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Cartilaginous Metaplasia and Overgrowth of Neurocranium Skull After X-Irradiation in Utero*

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Summary. Prenatal X-irradiation of mice in the late organogenesis stage either with a fractionated or a single exposure dose (3×160 R or 200 R) leads to remarkable, previously undescribed malformations of the skull. These malformations range from mild hyperostotic nodule formation in about 90% of the offspring to excessive formation of desmal bony tissues, which extend deep into the forebrain and are thus only detectable in histological sections. Metaplastic and hyperplastic formation of cartilage in all the neurocranial regions is observed in about 10% of the offspring. The pathogenesis of these overgrowth phenomena is presumably related to a growth disturbance of both the mesenchymal skull primordium and the brain. While malformation of the latter leads to a decrease of intracranial pressure and consequently to altered growth activity of the skull sutures, the reparative and proliferative capacities of the mesenchyme are also stimulated, in a hyperplastic direction, by X-irradiation.

Key words: Overgrowth – X-irradiation – In utero.

Introduction

In the past numerous investigators have been concerned with the description of skeletal abnormalities produced following prenatal X-irradiation. These studies were performed mainly in mice and rats (Russell, 1950; Levy et al., 1953; Kriegel et al., 1962; Murakami et al., 1962, 1963), and were evaluated macroscopically by staining the fetal skeleton with alizarin red S. This method allows the quantitation of teratological effects in a large number of fetuses and thus provides distinct dose/response correlations. Russell (1950) was the

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first investigator to determine the whole spectrum of skeletal malformations in detail and reported on how different malformation patterns depend on the time of prenatal X-irradiation (phase specificity). Malformations of the head in the mouse can be induced over an extensive period of gestation – between day 6 post conception (p.c.) and day 14 p.c. – and include numerous anomalies of the nose, snout, palate, pharynx, and also of the dorsal regions of the skull. (Russell, 1950; Kriegel et al., 1962; Dagg, 1964; Murakami et al., 1963; Dekaban, 1969; Nash, 1971). The basis of all these anomalies is the occurrence of hypoplasia or aplasia of the sensitive tissues in response to the prenatal irradiation injury.

We have been concerned mainly with the developmental pathology of the CNS after fractionated or single-exposure X-irradiation at different gestational stages in the mouse (Schmahl et al., 1977, 1979). As the development of the brain and the skull are two interrelated events (Jordaan, 1976), we extended our investigations to simultaneous studies of CNS and neurocranial malformations. By combining macroscopic observations with histological studies we detected not only malformations reducing skull size but also a considerable number of newborn with anomalies of the neurocranial skull, indicating an excessive proliferation of the primordial mesenchyme throughout the gestation period in response to the radiation insult.

Material and Methods

Female, virgin, 8-week-old NMRI mice were mated between 8 a.m. and 10 a.m. and subsequently examined for vaginal plugs. The next morning was regarded as day 1 post conception (p.c.). The animals received standard diet (Altromin) and water ad libitum and were housed in single cages in air-conditioned animal rooms at 23° C with artificial light from 6 a.m. to 6 p.m. Irradiation of the pregnant mice was effected by a therapeutic X-ray unit at 180 kV and 15 mA and a dose rate of 1 R/min. We used an 0.3 mm thick copper plate as a filter. The distance between the animals and the X-ray unit was 40 cm in all cases. For irradiation purposes, five pregnant animals were caged together in a round, flat plastic restrainer. The radiation dose applied was registered by a Victoreen dosimeter at a central hole in this restrainer. The dams came to autopsy on day 18 p.c. The number of dams used in the various groups at different irradiation times, and the number of offspring observed, are shown in Table 1. The number of implantations, resorptions and macerations was counted and the weight of the fetuses and placentas determined. The fetuses were fixed in 70% ethanol for subsequent alizarin red S-staining according to Dawson (1926). From each dose group we took another 30 fetuses at random for histological serial sectioning of the whole head at 6 µm and subsequent staining of the sections either with hematoxylin and eosin or with alizarin red S.

Results

1. Macroscopic Findings

The number of implantations per animal, the percentage of live and dead fetuses, macerations and resorptions, as well as the fetal and placental weights are listed in Table 1.

Table 1. Macroscopic findings at irradiated animals and controls at day 18 p.c.

