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Early Effects of X-Irradiation of the Cerebellum in Infant Rats: Decimation and Reconstitution of the External Granular Layer

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The cerebellum of rats was irradiated daily with 200 r x-ray from the day of birth, with the number of exposures ranging from one to ten. The animals were killed 2 and 24 hours, and 4 days after the last exposure, and at the constant ages of 8 and 10 days. The cell population of the external granular layer was drastically reduced 24 hours after irradiation with a single dose of 200 r, and it was subtotally and maximally destroyed 24 hours after the second exposure. In animals exposed up to 5 × 200 r, recovery of the external granular layer was evident 4 days after the last irradiation; the extent of recovery was inversely related to the number of doses received. The width of the external granular layer was normal or supernormal by 10 days of age in all these groups, though planimetric measurements showed that the total area occupied by this proliferative matrix was subnormal (due to the decreased surface area of the cerebellum) and it was inversely related to the number of doses received. Some evidence was obtained that after some delay the recovered cells differentiated, and led to the development of the molecular and internal granular layers. Several abnormalities were noted in the morphology of the developing cerebellar cortex after exposure to a higher number of x-ray doses, such as abnormal folding of the cerebellar cortex and the massing and disoriented growth of Purkinje cells, and explanations were offered for these effects.

Introduction

This study deals with the early consequences of exposure of the cerebellum of infant rats to single or multiple doses of low-level x-ray. Attention is paid, first, to the destruction of the radiosensitive cells of the external

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granular layer, the proliferative matrix of the cerebellar cortex (2, 4) and, second, to the conditions of the subsequent recovery of this germinal system (5).

In a previous study (3), we described the gross morphological consequences of irradiation of the cerebellum in infant rats on successive days after birth with daily doses of 200 r, and with total number of exposures ranging from 1 to 10 days. After exposure to a single dose or to multiple doses of x-ray, the length of the cerebellum was appreciably reduced by 10 days, indicating retardation in cerebellar growth during this period with all radiation schedules. However, this effect was not seen at 30 and 90 days of age in the animals exposed to one or two doses of 200 r, although in the animals that received 4–5 × 200 r, the length of the cerebellum was reduced to that of controls 10 days of age, and after 10 × 200 r to that of neonates. In another pilot study, we irradiated the entire head of 3-day-old rats with a single dose of 200 r and allowed the animals to live for periods ranging from 10 min to 5 days. Between 4 and 12 hours after irradiation a large proportion of the cells of the external granular layer became pyknotic; these pyknotic cells were abundant both in the proliferative and migratory zone of the external granular layer (4). Between 24 and 48 hours after irradiation all the pyknotic cells disappeared, and there was a drastic reduction in the cell population of the external granular layer. However, by the third day after irradiation the external granular layer began to increase in width, and by the fourth day it was indistinguishable from normal. Partial recovery of the external granular layer was also indicated in another experiment in which the cerebellum was irradiated with 200 r on 5 successive days after birth (5).

These observations indicated the possibility of *developmental reparation* of the precursors of a neuronal system (the stellate and basket cells of the molecular layer and the granule cells of the internal granular layer) to which the external granular layer gives rise (1). The present study represents our first attempt to specify the reconstitutive capacity of the external granular layer after daily irradiation of the cerebellum with 200 r, number of exposures ranging from one to ten from the day of birth onward.

Materials and Methods

Laboratory-bred, Long-Evans hooded rats were used in these experiments. The technique of cerebellar irradiation and details of the radiation procedure and dosimetry were given in an earlier paper (3). From the day of birth the skull overlying the cerebellum was irradiated with a 6-mm wide pencil-shaped x-ray beam (from a 2 MeV source) with daily doses of 200 r (at a rate of about 50 r/min). The number of exposures ranged from one (irradiation on the day of birth only, called day 0) to ten (last irradia-

tion on day 9). Survival periods after irradiation were 2 and 24 hours, 4 days, and the constant ages of 8 and 10 days. For each condition at least two brains were processed, with a total of 91 brains. A summary of the radiation and survival schedules is given in Table 1.

These brains were embedded in Paraplast, cut sagittally or coronally at 6 μ and stained with cresyl violet and gallocyanin chromalum. (In the 8-day-old group only coronally sectioned brains were available.) For control material we used brains from 54 animals, ranging in age from 0 to 13 days. These brains were processed in a manner similar to the experimental brains. A summary of the normal cerebella analyzed is given in Table 2. In the quantitative evaluation of this material, we concentrated on matched parasagittal sections that were cut about 900 μ from the midline.

TABLE 1
IRRADIATION AND SURVIVAL SCHEDULE

No. expos.	Age in days when animals were killed													
	0	1	2	3	4	5	6	7	8	9	10	11	12	13
1×	2	2			2				4		4			
2×		2	2			2			2		4			
3×			2	2			2		2		4			
4×				2	2			2	2		4			
5×					4	4			3		4			
8×								4	4		4	2		
10×										5	5			2

QUALITATIVE OBSERVATIONS

Effect of 1 × 200 r. In the rats in which the cerebellum was exposed to 200 R on the day of birth and were killed 2 hours after irradiation (0 days of age) the cells of the external granular layer were not visibly affected. In the animals killed 24 hours after irradiation there was a considerable reduction in the thickness of the external granular layer and pyknotic cells were numerous both in the proliferative and migratory zones of the layer. The damage was more pronounced in the anterior vermis than in the posterior vermis; this difference, seen in practically all the irradiated animals in this study (Figs. 1, 6), was attributed to inadequate irradiation of the posterior parts of the cerebellum. In the animals killed 4 days after irradiation only an occasional pyknotic cell remained and the structural appearance of the external granular layer, though not its width, was normal. In the animals killed at 10 days of age (Fig. 6a) the external granular layer was normal both in appearance and width, and there was no longer any difference between the anterior and posterior vermis, indicating that the external granu-

