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Lipid Composition in Postnatal Methylazoxymethanol-Treated Swiss Albino Mouse Cerebellum (39092)

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(Introduced by O. Mickelsen)

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Methylazoxymethanol is the toxic component of the cycad seed which is used in some parts of the world for food (1). The unusually high incidence of amyotrophic lateral sclerosis and a neurological disorder in cattle in these areas of the world prompted several studies designed to clarify any relationship which might exist between this toxic compound and neurological diseases (2, 3). In the course of these studies, both the glucoside and acetate of methylazoxymethanol were shown to have a variety of teratogenic, neurotoxic, carcinogenic, and hepatotoxic effects (4–7). Lesions similar to those in amyotrophic lateral sclerosis were never observed; however, lipid compositional changes were shown in the spinal cord of the mouse following treatment with the glucoside of methylazoxymethanol (8). Of particular interest were the effects on the differentiating cells of the central nervous system. The destructive effects of methylazoxymethanol on differentiating cells at various stages of gestation and postnatal development resulted in micrencephaly or cerebellar hypoplasia depending on the time of administration of the compound (4). In this respect, the effects of the agent were quite similar to those of irradiation, certain viruses, certain genetic mutations, and other toxins (9–13). The cerebellar hypoplasia which resulted from postnatal interruption of cerebellar development by these agents differed, however, from the effects of endocrine abnormalities or malnutrition (14, 15).

Previous studies in this laboratory have documented the histological, behavioral, and ultrastructural changes that follow administration of methylazoxymethanol in the early postnatal period of the Swiss albino mouse (16–21). It was substantiated that necrosis of the differentiating cells of the external differentiating cell layer of the cerebellum followed methylazoxymethanol administration on the first day of postnatal life and that varying degrees of granuloprival cerebellar hypoplasia resulted. At the ultrastructural level, postsynaptic differentiation proceeded although disorientation and dislocation of cerebellar Purkinje cells was common (17, 18). Ataxia was the usual behavioral concomitant of the composite of cerebellar changes (22). These studies have permitted correlation with biochemical changes that accompany altered synaptogenesis. For example, Spatz suggested that the lipid and protein changes in the micrencephalic rat, after antenatal methylazoxymethanol administration, could be related to morphologic or behavioral characteristics (4). Wong *et al.* (23) suggested that behavioral changes observed in the chick might be correlated with the reduction in adenylcyclase which is known to be concentrated at synaptic connections. Such morphologic changes and adenylcyclase reduction were substantiated in a similar study in the Swiss albino mouse (24). Young, Oster-Granite, *et al.* (25) reported that glutamate was selectively depressed in the hypoplastic cerebellum of the virus infected animal and suggested that glutamate is the putative transmitter of the granule cell. Chanda *et al.* (26) have demonstrated that recovery following methylazoxymethanol treatment is paralleled by expected changes in DNA, RNA, and protein.

As mentioned previously, these models have permitted assessments of myelin biochemical changes during development. Preliminary examination of the lipid composition of the spinal cord following methylazoxymethanol, for example, showed that phospholipid concentration was lower and

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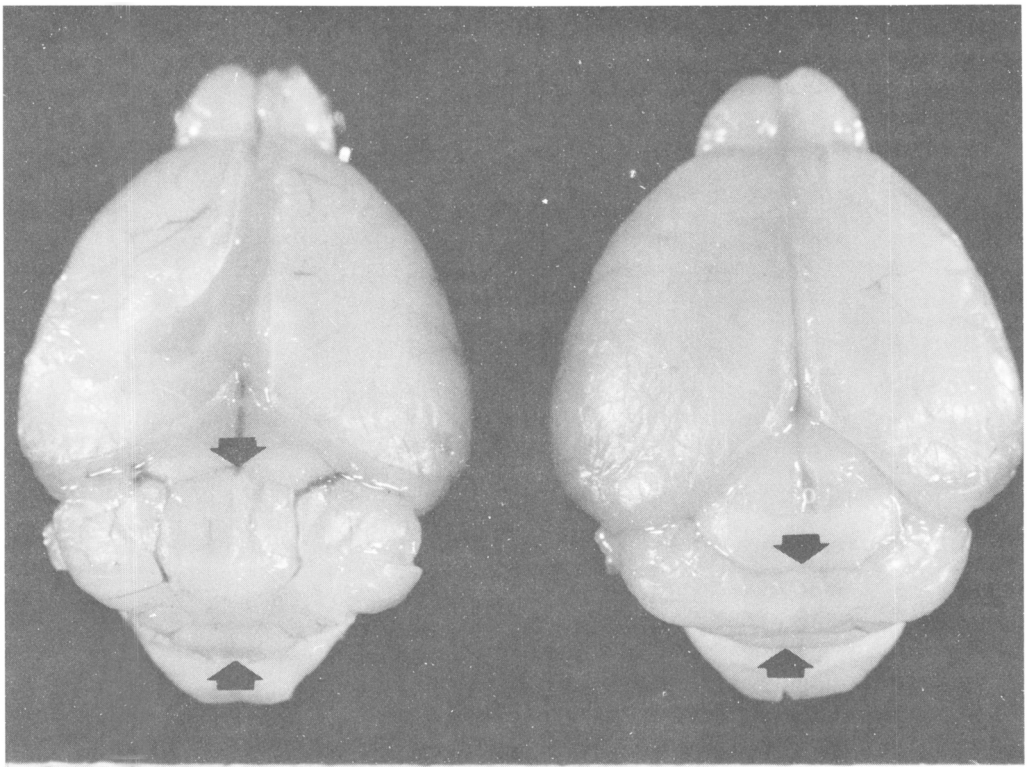