Group No	Day p.c. of irradiation	Dose (R)	Dams of group	Implantations %	Fetuses alive		Dead fetuses %	Macerations %	Resorptions %	Fetal weights (g)	Placental weights (g)
					Nos	%					
1	Control a	–	10	13.3	138	91.5	0	0	8.5	1.31 ± 0.11	0.0877 ± 0.01
2	11–13	3×80	13	14.1	177	91.8	0	0	8.2	1.05 ± 0.11[a]	0.0771 ± 0.01[a]
3	11–13	3×120	17	12.3	181	86.8	0.5	3.3	9.6	0.81 ± 0.10[a]	0.0726 ± 0.01[a]
4	11–13	3×160	14	12.7	138	77.1	1.7	6.2	15.0	0.64 ± 0.09[a]	0.0662 ± 0.01[a]
5	14–16	3×80	18	12.3	200	90.5	0	0.5	9.0	1.20 ± 0.10[a]	0.0875 ± 0.01 n.s.
6	14–16	3×120	14	12.6	168	94.9	0	0.6	4.5	1.15 ± 0.11[a]	0.0794 ± 0.01[a]
7	14–16	3×160	13	13.0	158	93.5	0	0.6	5.9	1.05 ± 0.11[a]	0.0769 ± 0.01[a]
8	11–16	6×80	14	13.2	174	93.5	0.5	0	5.9	0.92 ± 0.11[a]	0.0774 ± 0.01[a]
9	11–16	6×120	14	13.2	164	90.1	0	2.6	7.3	0.66 ± 0.11[a]	0.0683 ± 0.01[a]
10	11–16	6×160	13	12.0	97	62.2	2.6	16.0	19.2	0.54 ± 0.07[a]	0.0639 ± 0.01[a]
1	Control b	–	33	13.6	398	92.0	0	0	8.0	1.32 ± 0.10	0.0889 ± 0.01
11a)	12	200	14	13.1	171	91.4	0.23	0.23	8.12	0.91 ± 0.10[a]	0.0975 ± 0.01[a]
11b)	12	200	19	12.9	223	91.2	0.17	0.52	8.11	0.94 ± 0.11[a]	0.0892 ± 0.01[a]

[a] Significance level against controls: $P < 0.0001$

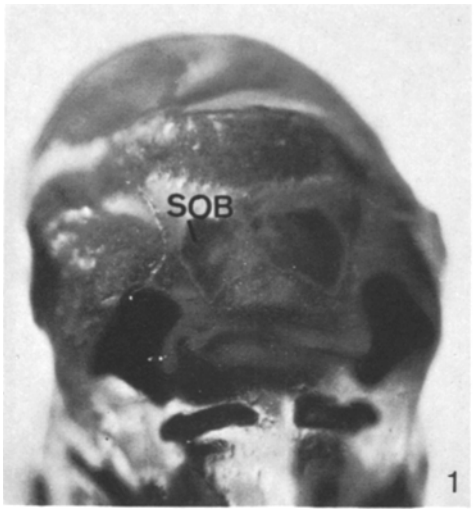
Table 2. Abnormalities of the *supraoccipital* bone at irradiated animals and controls at day 18 p.c.

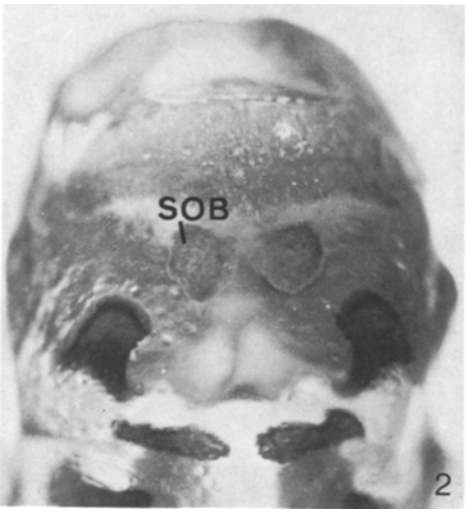
Group No	Nos. observed	Day p.c. of irradiation	Dose (R)	Macroscopic findings (%)			Microscopic findings (%)	
				rudimentary	incomplete closure	additional ossification center	abnormal chondrification	hyperplastic osteoid nodules
1	138	Controls	–	0	0	0	0	0
2	177	11–13	3× 80	0	0	0	0	0
3	181	11–13	3×120	0.6	3.6	0	0	23.3
4	138	11–13	3×160	1.5	26.3	6.6	10.0	93.3
5	200	14–16	3× 80	0	0	0	0	0
6	168	14–16	3×120	0	0	0	0	6.6
7	158	14–16	3×160	0	0	0	0	0
8	174	11–16	6× 80	0	1.2	0	0	0
9	164	11–16	6×120	1.5	0.8	0	0	6.6
10	97	11–16	6×160	1.1	15.6	3.1	0	13.2
11	171	12	200	1.1	12.4	4.8	13.2	86.6