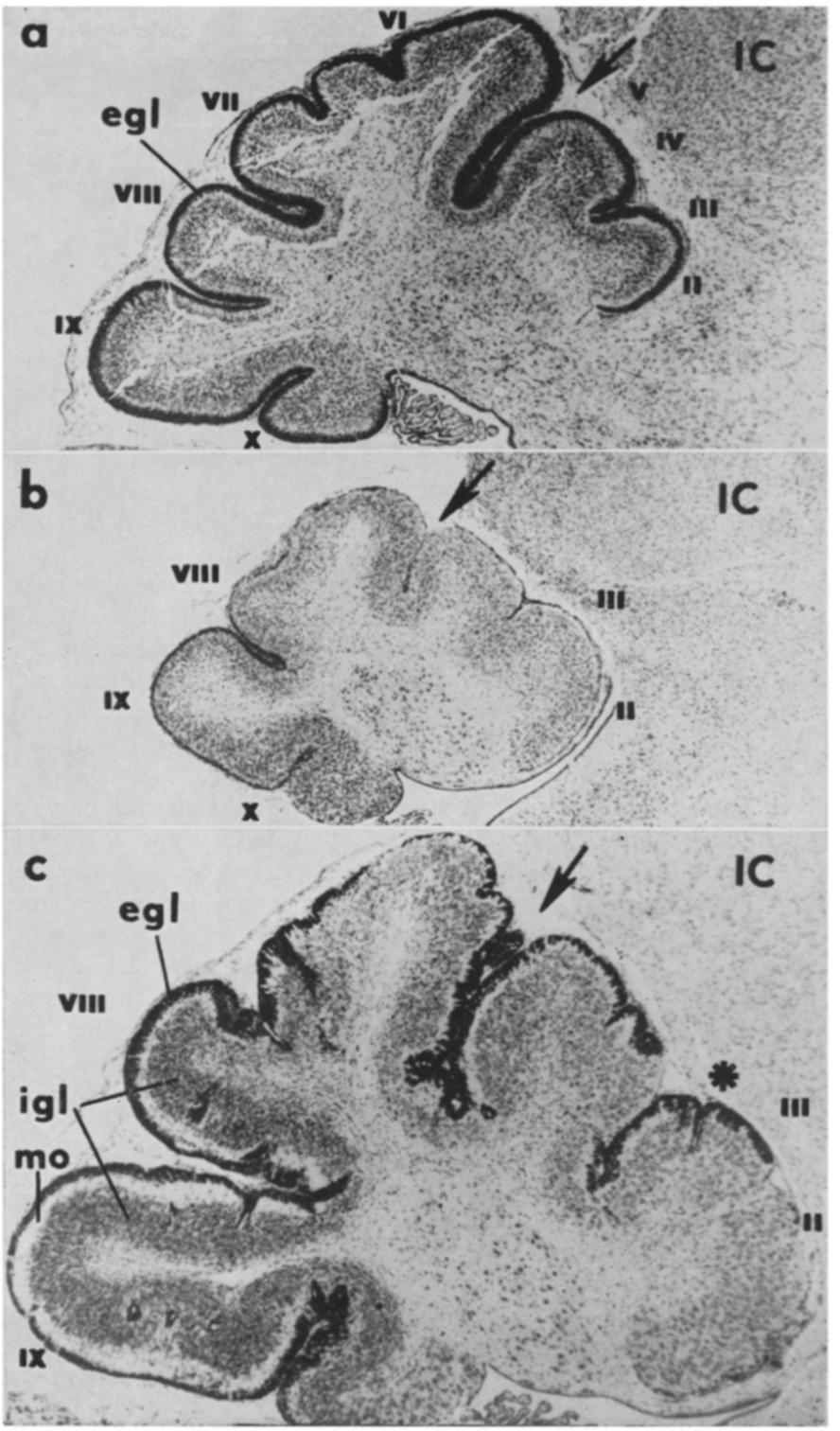
TABLE 2
CONTROL CEREBELLA ANALYZED

Age (day)	No. animals	Age (day)	No. animals
0	3	7	4
1	4	8	4
2	2	9	3
3	2	10	11
4	4	11	4
5	3	12	5
6	0	13	5

lar layer has recovered entirely. However, in these animals the molecular layer was less developed than in normals of this age, being thin with few spindle-shaped cells; this retardation of cerebellar cortical development was conspicuous in the more affected anterior lobes of the vermis.

Effect of 2 × 200 r. In the animals exposed to 200 r on days 0 and 1 and killed 2 hours after the second irradiation, the thickness of the external granular layer was greatly reduced, and pyknotic cells were abundant over the entire anterior vermis and parts of the posterior vermis (the pyramis and uvula were commonly spared; for a designation of lobules, see Fig. 1). Reduction in the width of the external granular layer was more pronounced 24 hours after the second exposure and many pyknotic cells were present. In the animals that lived for 4 days after the last irradiation (5 days of age) few pyknotic cells remained and the external granular layer had partially recovered: It was about half the width in the more affected anterior vermis than in the partially irradiated posterior vermis. In the 10-day-old animals reconstitution of the external granular layer was complete over the entire vermis. However, retardation in cerebellar development was evident in the other layers of the cerebellar cortex. In the more affected anterior vermis, the molecular and internal granular layers were quite undeveloped and the Purkinje cells were "massed" (many to a single row), whereas in the posterior vermis (as in the cerebellum of unirradiated animals) the molecular layer was beginning to grow in width with many spindle-shaped migratory cells embedded in it, there was a clearly recognizable internal granular layer, and the Purkinje cells were arranged singly in a row.

Effects of 3 × 200 r. In the animals exposed to 200 r on days 0, 1, and 2, and killed 2 hours after the last irradiation, the external granular layer was no longer present as a continuous sheet of cells over most of the vermis, excepting the depth of some of the fissures and in the most caudal parts of the vermis (particularly the pyramis). Scattered isolated cells, resembling those of the external granular layer, were seen even in the most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affected regions over the layer of Purkinje cells, together with a few pyknotic ones. The same pattern was observed 24 hours after the last exposure. In the animals killed 4 days after the last irradiation (6 days of age) slightly different results were obtained. In one rat the external granular layer was absent over most of the anterior vermis. In this animal, wherever the external granular layer was present, the apical cones of developing Purkinje cells (Figs. 2b, c) were oriented toward it (or perpendicularly to the surface of the cortex), while the orientation of the apical cones was random where the external granular layer was absent (Figs. 3b, c). In the other animal, the external granular layer formed a continuous sheet over the entire vermis. In all the animals of this group that lived to 10 days of age, the external granular layer formed a continuous sheet over the entire vermis. It was thinner and normal-looking over the posterior vermis; thicker and corrugated in appearance over the more affected anterior vermis. In the posterior cerebellum the molecular and internal granular layers were developing and Purkinje cells were distributed in a single row; in the anterior cerebellum, the molecular layer was poorly developed, a clearly recognizable internal granular layer was absent, and the Purkinje cells, though of normal size, were massed. Apparently, the differentiation of the reconstituted cells of the external granular layer has not begun at this age in the more affected portion of the vermis.

