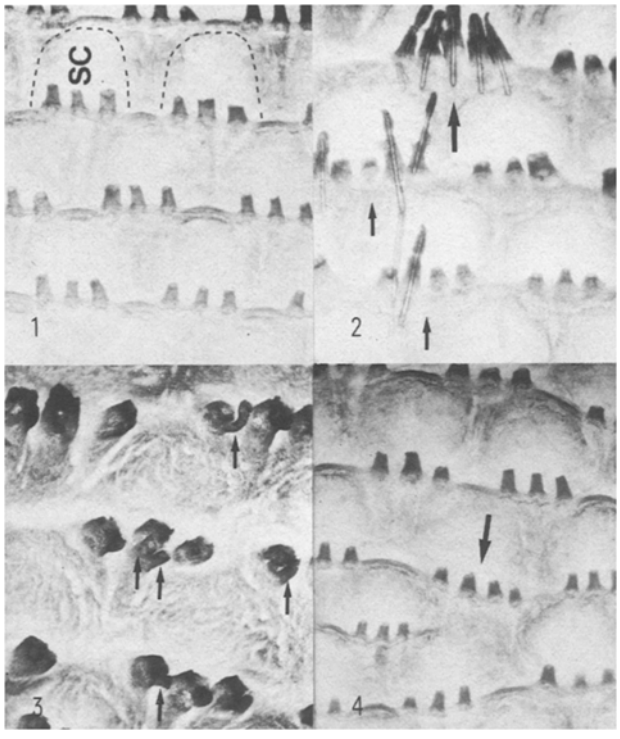

Fig. 1. Whole mount of depilated tail epidermis of an untreated adult mouse, showing triads of hair follicles associated with each scale (SC). During the separation of the epidermis from dermis, hair follicles are ruptured at the level of the sebaceous glands, so that only follicular necks can be seen. Note the absence of sequences with more than 3 hairs per scale. Hematoxylin; × 50.

Fig. 2. TPA-treated mouse, showing 1 sequence with 4 follicles and 2 sequences with 5 follicles per scale (arrows). Despite depilation, 1 set of 5 follicles has retained the hairs (large arrow). × 50.

Fig. 3. PDD-treated mouse, showing numerous buds and rudimentary follicular outgrowths (arrows) in the vicinity of parent follicles. × 75.

Fig. 4. Untreated mouse. Note the occurrence of a sequence with 4 follicles (arrow) at the site of disturbance of the parallel order of the scale rings. × 50.

sites that the rare sequences with more than 3 hairs are encountered (figure 4). It is only reasonable to assume that these disorders are the consequences of wounding.

Both promotion of epithelial tumour growth[13] and hair neoformation[8] in adult animals after wounding are dependent on concomitant dermal injury. On the other hand, normal hair development in mammals also requires the symbiotic action of both embryonic dermis and epidermis[8]. It may therefore be speculated that tumour promotion and hair neogenesis in adult animals is accompanied by specific dedifferentiation processes not only in the epidermis but also in the dermis, regardless of the nature of the promoting stimulus. The present knowledge of dermal alterations after treatment of skin with a tumour promoter is more or less restricted to the descriptive level of rather drastic events (i.e. edema, leucocyte infiltration), and in general alterations of this kind are produced also by non-promoting irritants. It is, however, noteworthy that even in long-term tests the strongly hyperplasiogenic non-promoter 4-O-methyl-TPA, which does not induce formation of new hairs, leaves a morphologically essentially unchanged dermis[14].