The fetal weight reduction was less pronounced after fractionated X-irradiation during the fetal period (days 14–16 p.c.), more severe after X-irradiation in the late organogenetic period (days 11–13 p.c.), and most drastic (> 50%) after X-irradiation between days 11–16 p.c. The degree of fetal weight reduction showed a clear correlation with the dose applied within a distinct developmental period. A similar tendency can also be shown with regard to the placental weights, although these differences were less pronounced than the fetal weight differences.

The malformations of the supraoccipital bone are listed in Table 2. We have confined ourselves here to this particular region; the complete malformation pattern of the skeleton is reported in detail elsewhere (Meyer, 1979).

In the control animals there was distinct communication between the bilateral major ossification centres of the supraoccipital bone, giving a butterfly-like appearance (Fig. 1). This bridge was lacking in 3.6% of the fetuses in group 3 (3 × 120 R on days 11–13 p.c.) (Fig. 2), and in 26.3% of the group 4 fetuses (3 × 160 R on day 11–13 p.c.). In this latter group an additional ossification centre was observed in 6.6% of the alizarin red-stained fetuses (Fig. 3). Similar results were obtained by irradiation with 200 R on day 12 p.c. which caused an incomplete closure of the supraoccipital bone anlage in 12.4% and an additional ossification centre in 4.8% of the animals observed. Irradiation in the early fetal period (days 14–16 p.c.) did not influence the macroscopic development of the neurocranium, irrespective of the dose applied. Furthermore, irradiation in the early fetal period showed no enhancement of the effects observed after irradiation during late organogenesis, as was shown by the malformation frequency of groups 8–10. In fact, there was a decline in both the incidence





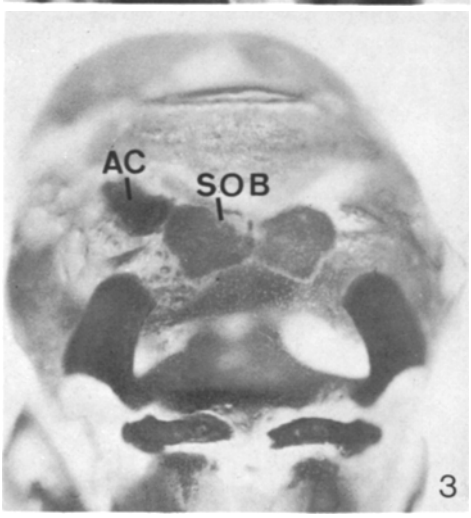


Fig. 1. Occipital view of the head of a control fetus at day 18 p.c. with large conjoint ossification centres of the supraoccipital bone (*SOB*). Alizarin stain, 12×