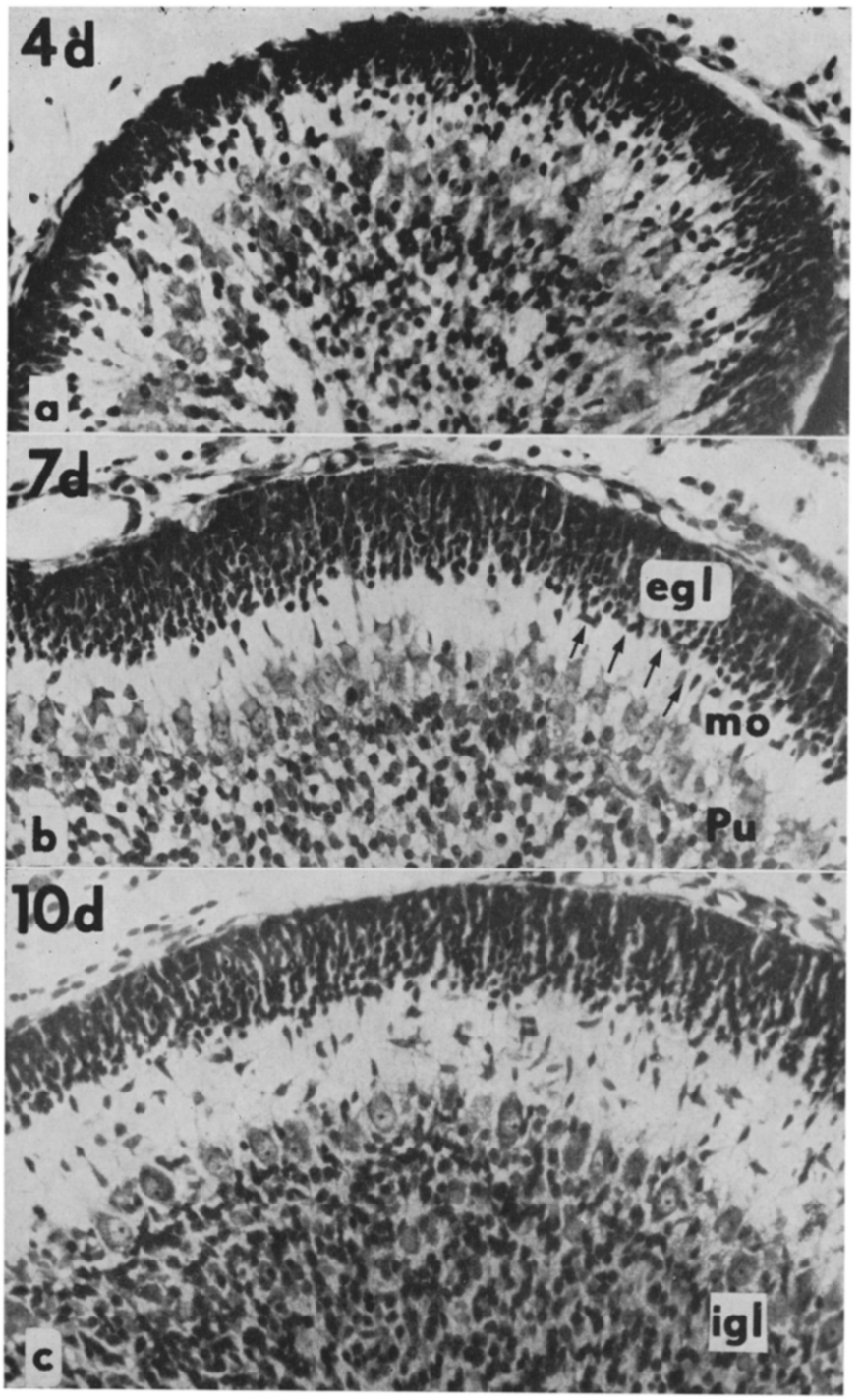
Effects of 4 × 200 r. In the animals that were irradiated with 200 r on days 0, 1, 2, and 3, and were killed 2 hours after the last irradiation, the external granular layer was absent over the vermis as a continuous sheet of cells, excepting for clusters of cells that were seen in the depth of some of the fissures and over part of the uvula. Isolated cells, presumably representing surviving, radio-resistant elements of the external granular layer, were seen over the entire vermis. Few or no pyknotic cells were encountered. The same pattern was essentially observed in the animals killed 24 hours after the last irradiation (Figs. 1b, 3a). In the rats killed 4 days after the

FIG. 1. Low-power photomicrographs of sagittal sections of the cerebellum (about 900 μ from midline). Designation of vermian lobules, according to Larsell (7): II, centralis ventralis; III, centralis dorsalis; IV, culmen ventralis; V, culmen dorsalis; VI, declive; VII, tuber; VIII, pyramis; IX, uvula; X, nodulus. Arrows point to fissura prima; egl, external granular layer; IC, inferior colliculus; igl, internal granular layer. *a,* cerebellar vermis of a normal 4-day-old rat; *b,* of a 4-day-old rat irradiated with 200 r on days 0, 1, 2, and 3 and killed 24 hours later. Note the absence in *b* of an external granular excepting as a thin sheet over uvula. *c,* from a 10-day-old rat, irradiated as *b*. Note presence of a quasi-normal external granular layer and developing molecular and internal granular layers caudally in uvula, to lesser extent in pyramis. More rostrally the external granular layer is reconstituted excepting over centralis ventralis. There is no internal granular layer in the rostral lobules. Area indicated by asterisk shown at higher magnification in Fig. 3c, Cresyl violet, ×32.

last exposure (7 days of age) the external granular layer was entirely reconstituted over the posterior vermis (it was thicker here than in normal animals of this age) and there was a gradient of decreasing thickness in the rostral direction, being altogether absent in the anterior vermis (Fig. 3b). The molecular and internal granular layers were present only in the uvula and pyramis. Again, the apical cones of developing Purkinje cells tended to be oriented toward the external granular layer where it was present; they were randomly oriented where the layer was still absent. In the animals that lived to 10 days of age the external granular layer covered the entire vermis; it was now thinner and nearly normal in appearance over the posterior vermis, thicker and corrugated in appearance over the more affected anterior vermis (in one animal the external granular layer was missing over parts of centralis ventralis; Fig. 1c). The development of the molecular and internal granular layers showed a clear caudorostral gradient. In the posterior vermis, particularly in the uvula and pyramis (Fig. 1c), these layers showed early signs of development, but in the rest of the vermis the molecular and internal granular layers were still absent indicating that the differentiation of the cells of the external granular layer has not begun (Fig. 3c). Here the Purkinje cells were normal in size but their growing apical cones were randomly oriented with respect to the surface of the cerebellar lobules.

Effects of 5 × 200 r. In the rats that were exposed to 200 r on days 0, 1, 2, 3, and 4 and were killed 2 hours or 24 hours after the last irradiation, the external granular layer was absent as a continuous sheet of cells over the entire vermis, except over parts of the uvula. In these animals pyknotic cells were not generally seen. The sparing of the caudal tip of the vermis (uvula), even after five daily exposures to 200 r, indicated the systematic sparing of this region by the narrow x-ray beam used. Isolated dark cells, presumed to be surviving, radio-resistant elements of the external granular layer, were still commonly seen. In the animals that survived for 4 days after the last irradiation session (8 days of age), a continuous band of thick, corrugated external granular layer was present over the uvula and pyramis, a fragmented layer over tuber, declive and parts of centralis dorsalis, and it was absent in the anteroventral lobes of the vermis. Initial signs of the development of the molecular and internal granular layers were seen only in the uvula. In the animals that were killed at 10 days of age, a thick, corrugated external granular layer was present over the entire vermis. It was

