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Postnatal Cerebellar Hypoplasia and Dysfunction following Methylazoxymethanol Acetate Treatment (38495)

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(Introduced by O. Mickelsen)

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Methylazoxymethanol (MAM) is a toxic aliphatic azoxy compound which occurs naturally as a glucoside in the members of the botanical family Cycadaceae (1). The glucoside, cycasin, first received attention because of its potential etiological importance in the disease amyotrophic lateral sclerosis. This disease occurs in high indidence where foodstuffs from the cycad are consumed by the natives (2). A link between neurological disorders and the compound was suspected because cattle often developed neurological disability after consuming the palm-like cycad plant (3). Neurotoxic effects of the compound were never demonstrated during the earlier investigations, although MAM was found to be carcinogenic (4). Studies were then initiated in postnatal animals which were designed to clarify the carcinogenic properties of MAM. Treated mice developed signs and symptoms of cerebellar dysfunction (5). Hirono demonstrated that the neurological dysfunction which followed administration of MAM was caused by its toxic effect on the cells in the external differentiating cell zone of the cerebellum (6). These facts were also substantiated by Shimada (7). Extensive necrosis of differentiating cells also appeared to be the basis for the microencephaly induced by MAM in the offspring of pregnant rats treated at specific times during gestation (8). Cells differentiating in the subependymal zone of the lateral ventricles were the target of MAM at this earlier stage of development (9). Studies in our laboratory on the effects of the glucoside and the acetate of MAM (MAM-ac) were initiated on Swiss albino mice after cerebellar lesions in another strain of postnatally treated mice were confirmed by Sanger *et al.* (10). At 25-days of age, cerebellar hypoplasia was profound and was accompanied by completely disordered arrangement of the molecular, Purkinje cell and granule cell layers. Diminution of granule cells, which arise from the susceptible external differentiating cell layer, was the most prominent feature (11). At the ultrastructural level, absence of granule cell-Purkinje cell synaptic contacts was substantiated (12, 13). The pathogenesis of the lesions leading to these gross, microscopic and ultrastructural changes as well as their biochemical and behavioral correlates were substantiated in a series of reports (12, 13, 14, 15, 16, 17, 18). The purpose of the present investigation was to document the extent of cerebellar hypoplasia and neurological dysfunction at various points of development following postnatal administration of MAM-ac to Swiss albino mice.

Procedures. Six litters of newborn Swiss Webster albino mice were obtained from Spartan Research, Inc., for each sacrifice date. Average body weights were recorded for each litter. Three litters were injected subcutaneously with 0.05 μl of MAM-ac/g body weight and three with 0.05 μl of physiological saline/gram body weight. After injection, litters were housed individually in clear plastic shoebox type cages on pine chip litter. They were fed Purina Rat Chow *ad libitum*. Tap water was available at all times. Room temperature was kept at 70° ± 2° F, with 40–50% relative humidity. Lights were kept on from 8 AM to 8 PM. At 0, 5, 10, 15, 20 and 25 days, 18 mice were randomly selected from the three control litters and 18, when possible, were selected from the survivors of the treated litters. As each mouse was selected general observations of behavior were made about motor activities e.g., righting and walking abilities. In addition, such things as ap-

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TABLE I. SWISS ALBINO MICE CEREBELLAR, WHOLE BRAIN AND BODY WEIGHTS FOLLOWING INJECTION OF 0.05 μl OF MAM-ac/g BODY WEIGHT AT 1 DAY POSTNATAL LIFE.

Treatment	Age of mice (days)					
	0	5	10	15	20	25
	Cerebellar (mg)					
Saline	7.8	14.8	31.7	47.1	54.2	59.2
MAM-ac	6.5	7.1	14.7	23.5	32.3	35.7
	Whole brain (mg)					
Saline	96.7	213.2	322.9	405.3	441.3	472.6
MAM-ac	94.4	169.5	263.4	376.7	410.9	411.8
	Body (g)					
Saline	1.62	3.58	5.84	7.40	9.98	17.0
MAM-ac	1.62	2.68	4.40	7.44	9.75	8.9

pearance of fur, eyes and tremors were noted. The body weights were recorded for each mouse. The mice were then decapitated and the individual fresh whole brain and cerebellum weights were determined. The data was then analyzed statistically using *t*-distribution for unequal samples.

Results and Discussion. By testing the motor functions, it was found that the 0-day treated mice were indistinguishable from those in the control group. But, by 5 days the treated mice had an impaired and tremorous gait, and were slow at righting themselves. The 10-day treated mice continued to show an altered gait but seemed to have improved their righting. This trend continued and by day 25, the treated mice had severe tremors and paralysis of the hindquarters but nearly normal righting abilities. In addition to motor dysfunction it was observed that the MAM-ac mice had delayed postnatal development. Eye opening and fur production were delayed as much as 5 days. Table 1 gives the data concerning whole brain, cerebellum and body weights for the six time periods. It can be seen that for the first five time periods the body weights of the treated mice are not substantially different from those of the control mice. However, at day 25 the control mice show evidence of growth while the treated mice showed a reduction from the weight of 20 days. Also it can be seen that the whole brains of treated mice had only a slight reduction in weight when compared to the whole brains of control mice. It should be noticed that this reduction is mostly due to the diminished cerebellar weight. Cerebellar growth rates (as defined by the formula rate = $dW/dt \cdot 1/W$) were computed as shown in Fig. 1. The control mice exhibited a high rate of cerebellar development from day 0 to day 10 followed by a continuous decrease from day 10 to day 25. The treated mice had a very low rate of cerebellar development from day 0 to day 5, but the rate of growth from day 5 on is nearly equal