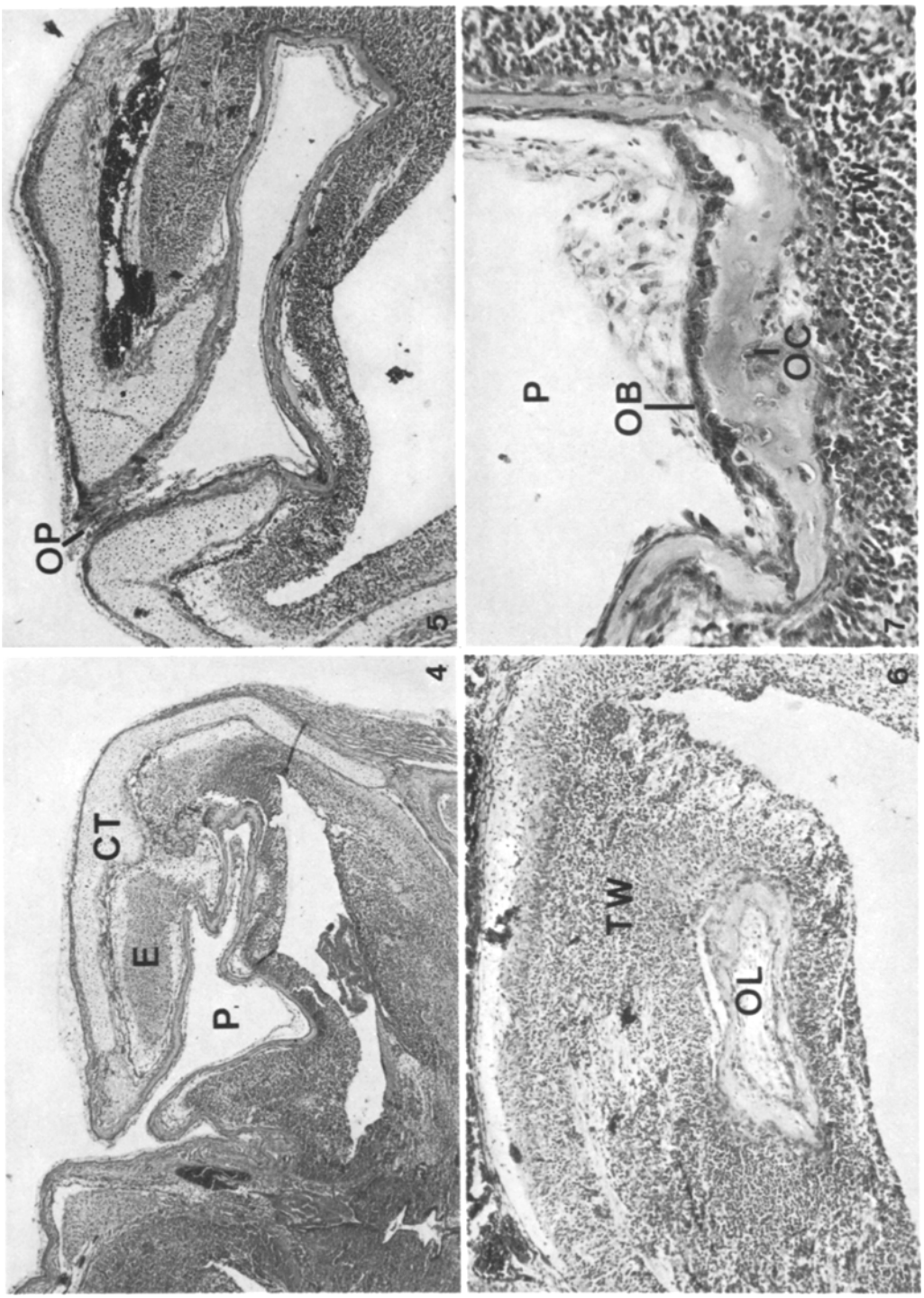
Fig. 2. Occipital head region of a fetus (day 18 p.c.) irradiated with 3×120 R at days 11–13 p.c. (group 3). The supraoccipital ossification centres (*SOB*) are smaller than in controls and devoid of a midline communication. Alizarin stain, 12×

Fig. 3. Occipital view of the head from a fetus at day 18 p.c. from the irradiation group 4 (3×160 R at days 11–13 p.c.) with only a small bridge between the ossification centres of the supraoccipital bone (*SOB*). An additional ossification centre (*AC*) appears parietolaterally from the left genuine supraoccipital anlage. Alizarin stain, 12×

of incomplete closure and in the appearance of an additional ossification centre in the group 10 animals, in comparison to the effects in the group 4 animals.