FIG. 2. High-power photomicrographs of lobulus centralis from normal rats, *a*, 4 days, *b*, 7 days, and *c*, 10 days of age. Abbreviations: egl, external granular layer; igl, internal granular layer; mo, molecular layer; Pu, Purkinje cells. Arrows show the orientation of the apical cones in normal cerebellum in direction of external granular layer. Cresyl violet, ×640.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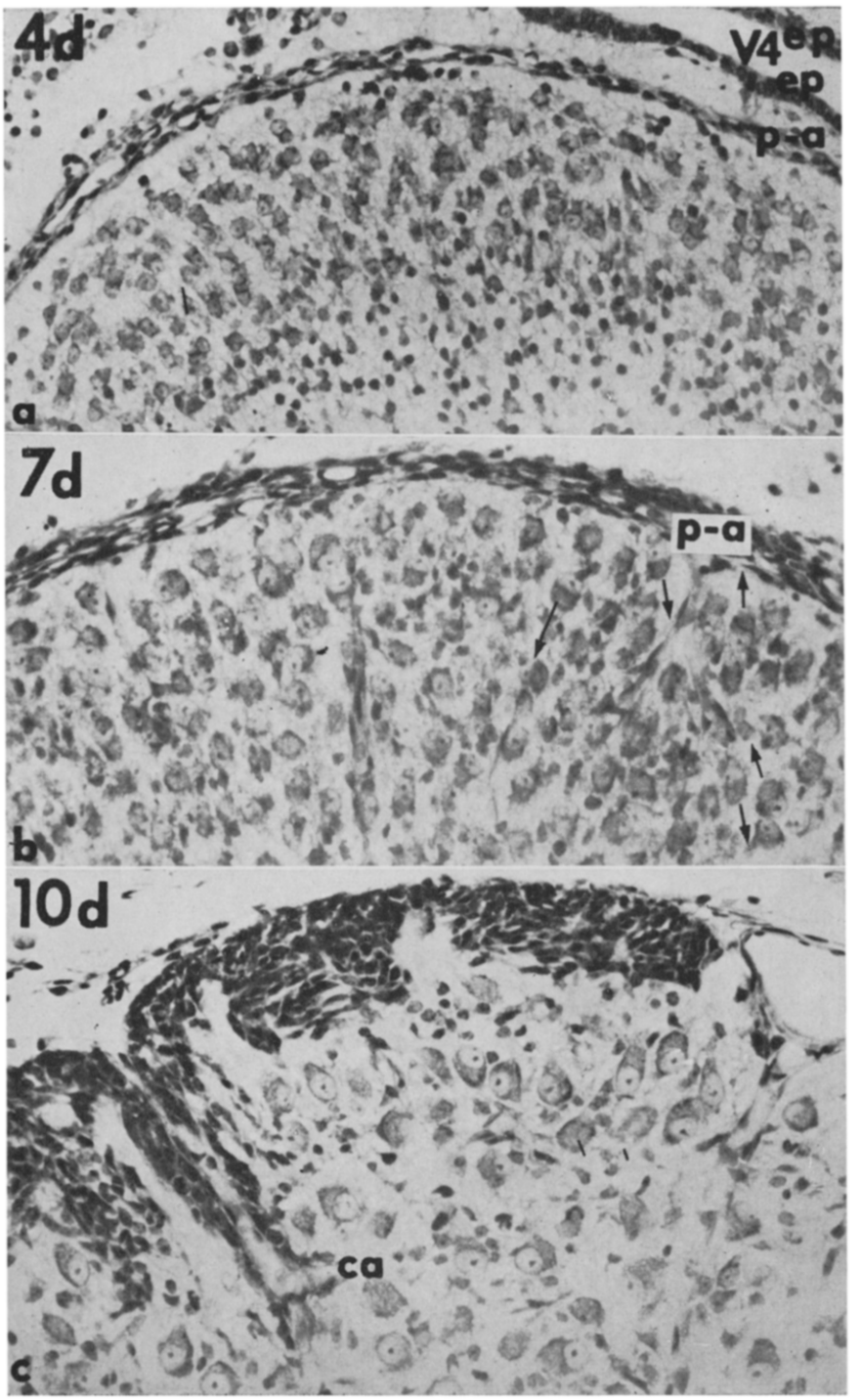
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more bizarre in appearance (with islands or rosettes, and its cells haphazardly oriented) in the anterior than in the posterior vermis. The molecular and internal granular layers showed incipient signs of development in uvula and pyramis; elsewhere, the differentiation of the reconstituted cells of the external granular layer has not begun. Throughout the vermis, Purkinje cells formed a several cell thick zone and their conspicuous apical cones were randomly oriented, except in the maturing uvula and parts of the pyramis where the majority of Purkinje cells were normally oriented.

Effects of 8 × 200 r. In contrast to the relatively consistent pattern of destruction and recovery seen with fewer doses of 200 r, considerable variability was seen among the animals that were exposed to 200 r from day 0 to 7. This variability is attributed to increased probability of sparing of the posterior cerebellum (particularly in bigger animals with larger skulls) as a result of our use of a constant width (6 mm) x-ray beam.

In one animal of this group that survived for 2 hours after the last exposure, the external granular layer was absent over the entire vermis. In this animal there were no isolated cells seen resembling those of the external granular layer and essentially the entire cerebellar cortex was composed of large, densely packed Purkinje cells. In contrast, in two other animals the external granular layer was present over the pyramis and uvula, and at these sites the molecular and internal granular layers were developing, indicating that these posterior lobules of the vermis were spared for several days. Likewise, in the group that survived for 24 hours after the first exposure (8 days of age), in one rat the external granular layer was gone over the entire vermis, while in two others the external granular layer, together with developing molecular and internal granular layers, were present in the posterior lobules. This variability among animals was also characteristic of the 10-day-old animals. In two rats a semi-normal looking external granular layer was present over several of the lobules of the posterior vermis, and there was a developing molecular layer (studded with migratory cells) and a recognizable internal granular layer. In these animals a bizarre external granular layer was present (thick, corrugated and with randomly oriented cells) in the adjacent lobules in the rostral direction (tuber and culmen).

FIG. 3. High-power photomicrographs of lobulus centralis from rats irradiated with 200 r on days 0, 1, 2, and 3 and killed at the age of *a*, 4 days, *b*, 7 days, and *c*, 10 days. External granular layer is absent as a continuous sheet in *a* and *b*, although a few cells belonging to this proliferative are present below the pia-arachnoid membrane (p-a). Note in *b* and *c* the random orientation of the apical cones of Purkinje cells (arrows). Note corrugated appearance of reconstituted external granular layer in *c*, also the invasion of its cells along a capillary (ca). The absence of a molecular layer with migratory cells, and of an internal granular layer, indicates that the differentiation of the reconstituted cells of the external granular layer has not begun. Other abbreviations: ep, ependyma; V4, cerebellar recess of fourth ventricle. Cresyl violet, ×640.

and here the molecular and internal granular layers were absent. In another animal there was an apparently recently regenerated, bizarre external granular layer over uvula and pyramis, but there was neither an external granular layer more rostrally, nor an internal granular layer, and the anterior lobe was composed almost exclusively of Purkinje cells (Fig. 4). In the animals killed 4 days after the last irradiation (11 days of age), the external granular layer was semi-normal over the posterior vermian lobules, it was bizarre but thick and continuous in the lobules around fissura prima, and it was present in fragmented patches over the anterior vermis. The invasion of the more affected anterior vermis by cells from the posterior vermis was suggested by this gradient in recovery and also by the presence of superficial spindle-shaped cells, apparently in motion between recovered and not yet recovered regions.

