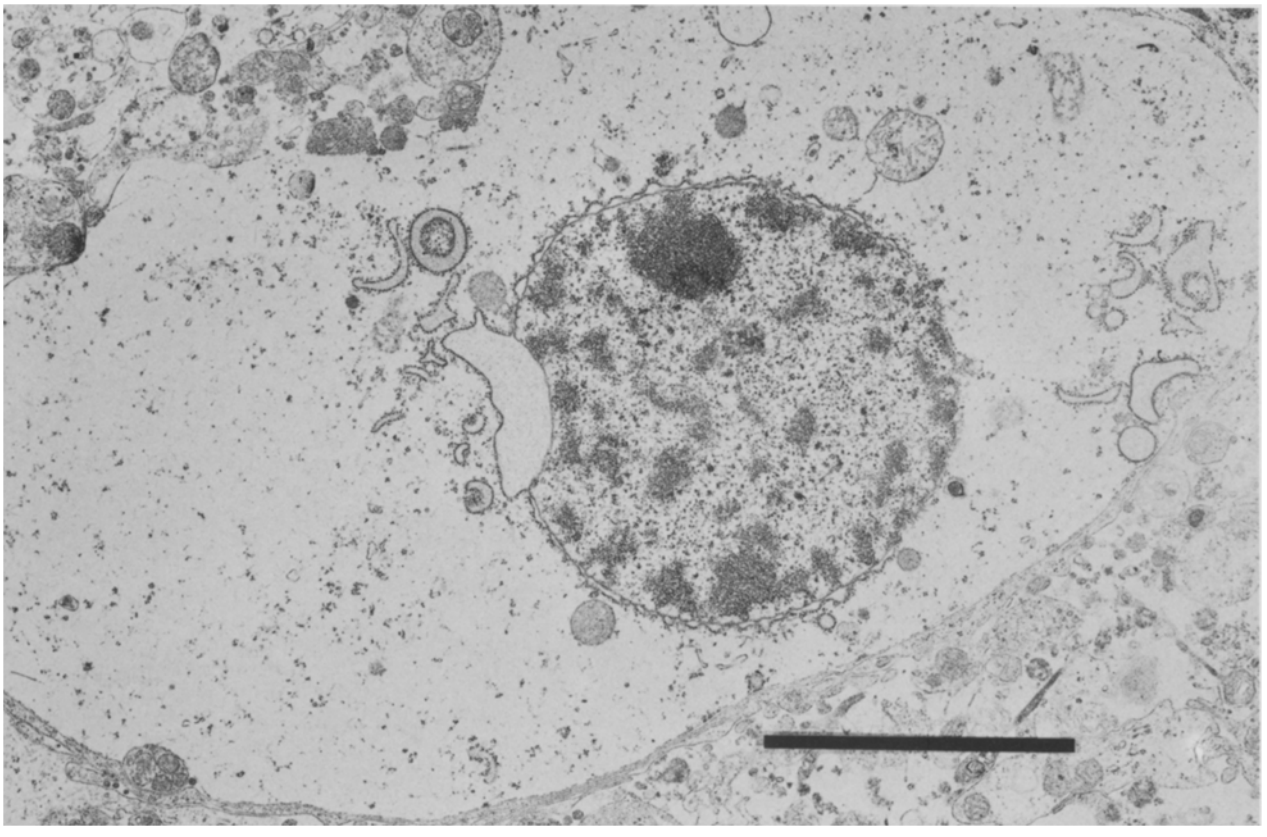

Fig. 2. Electronmicrograph of neuronal cell, 4 after gestation, in cerebral cortex of mouse. Addition of 0.01 AFS from control to feeding medium results in normal appearance. × 800. Bar = 2 μm.

addition, variability of donors contributing amniotic fluids, should give caution at oversimplified interpretations of the foregoing report.

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Lactate dehydrogenase polymorphism in *Mus musculus* L. and *Mus spretus* Lataste[1]

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Summary. First variation at the *Ldh-A* locus and a new allele at the *Ldh-B* locus are reported in a *M. musculus* population dimorphic at the *Ldh-A* locus and in a *M. spretus* population trimorphic at the *Ldh-B* locus.

Biochemical population genetics of Southern European mice appear promising in solving recurrent problems in systematics and in providing new variants needed for research. For instance, lactate dehydrogenase (LDH) was used to demonstrate reproductive isolation of *Mus musculus* and *Mus spretus* in nature. In Southern France, the latter species has a new allele fixed at the LDH B chain locus (*Ldh-B*); this was the 1st report of allelic variation of this outstanding enzyme in the genus *Mus*[4,5]. Extending this investigation geographically, we are now able to assert that LDH variation in *Mus* occurs not only at the interspecific level but also at an intrapopulation level and that the LDH A chain locus (*Ldh-A*) is involved as well as *Ldh-B*.

Material and methods. This report deals with the electrophoretic survey of 2 mice populations, one from Southern Spain (18 individuals trapped in cultivated fields at Santa Teresa, Province of Granada), the other from Hungary (9 individuals trapped inside houses at Farkasgyepü, Veszprem County). Allelic variation was investigated at 20 loci by starch gel electrophoresis. Techniques and symbols used are indicated in Britton and Thaler[5].

Results. The following loci for which no variation is known