2. Microscopic Findings

The most outstanding feature of abnormal skull development in X-irradiated fetal mice is a heterotopic chondrification of the neurocranial capsule, which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will be described in detail later. This anomaly is seen only after application of 3×160 R on days 11–13 p.c. (group 4: 10%) or after 1×200 R on day 12 p.c. (group 11: 13.2%). Another remarkable anomaly is the occurrence of small osseous nodules of the neurocranium which are completely concealed by the overlying skin (see also the description below). These nodules are very frequent after 3×120 R or 3×160 R irradiation exposure in late organogenesis (group 3: 23.3%; group 4: 93.3%) as well as after single exposure with 200 R on day 12 p.c. (group 11: 86.6%). They seldom occur after early fetal X-irradiation (group 6: 6.6%) or after X-irradiation between days 11 and 16 p.c. (group 9:6.6%; group 10:13.2%). Abnormal chondrification is manifest by a neurocranial capsule consisting a mass of cartilaginous tissue instead of the individual squamosal, parietal, interparietal, and occipital bones. Apart from this there is fusion of the peri-otic cartilage with the cartilaginous tissue of the occipital area. This malformation complex occurred in association with completely unaltered development of the other cranial and facial bones. The cartilaginous structures extend circumferentially from both the sphenoids to the midline of the head, ending a small distance in front of the sagittal suture (Fig. 4). Marked perichondral bone formation, originating from the outer perichondrium of the outmost medial cartilage, then appears in close continuity. These bony lamellae have a very large extension into the region of the median suture (parieto-parietal suture). Usually, the extension is in both a basolateral and rostral direction towards the brain cortical surface, leading to a severe compression and displacement of the telencephalon. This protrusion originates from around the supra-optic region and extends to the level of the fronto-parietal suture approximately. The most laterally-oriented part of this cranial vault protrusion is closely connected in one instance with a prominent thickening of a laterally-situated cartilage district (Fig. 4 "CT"). The most dorso-medio-occipital parts of the telencephalon thus appear to be somehow "evaginated" in a manner similar to aneurysms (see Fig. 4 "E"). This all occurs, however, within a head shape which, is generally unaltered.

Fig. 4. Coronal section of the head of a newborn mouse through the zygomatic region. The neurocranium consists in its lateral and dorsal parts of an uninterrupted cartilage with prominent thickening (*CT*) in the dorsolateral district. From the midline area a marked perichondral bony protrusion (*P*) extends into the brain in a lateral direction, the most dorsomedial parts of the brain thereby having a rattle off-appearance (*E*). H.E., $31{,}5\times$

Fig. 5. Osseous protrusion into the brain originating from the dorsolateral region. The hyperplastic cartilaginous capsule shows an opening (porus=*OP*) into the skull invagination. H.E., $40{,}5\times$

Fig. 6. Coronal section of the head of a newborn mouse through the eye region. There is a marked extension of the dorsal telencephalic wall (*TW*) with an "ectopic" osseous double lamella (*OL*). Note the completely irregular arrangement of the neuronal cell layers. H.E., $165\times$

Fig. 7. Protrusion area (*P*) into the dorsal telencephalic wall (*TW*) of a newborn mouse. Note the broad lining of osteoblasts (*OB*) at the inner surface of the protrusion, the marked and irregular thickening of the bony lamella and also the lack of osteoclastic cells (*OC*) at the outer surface of the protrusion. H.E., $262{,}5\times$