Effects of 10 × 200 r. The variability observed in the previous group was also characteristic of the animals that were exposed to 200 r on days 0–9. In all animals that survived for 2 and 24 hours after the last irradiation, the external granular layer (including isolated cells) was totally eradi-

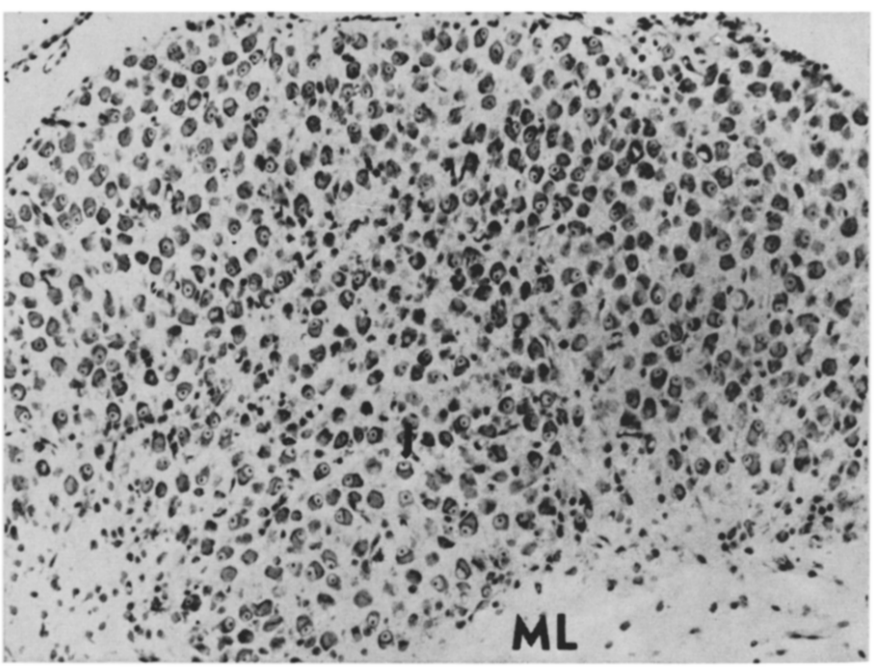


FIG. 4. Photomicrograph of lobulus centralis from a rat irradiated with doses of 200 r on days 0–7 and killed when 10 days old. Note the absence of external granular, molecular, and internal granular layers; the entire lobule is composed essentially of unaffected Purkinje cells. ML, medullary layer. Cresyl violet, ×101.

cated over the anterior vermis. However, a near-normal looking external granular layer was present in some of the animals over a few of the lobules in the posterior cerebellum with maturing molecular and internal granular layers, indicating that sparing of these regions must have gone on for several days. In two animals numerous pyknotic cells were seen in the external granular layer in these regions, suggesting that the sparing of this proliferative matrix at this age is not attributable to changed radio-sensitivity (development of greater radio-resistance with age of the animal in areas where cells have survived) but rather to inadequate exposure to x-ray. In other animals the external granular layer was gone over the entire vermis. In the animals that lived for 4 days after the last irradiation (13 days of age) a partially abnormal, recently reconstituted external granular layer was seen over nodulus, uvula, pyramis, tuber and declive except in uvula, the molecular and internal granular layers were absent. Over the lobus centralis ventralis and lingula the external granular layer was still missing. Where this layer was present, it was typically bizarre in appearance with its cells oriented in all directions and apparently penetrating into all available spaces. Most striking was the invasion along perivascular spaces into the interior of the cerebellum, which accounts for the numerous fingerlike intrusions by the external granular layer seen in these animals. The task of accommodating these profusely proliferating cells over the surface of the relatively small, undeveloped cerebellum may be the major cause of the excessively wrinkled and folded appearance of the cerebellar cortex in these animals.

Results

Because of the considerable sparing of the external granular layer in many animals in the posterior lobules of the vermis (particularly after 8–10 $\times$ 200 r), quantitative analyses were restricted to the more consistently affected anterior lobe in the groups exposed to 1–5 $\times$ 200 r. The first analysis was concerned with changes in the thickness of the external granular layer at different periods after the last irradiation. The "cell thickness" of this layer over a lobule was established by counting (at 625$\times$ magnification) the number of cells bisected by an ocular grid line oriented perpendicularly to the surface of the cortex. In every lobule, 20 random measurements were made and the means were tabulated. The two lobules particularly selected were culmen dorsalis, near the anterior border of fissura prima, and the more rostrally situated lobus centralis dorsalis. In these two lobules the mean cell thickness between 0–10 days was determined for the control and irradiated animals (Figs. 5a, b). For the latter group, the data were plotted to indicate, in addition to the age of the animals, the number of daily exposures and survival period after the last irradiation.