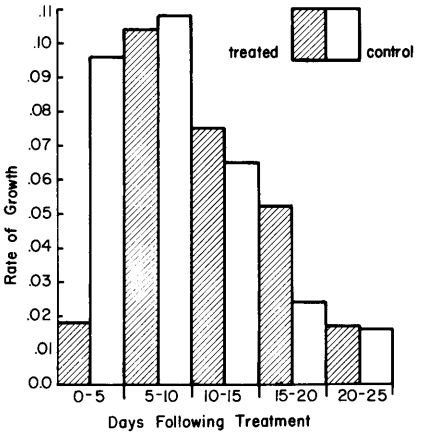

FIG. 1. Cerebellar growth rates as determined by the formula Rate = $dW/dT \cdot 1/W$. From 0 to 5 days, the treated mice showed a drastically lower growth rate than control mice. However, from 5 to 25 days, the treated mice exhibited cerebellar growth which was nearly equal to or higher than cerebellar growth in control animals.

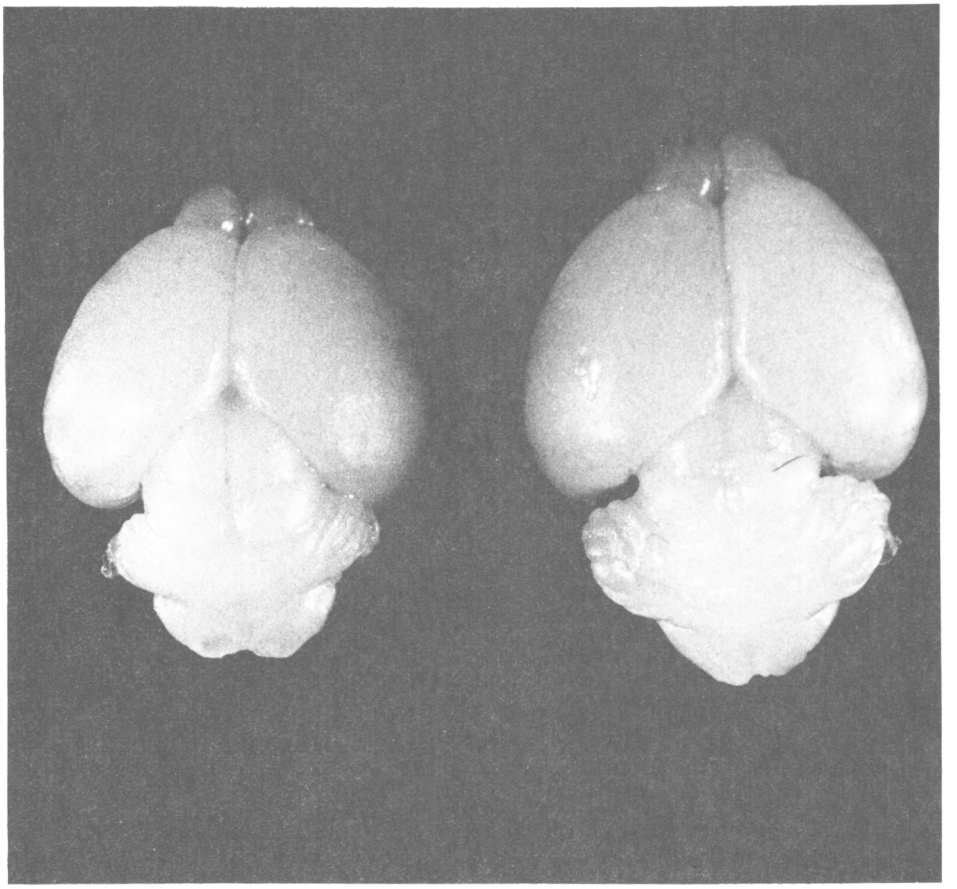

FIG. 2. The gross morphological variations between treated (left) and control (right) brains are shown here. Notice the drastically reduced cerebellum exhibited by the treated specimen as compared to the control specimen.

or higher than the rate of growth in control animals. Also, any reduction in cerebellar weight from day 10 on appears to be due to the destruction during the first 5 days, and not to continuous toxicity. Figure 2 shows the gross morphological structures of both control and treated mice at 10 days. The conclusion is that MAM-ac injected postnatally inhibited the rapid formation of the cerebellum through selective destruction of differentiating cells and thus altered growth and functional development of the cerebellum.

Summary. Swiss albino mice were injected postnatally with 0.05 μl of MAM-ac/g body weight. The mice were sacrificed at 0, 5, 10, 15, 20 and 25 days. Cerebellar, whole brain and body weights were determined. The treated mice generally showed smaller body weights reflecting the general toxicity of MAM-ac. There was no statistical difference between the whole brain weights of the two groups, when the loss of cerebellar weight was taken into account. Significant differences between the cerebellar weights of control and treated animals were shown at each sacrifice time. It is the conclusion of this study that those differences in weight reflect the destruction induced by MAM-ac during the first 5 days of postnatal life and are not due to a continued degeneration. As for general developmental and behavioral dysfunctions, the treated mice showed delayed eye opening and fur appearance, and by day 25, exhibited a tremorous altered gait.

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