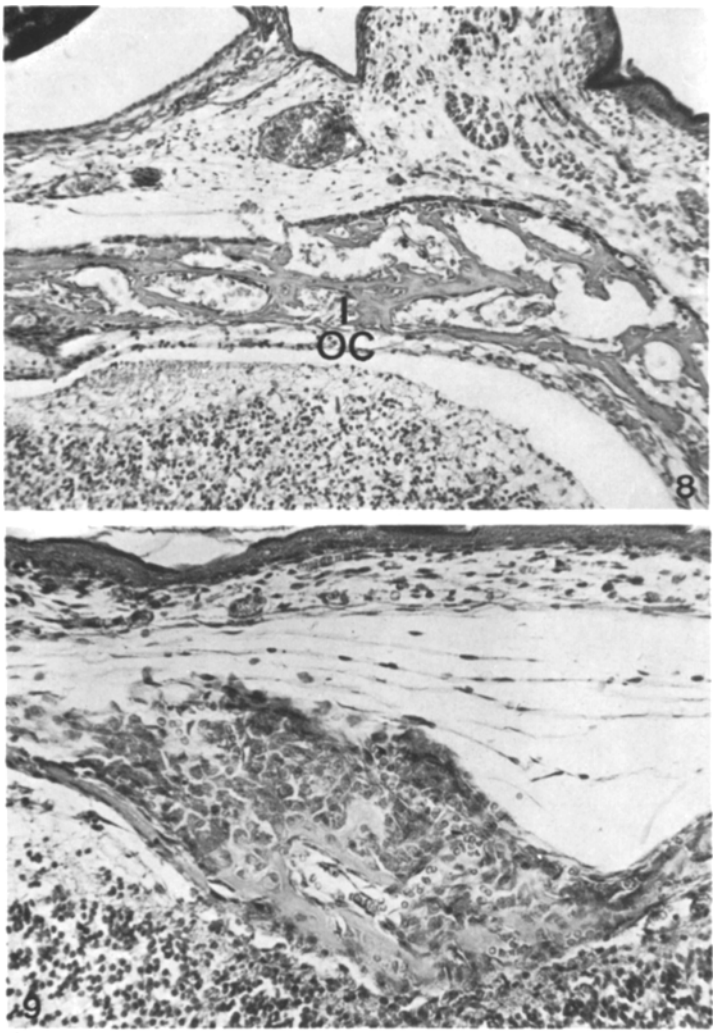

Fig. 8. Parietal bone of a newborn mouse (control animal). There is a marked osteoclastic (*OC*) activity leading to a distinct lamellation and diploe formation. H.E., 165 ×

Fig. 9. Irregular, nodule-like parietal bone formation of a newborn animal from the X-irradiated group. Ther is a severe hyperplasia of the osteoblastic tissue, but without a marked brain impression. H.E., 262,5 ×

In another instance the osseous protrusion of the skull originates from a similar region of cartilaginous hyperplasia of the dorsolateral skull (Fig. 5). Here the cartilaginous capsule shows a definite discontinuity, with an opening into the osseous protrusion "distally" and also a clear angulation of the entrance sites, thus the question arises as to whether this region represent the original squamosal-parietal suture.

In all cases the protrusion of desmal bones originate from the occipital regions of the neurocranium and extend almost horizontally in a rostral direction into the hemispherical walls of the severely injured brains. Thus, a marked extension of the dorsal telencephalic wall occurs in the more rostrally situated coronal sections of the brains, which show a completely irregular arrangement of the neuronal cell layers in the periphery of an "ectopic" osseous double lamella (Fig. 6). In general, fetuses with these skull anomalies reveal the most severe histopathological alterations of the CNS. The mean dorsal forebrain cortex diameter is reduced to about a third of the control value, in some cases there is porencephaly in the dorsolateral regions. (These neuropathological findings are presented in detail by Schmahl et al., 1979.) The invaginations of the skull into the forebrain reveal a marked production of osteoid and concomitantly a rather broad lining of osteoblasts at the inner surface (identical with the outer surface of the skull) (Fig. 7). Osteoclastic cells are seldom found at the border with the compressed brain. The bone cells are of an irregular shape and magnitude. Due to the lack of osteoclastic activity no distinct lamellation or diploe formation as seen in the parietal bones of control animals of the same age, is found (Fig. 8). Figure 8 also shows the normal relationship between the skull and underlying brain and meninges of untreated fetuses, in contrast to the severe CNS compression in Figs. 7 and 9.

Most of the fetuses exposed to 3×160 R or 1×200 R in late organogenesis show so-called "hyperplastic" nodules (see Table 2). These are irregularities in the formation of the parietal bones, similar to the events described above. However, there are no marked invaginations of the skull vaults in most cases (Fig. 9). Osteoid formation occurs in close proximity to the dura mater, but there is also excessive mesenchymal tissue outlining these nodules; this consists mainly of fibroblasts and osteoblasts. In addition, there is often a broad oedematous cleft between the skull and the skin.

Discussion

There are two main findings in the skulls of those mice X-irradiated during the period of late organogenesis. The first is, a displacement of the squamosal, parietal and interparietal region by intervening cartilaginous tissue and the second, a greatly extended growth of desmal bone tissue in the sutural areas. Both findings are those resulting from metaplastic and hyperplastic events. These observations are in contrast to the general view of delayed, diminished, or absent bone formation after X-irradiation (Warkany et al., 1947; Hanson, 1923; Roizin et al., 1962; Rugh et al., 1964; Murakami et al., 1963) also confirmed – especially for skull formation – by Murakami et al. (1962), Esaki (1963) and Rajtova et al. (1974).