FIG. 1. Control mouse cerebellum (left, between arrows) shows degree of development of cerebellar vermis and hemisphere folia by 25 days postnatal.

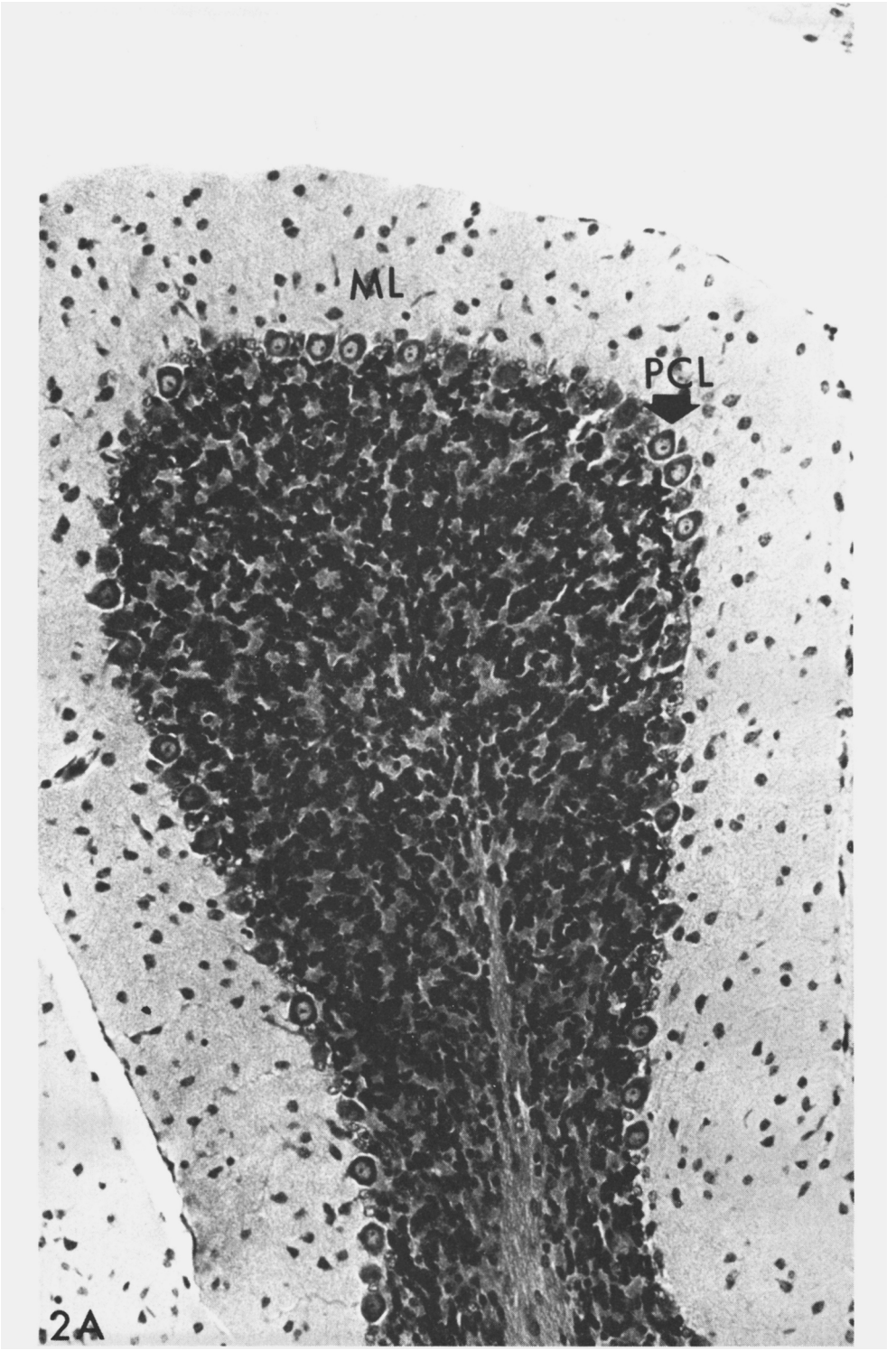
Cerebellum of mouse treated during the first day of life with MAM-acetate shows diminution in size of cerebellar vermis (right, between arrows) and relatively little development of folia.