These data indicate unequivocally that following decimation of the

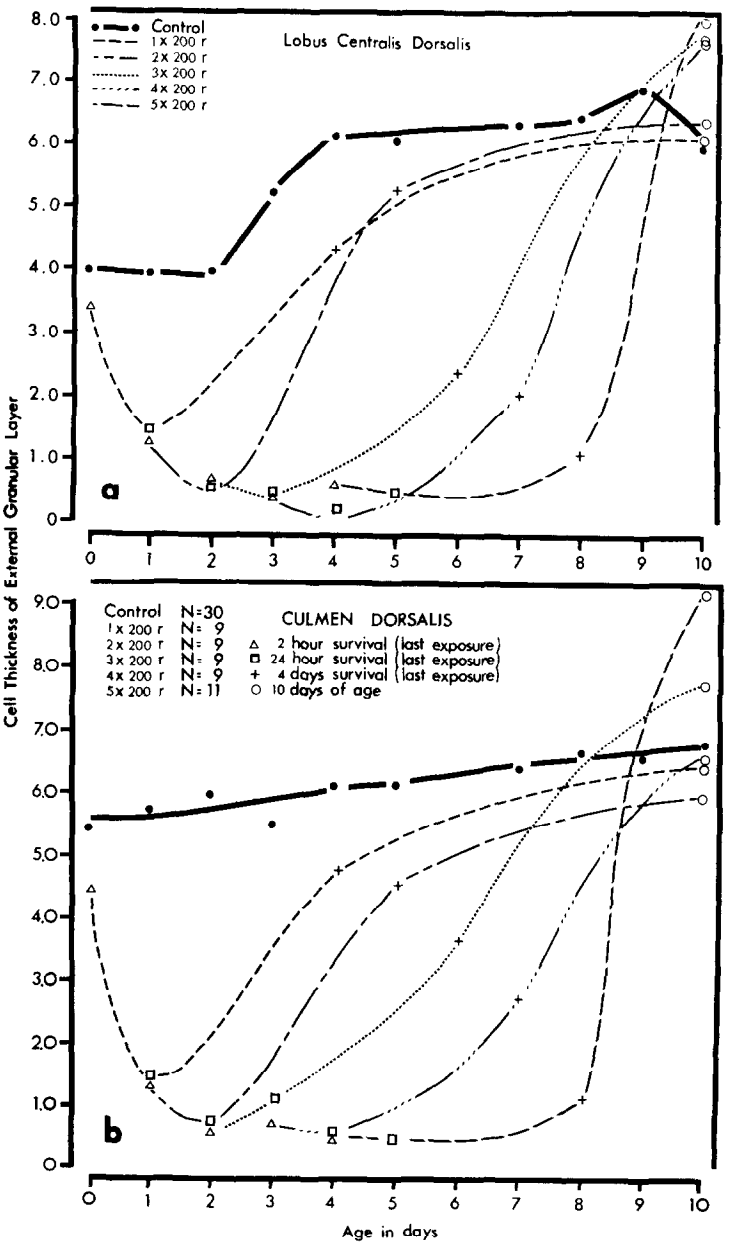


FIG. 5. Cell thickness of external granular layer in lobulus centralis (dorsalis) and culmen dorsalis as a function of number of daily exposures, age, and survival period after last irradiation.

external granular layer with 1–5 × 200 r, there is always a recovery of this proliferative matrix by 10 days of age (though this recovery is not as complete as a superficial interpretation of Fig. 5 would indicate; see below). With regard to the differential consequences of the different radiation schedules used the following results emerged: (*i*) Reduction in the thickness of the external granular layer was minimal 2 hours after irradiation. This was indicated, first, by slight decreases in the two lobules in the animals exposed to a single dose (1 × 200 r) when compared with unirradiated controls; second, by the fact that the thickness of the external granular layer in animals that received one additional daily dose of 200 r but lived only for 2 hours after the last exposure, was comparable to that obtained in animals that received one less daily exposure but lived for 24 hours. This result is reconcilable with our present qualitative and earlier quantitative observation (5) that cell pyknosis does not become evident within 2 hours after irradiation. (*ii*) Reduction in cell thickness was pronounced 24 hours after irradiation with a single dose and was maximal 24 hours after last irradiation with two successive daily doses of 200 r, in the sense that additional daily exposures did not further reduce the cell thickness (which was less than 1). In this context we may recall our previous observations that there was a reduction in the number of scattered, apparently radio-resistant small, dark cells as a function of additional number of daily exposures to 200 r. (*iii*) The major effect of additional daily exposures of the cerebellum, as revealed by these quantitative data, was the interference with or postponement of recovery of the external granular layer. While in all groups there was a recovery by the fourth day after the last irradiation, the extent of recovery in terms of cell thickness in the two lobules (with one exception) was an inverse function of the number of daily irradiations. (*iv*) Once recovery has commenced, the cell thickness of the external granular layer increased suddenly and it reached the normal level, or went beyond it, by 10 days of age in all groups. The greater width of the layer in most animals that received 3–5 × 200 r might partly reflect compensatory growth but we must take into account the fact that the area of the cerebellum is greatly reduced as a function of increased number of daily irradiations (mainly because the development of the molecular and internal granular layers is prevented) with a consequent reduction in the surface area of the cortex (Fig. 6). The great reduction in the total area occupied by the external granular layer as a function of different irradiation schedules is indicated by the planimetric determinations summarized in Fig. 7.

In this evaluation, tracings were made at 65× magnification of the cerebellar cortex in matched sagittal sections (cut at about 0.9 mm from the midline) and the area occupied by the external granular layer was deter-

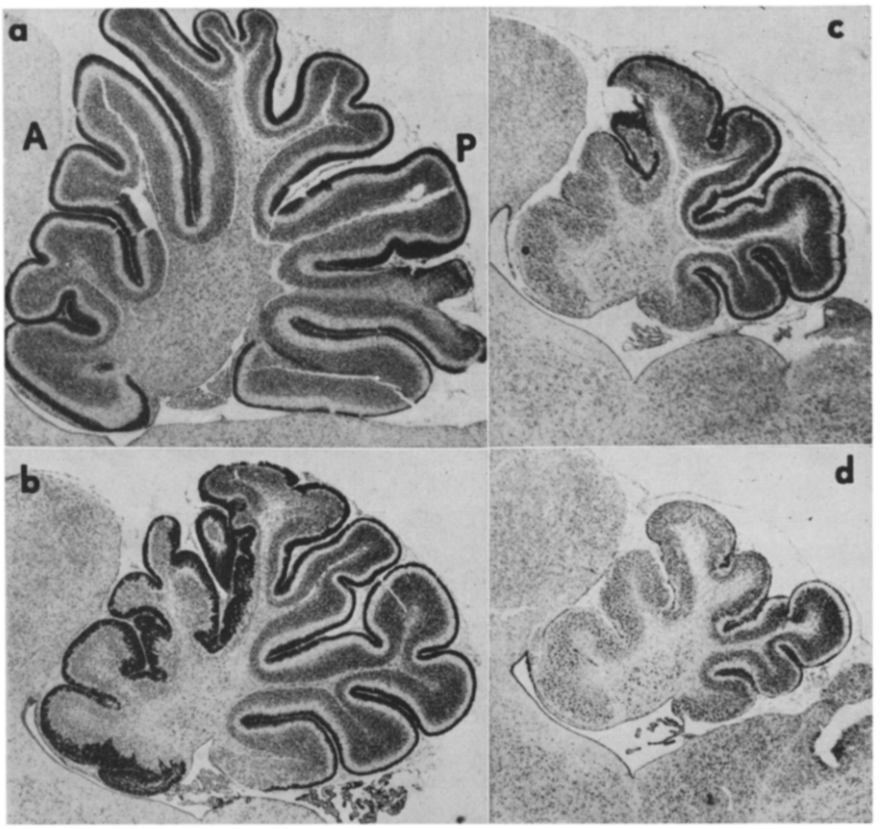


FIG. 6. Low-power photomicrographs of the cerebellar vermis from 10-day-old rats with the following antecedent irradiation history: *a*, 1 × 200 r; *b*, 5 × 200 r; *c*, 8 × 200 r; *d*, 10 × 200 r. Note typical gradient in retardation of cerebellar development from the posterior (P) to anterior (A) lobules. Vermis nearly normal in *a*. The posterior lobe is nearly normal in *b*, with a bizarre external granular layer and no internal granular layer in the anterior lobe. In *c*, with only 3 days for recovery after last exposure, the external granular layer is still absent over the anterior lobe. In *d*, with 24-hours recovery period after the last exposure, external granular layer is absent over the vermis, excepting as a broken, thin sheet in the most posterior lobules together with an ill-developed internal granular layer. The retardation in the areal development of the cerebellum is obvious. ×17.