The formation of the ectopic cartilage may be explained by the experiments of Markens et al. (1978): Transplantation of bone fragments of fetal rat skull, containing the presumptive coronal suture onto the intact dura mater of adult rats, results in all cases in the production of ectopic cartilage. This chondrogenic activity is located only on the cerebral side of the transplanted fetal dura mater

and only in the region of the presumptive suture. As this response is only observed prior to the 20th day of gestation, these authors conclude that cartilage originates from undifferentiated mesenchymal cells which outnumber the fibroblasts at earlier gestational stages and which are preferentially located in the sutural area. This undifferentiated group of cells "is supposedly capable of responding to environmental circumstances, such as changing mechanical and chemical qualities, by forming cartilage" (Markens et al., 1978). We assume that in our animals the responsible "environmental change" is caused by X-irradiation, leading to the ectopic differentiation of sutural mesenchymal cells to cartilage, similar to the abnormal chondrogenesis of the young animal's tibia after X-irradiation with 400 R, observed by Melanotte and Follis (1961) in the rat, and by Morse et al. (1974), in the mouse at a dose range between 300 and 900 R.

The simultaneous appearance of ectopic osteoid clearly corroborates the experimental findings of Nijweide et al. (1978). These authors quantified the growth capacity of the radii and calvaria of 15-day-old fetal mice and 18-day-old fetal rats after X-irradiation with doses between 500 and 1,000 rad. As one of the most interesting results of their investigation, they describe the formation of ectopic osteoid, although at the same time the number of osteoblasts lining the bone collar decreased. Moreover, while most of the reactions to irradiation increased in severity with increased doses, ectopic osteoid formation was generally more stimulated in the lower dose range than in the higher range. We observed similar osteoid formation in close relationship to the fetal dura mater, and therefore suggest that it represents an alternate pathway of aberrant differentiation of the primitive mesenchyme in response to radiation injury.

The thickness of the skull cartilage and the notable appearance of osteoblasts in the hyperostoses and the invaginated osseous tissue, is indicative of a hyperplastic "overgrowth" process. This term was used by Russell (1950) for increased bone growth in the hind feet of mice after X-irradiation with 200–300 R on day 10 p.c., and was also applied by Nash (1971) to the same finding obtained with doses between 160–320 R on day 11 p.c. Surprisingly, these authors only mentioned these findings but did not discuss it.

Overgrowth of the calvarium was studied especially by Moss (1975) and Melsen et al. (1977) and was attributed to a decreased intracranial pressure of the skull contents. Following microencephaly induced by methazoxymethanol, overgrowth of skull sutures developed parallel to a decreased proliferation rate of the cerebral cortical tissue. As fetal X-irradiation has drastic volumereducing effects on the mouse brain (Schmahl et al., 1979), the overgrowth in our experimental animals may be explained by analogy with the discussions of Melsen et al. (1977) who also describe an anomalous angulation of skull synchondroses and discuss a decreased resorption activity at the inner surface of these bones, due to the retarded development of the brain. A main indicator of the resorption activity is the appearance of osteoclasts, which were only seen in moderate numbers in our skull sections. According to Schramm, Melsen and LaCour (1974) it also seems possible that not only the amount of cartilaginous growth but also the regulation of its growth activity in some skull regions may be influenced by brain development. Taking this into account, the enormous skull

invaginations into the cerebral hemispheres in our experimental animals are more readily understood.

Our findings thus represent a type of malformation which originates from a growth disturbance caused by X-irradiation of both the mesenchymal skull primordium and the brain. These two developmentally interdependent tissues (Young, 1959; Jordaan, 1976) are affected by our irradiation dose in a "balanced way": the mesenchymal component is injured only moderately so that necrotic processes will not predominante and consequently no definite exencephalies will develop. Parallel to this, however, there is also a decrease of intracranial pressure caused by microencephaly. There are still proliferation and reparative capacities in the mesenchyme, which is presumably stimulated in a metaplastic and, in sutural areas, in a hyperplastic direction.

It is quite remarkable that these events are most readily induced only in the period of late organogenesis, and hardly at all in the fetal stages. Irradiation at a later point in time seemed to suppress the induction of the hyperplastic events originating from former irradiation exposure.

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