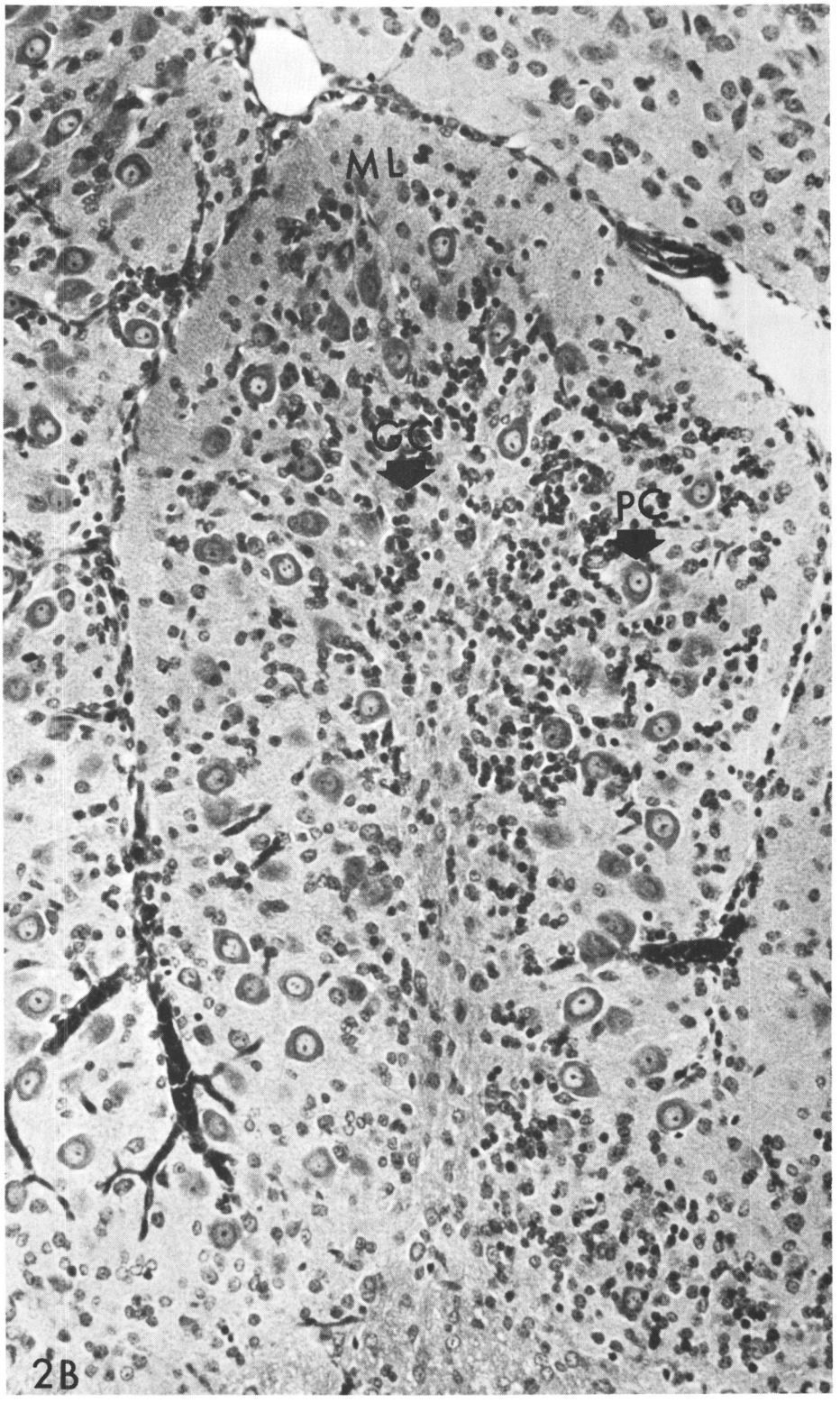
that phospholipid compositional changes were also present. The extent to which such biochemical alterations during development may alter the susceptibility to disease was not documented, but was postulated as a contributing factor to the development of diseases such as amyotrophic lateral sclerosis. Certain deductions about normal developmental mechanisms as well as about the biochemical correlates of differentiation have been possible as the result of these studies. This investigation was directed toward extending these observations.