mined with a compensating planimeter. The reduction in the area occupied by the external granular layer (notwithstanding its greater thickness) is indicated for the animals irradiated with 2–5 × 200 r, which is due to the reduced area of the cerebellar cortex as a whole. Retardation in the growth of the cerebellar cortex is indicated by the measurement of the width of the molecular layer (in a manner similar to that described earlier for measuring

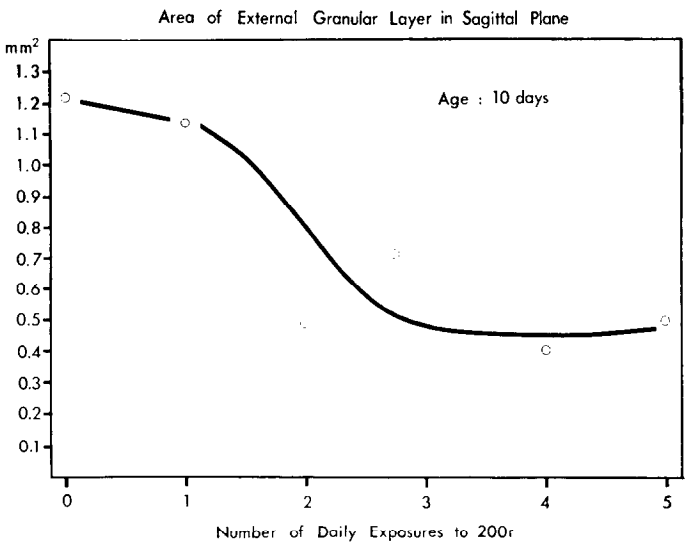


FIG. 7. Planimetric determinations of the area occupied by the external granular layer in matched sagittal sections in 10-day-old rats as a function of irradiation history.

the cell thickness of the external granular layer), as summarized in Fig. 8. This effect is a very drastic one, affecting even the animals that were exposed to only one or two doses at 0 and 1 days of age.

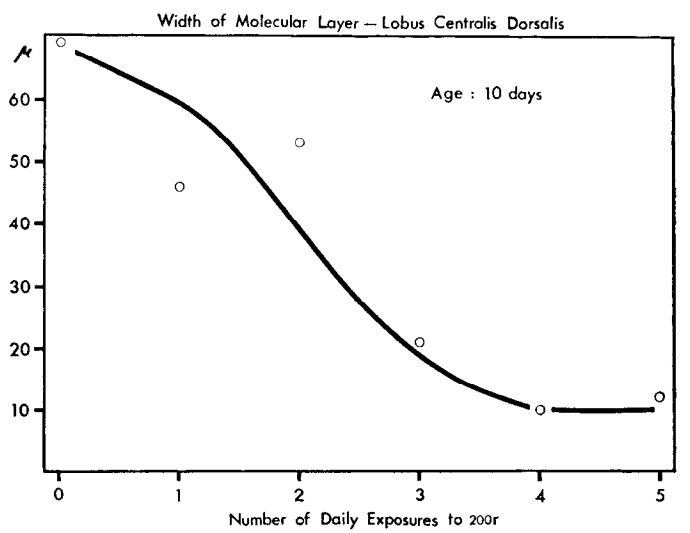


FIG. 8. Planimetric determination of the width of the molecular layer in lobulus centralis in 10-day-old rats as a function of irradiation history.

Discussion

The results of this study confirm and extend our earlier finding (5) that after exposure of the cerebellum to low-level radiation and drastic reduction of the cell population of the external granular layer this proliferative matrix has the capacity to reconstitute itself. We were led to study the possibility of *developmental reparation* of the external granular layer by two, seemingly contradictory, earlier observations; namely, that exposure of infant rats to a singe dose of 200 r x-ray destroyed within a day an appreciable proportion of the cells of the external granular layer (4) while at 30 and 90 days of age there were no indications of retardation in cerebellar growth in rats that were irradiated with one or two such doses during infancy (3). Because the present study was limited to early effects of irradiation and to recovery occurring within a few days after irradiation, the question will remain open as to what extent the reconstitution of the external granular layer observed here leads to the actual production of basket, stellate, and granule cells. We did observe the formation of an internal granular layer and of a molecular layer in animals that were irradiated with a few daily doses and in which sufficient time was available for cellular differentiation by the time they were killed at 10 days of age. Also, our recent normative studies indicate that few cells of the external granular layer differentiate during the first week of life, and that the bulk of granule cells and all the stellate cells are formed after the eleventh day (1). Hence, reconstitution of the external granular layer during the first week of life could conceivably lead to the development of a normal cerebellar cortex if two conditions were satisfied: (*i*) That reconstitution represented not only a process of recovery but a compensatory production of sufficient primitive cells to replace the lost ones; (*ii*) if no drastic changes were wrought in the architecture of the cerebellum during the period when its cell population was decimated by irradiation. Our data indicated that except in the group that received a single dose, the area occupied by (that is, the cell population of) the external granular layer was lower at 10 days of age in the irradiated rats than in normal controls, suggesting recovery with higher doses but no compensatory growth. Secondly, as we shall describe in greater detail below, the cell losses produced by several doses of x-ray does radically alter cerebellar architecture, and this could conceivably prevent the development of normal structural organization.