Because gangliosides are highly concentrated in synaptic endings and the bulk of synapses in the cerebellum are related to the granule cell–Purkinje cell contacts, it was hypothesized that there would be a selective reduction in the cerebellar ganglioside concentration following deletion of the granule cell population by methylazoxymethanol. In order to substantiate whether myelin changes existed in the cerebellum like those that were found in a preliminary study of the spinal cord, the cerebroside/sulfatide ratios were also determined (27).

FIG. 2. (A) shows light microscopy from sagittal section of cerebellar vermis folium of 25 day control Swiss albino mouse. The molecular layer (ML), Purkinje cell layer (PCL), and granule cell layers (GCL) are well organized and in the normal relationships. × 100, Luxol fast blue-Nissl stain.

(B) shows sagittal section of folium from cerebellar vermis of Swiss albino mouse treated during first day of postnatal life with MAM-acetate. Note that molecular layer (ML) has an admixture of small granule type cells and Purkinje cells (PC). Granule cells (GC) are also scattered in the normal location of the internal granule cell layer.


ML
PCL
2A


ML
GC
PC
2B

Procedure. Methylazoxymethanol acetate or physiological saline (0.05 μl/g of body wt) was administered to Swiss albino mice pups immediately after birth. The litters were reduced to 10 animals each and were kept with the mothers until 25 days of age. Animals were decapitated, the cerebellum was removed, weighed and frozen at −30°C. Two to three cerebella were pooled to form 12 separate samples equally representing control and treatment animals. Approximately 100 mg of cerebellum were homogenized and extracted according to the method of Suzuki (28). The lipid insoluble residue was retained for protein determinations according to the method of Lowry (29). Aliquots from the combined upper phases of the lipid extracts were utilized for ganglioside sialic acid determinations according to the method of Svennerholm (30). The lower phases were separated into neutral lipid, glycolipid, and phospholipid fractions by silicic acid chromatography (31). After mild alkaline hydrolysis, the glycolipid fraction was applied to silica gel G plates. Sulfatides and cerebrosides were separated by thin-layer chromatography. These were quantified by determining galactose concentration (32). Hexoses were positively identified by gas liquid chromatography. Phospholipid concentration was determined according to the method of Bartlett (33).

Results and discussion. Fig. 1 illustrates the cerebellar hypoplasia that resulted from the destructive effects of methylazoxymethanol in the early postnatal period. The reduction in the size was related to granule cell deletion (Fig. 2). Table I shows that there was a significant reduction in cerebellar weight in the treated animals. Parallel reductions in *n*-acetyl neuraminic acid, phospholipid phosphorous, protein, cerebroside, and sulfatide were documented. There was no difference in the concentration of these components, however. This indicated that the granule cell deletion was not accompanied by selective reduction in any of these components. Calculation of the cerebroside/sulfatide ratio showed that this was not significantly different at the 0.05 level although some reduction is obvious (Table I). These studies suggest that neither gangliosides nor phospholipids are selectively depressed as might be expected if these constituents were specifically concentrated in the granule cell or its synaptic connections. However, it is possible that compositional changes in these two fractions might accompany granule cell deletion if a specific ganglioside or phospholipid were selectively associated with the granule cell and its connections. Compositional studies, utilizing the granuloprival cerebellar model, may be useful in order to clarify the roles of these compounds at the synapse.

Summary. Postnatal Swiss albino mice were treated either with methylazoxymethanol acetate (MAM) or saline and sacrificed at 25 days of age. Granule cell depletion resulted. Significant reduction in the cerebellar weight, protein, ganglioside sialic acid, cerebrosides, sulfatides, and phospholipids were documented. There was not, however, selective reduction of any component known to be associated with the synaptic structures. Specific association of cerebellar ganglioside with its granule cell population was not substantiated. Cerebroside/sulfatide ratios were not different in the two groups, indicating that significant alterations previously observed in spinal cord were not present in

TABLE I. COMPARISON OF CONTROL AND MAM-ACETATE TREATED MICE (MEAN VALUES)

	Control	Treated
Cerebellum weight	59.4 mg	33.2 mg
N-acetyl neuraminic acid/cerebellum	12.87 μg	7.89 μg
Phospholipid phosphorus/cerebellum	192.9 μg	112.9 μg
Protein/cerebellum	5.91 mg	3.15 mg
Cerebroside-sulfatide ratio	3.87	3.22
Cerebroside/cerebellum	64.9 μg galactose	35.7 μg
Sulfatide/cerebellum	18.3 μg galactose	11.7 μg

the cerebellum. It was concluded that the bulk of cerebellar granule cells and their synaptic connections could be deleted without affecting total ganglioside and phospholipid concentrations.

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