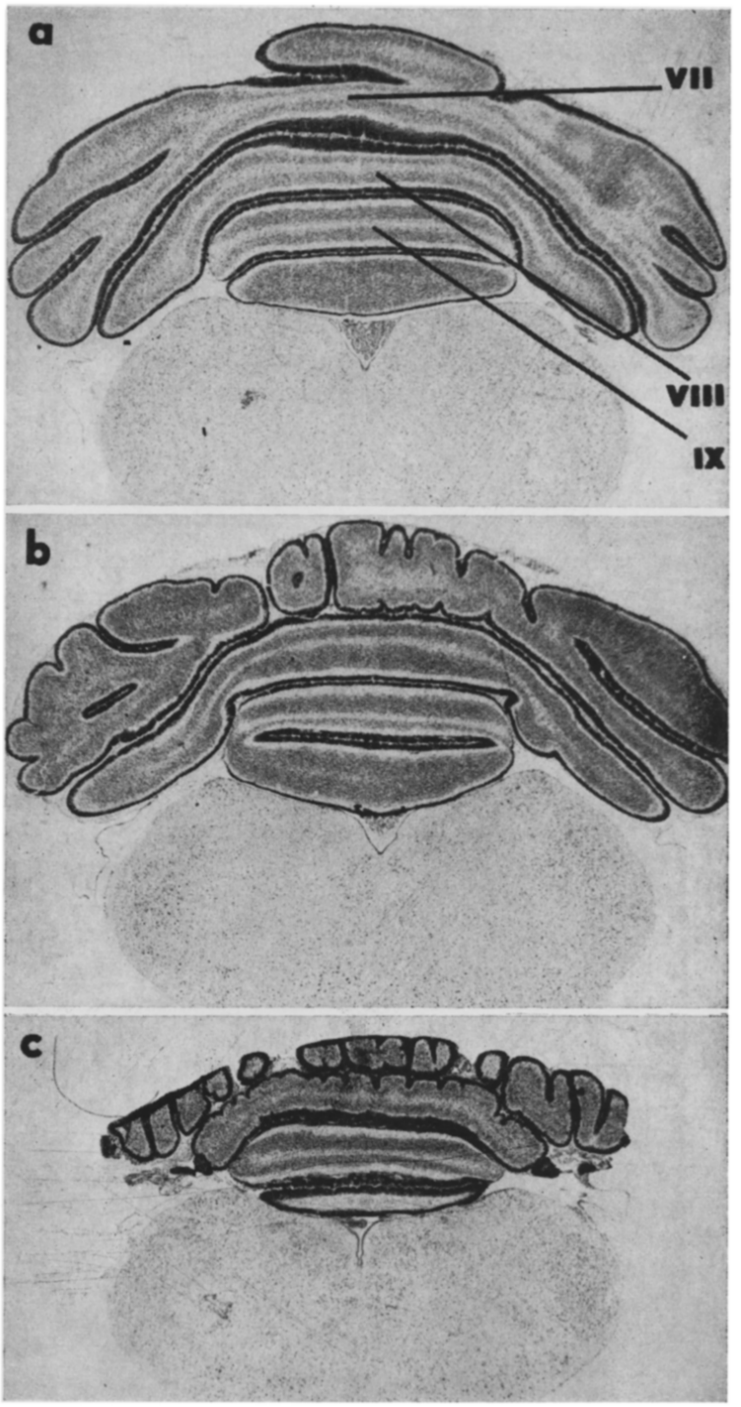
Our data indicate that 24 hours after exposure to a single dose of 200 r, the width of the external granular layer was reduced to one-fourth of its normal size and that destruction was maximal 24 hours after two successive doses. Additional exposures did not appear to produce more direct damage; only a few cells survived and these may have been the most

radio-resistant ones. Rather, additional doses prevented the recovery of the external granular layer and, in addition, also delayed its recovery beyond the days of irradiation. The latter claim is based on the observation that the degree of recovery of the external granular layer 4 days after the last irradiation was inversely related to the number of preceding exposures, with practically no recovery seen during this period in the animals that received 5 × 200 r. One possible explanation of this finding is that even the surviving radio-resistant cells succumbed to irradiation with increased daily exposures and fewer cells retained the capacity to initiate reconstitution of the proliferative matrix.

The assumption that we were making was that reconstitution of the external granular layer was dependent on surviving stem cells (the single, small and dark cells that we saw scattered over the layer of Purkinje cells in even the most affected regions). Another possibility is that reconstitution readily occurred in our material because in almost all instances we had some sparing of the external granular layer in the posterior lobe, and that cells migrated from the less to the more affected lobules. Our observation of a caudorostral gradient in recovery (not only of the external granular layer but also of the molecular and internal granular layers) is reconcilable with such an assumption, but does not prove it, because these observed gradients may have been the direct effects of a physical gradient in exposure to x-ray. To resolve this problem we will have to analyze material in which we had uniform distribution of x-ray over the entire cerebellum.

With increased number of daily exposures the structure of the cerebellar cortex became more and more bizarre. Several factors may be responsible for this. Two may be singled out: the distortion produced in the formation of folia by the rapid growth of the external granular layer after its recovery; and the autonomous growth of Purkinje cells in abnormal locations and directions.

A striking feature of the recovering external granular layer was that it was not forming a smooth sheet over the surface of the cerebellar lobules but was of variable thickness and highly folded, with finger-like processes ("rosettes") penetrating into the substance of the cerebellum. This is illustrated in Fig. 9 in coronal sections from 8-day-old animals that received different number of daily doses of 200 r. This abnormal folding, and the penetration of cells of the external granular layer into all available spaces, is most easily explained if we assume that this recovered matrix tends to produce a cell population that is typical of animals of that age, which now has to be accommodated over the surface of a cerebellum which is greatly reduced in size due to the continued destruction on previous days of a large proportion of its newly formed cell population.


a
VII
VIII
IX
b
c

Another factor to be considered here is the autonomous growth of Purkinje cells during and following irradiation. As we established earlier (2), exposure to 200 r does not visibly harm the prenatally formed (though undeveloped) Purkinje cells. We have now quantitative evidence (to be published) that even after ten successive daily doses of 200 r the number of Purkinje cells remains the same in adolescent and adult rats as that obtained in normal animals. However, the Purkinje cells in irradiated animals do display several developmental abnormalities. In normal infant rats the undeveloped Purkinje cells form a several cell thick zone below the external granular layer. As development proceeds, the Purkinje cells get strung out to form a row composed of single cells, with some spacing between cells. This characteristic distribution is attributable to the growth of the rich dendritic network of Purkinje cells which do not overlap and therefore force the cells further and further apart. The growth of this dendritic network is related, among others, to the establishment of synaptic contacts with the parallel fibers of differentiating granule cells. Because the granule cells do not develop during and immediately after the period of irradiation, the dendritic system of Purkinje cells presumably fails to develop. Our data indicate that, notwithstanding the absence of granule cells, the soma of Purkinje cells grow at the same rate in irradiated as in normal animals, displaying autonomy in this respect, with the only difference that they do not get strung out and that their apical cones grow randomly with respect to the surface of the lobules. Golgi material indicates (to be published) that this random orientation of the apical cones is associated with the disoriented growth of the main shaft of Purkinje cell dendrites which become extended and are moving in all directions, as if in search for synaptic contact with occasional granule cells present. The abnormal foliation produced by the rapidly growing external granular layer over the retarded cerebellum, the massed position of Purkinje cells beyond the normal period of development, and the abnormal pattern of growth of their dendrites, thus lead to an abnormal structural growth of the cerebellar cortex.

In summary, the results obtained in this study showed that after subtotal destruction of the external granular layer with low-level x-ray, this matrix reconstitutes itself. Evidence for postnatal developmental reparation in the brains of mammals, to our knowledge, has not been available before, although it was claimed to occur during embryonic development (6, 8). In the available material we could not determine whether occasional surviving cells in affected regions, or remnants of the external granular layer in less

FIG. 9. Low-power photomicrographs of coronal sections through posterior cerebellum of 8-day-old rats with the following irradiation history: *a*, 1 × 200 r; *b*, 3 × 200 r; *c*, 4 × 200 r. Cresyl violet. ×13.

affected regions, are responsible for recovery of the external granular layer over the entire surface of the cerebellum which was seen in animals exposed up to 5 × 200 r. We obtained some evidence that the reconstitution of the germinal cells is followed by cellular differentiation and formation of molecular and internal granular layers. In animals that were exposed to three daily doses of 200 r or more there was increasing abnormality in the structure of the cerebellar cortex. To what extent such structural abnormalities are associated with functional abnormalities remain to be determined. Also the variables thay may be controlling cerebellar recovery (such as exercise) deserve to be investigated.

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