1 Acknowledgment. I am grateful to Dr K. Goerttler and Dr F. Marks for helpful advice and discussions.
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Sexual dimorphism of mouse fetal brain lesions after X-irradiation prior to gonadal differentiation

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Summary. Fractionated X-irradiation of gestational days 11–13 in the mouse, with doses between 3 × 1.05 and 3 × 1.33 Gy resulted in rosette-like clusters of primitive ependym-resembling cells dispersed within the cortex walls. Quantification of these abnormalities showed a general prevalence in the female fetuses, especially due to the larger number of rosettes in the females than in the males. It was concluded that X-irradiation acts on sex-specific differentiation steps, which are fully developed at the beginning of the fetal period. At is was recently speculated that these are linked to an early divergence of gene expression between the sexes, we suggest that X-chromosome damage may be involved in the pathogenesis of the dimorphic lesion pattern. While, in principle, this will be valid for any fetal tissue, it only becames evident in the forebrain because of the outstanding relationship between cell necrosis and rosette development in this specific organ.

In a previous report[2] we presented quantitative data calculating the severity of pathological aberrations in mouse neocortex after a fractionated X-irradiation insult on days 11–13 of gestation. The main findings were rosette-like clusters of primitive neuroblasts dispersed throughout the telencephalic cortex. The incidence of these rosettes showed a clear dose dependency from 3 × 1.05 Gy upwards, and also increased in number linearly with a decrease of

Table 1. In utero distribution of the fetuses after fractionated X-irradiation (3 × 1.05 Gy)

Localization in the uterus	Fetuses (%)				Fetal weights				Placental weights of fetuses			
	Male		Female		Male		Female		Male		Female	
	Controls	X-irradiated	Controls	X-irradiated	Controls	X-irradiated	Controls	X-irradiated	Controls	X-irradiated	Controls	X-irradiated
Upper third	14.7	16.4	13.7	11.8	1.33	0.84	1.29	0.81	0.101	0.077	0.096	0.068
Mid region	20.9	19.9	18.0	18.3	1.32	0.88	1.28	0.85	0.104	0.080	0.094	0.074
Lower third	16.6	16.8	15.5	16.8	1.32	0.87	1.27	0.83	0.100	0.081	0.096	0.073

Fetal sex ratio	Controls	X-irradiated animals
Fetuses placed in:		
Right uterine horn	52.6:47.4	52.7:47.3
Left uterine horn	51.8:48.2	53.2:46.8
General	52.2:47.8	53.0:47.0

Table 2. Malformation pattern (number of rosettes/brain or diagnosis)

Number of fetus	3 × 1.05 Gy		3 × 1.14 Gy		3 × 1.33 Gy	
	♂	♀	♂	♀	♂	♀
1	n.p.			14		22
2	6			16		18
3	n.p.			12	14	
4		8	n.p.			40
5		n.p.	n.p.		10	
6		10		c.d.		c.d.
7	4			30		c.d.
8		4	n.p.		c.d.	
9		4		18	24	
10	n.p.		6		8	
11	n.p.			m.	m.	
12		n.p.		14		12
13		6	4			c.d.
14	4		14			c.d.
15		6		c.d.		36
16		4		m.	8	
17		n.p.		m.		c.d.
18	n.p.		n.p.			24
19	n.p.		6			c.d.
20	n.p.		n.p.			36

n.p. = no pecularities; c.d. = complete dysplasia; m. = severe microgyrie.

the cortical diameter within a distinct dose group. Further studies conducted on the carcasses of the same fetuses looked into the developmental pathology of the govads. In this process we determined, to our surprise, a prevalent occurrence of the above mentioned brain lesions in the female fetuses. In parallel to this finding was the observation that the mortality of female offspring was about 2-fold higher than that of males within the first 8 h post partum; this lasted, with a decreasing tendency, until 48 h of neonatal life[3]. We then attempted to confirm this by histological examination of another large number of fetuses from various irradiation groups.

Materials and methods. Female mice were X-irradiated on days 11, 12, and 13 of pregnancy with either 3 × 1.05, 3 × 1.14, or 3 × 1.33 Gy, as described earlier[2,3] (180 kV, 10 mA, 0.3 mm copper plate filter, focus target distance 40 cm; dose rate 0.01 Gy/sec). The animals were killed on day 18 of gestation, the fetuses removed and immediately fixed in neutral buffered formalin. 20 brains were taken at random from each experimental group and from the controls for histological processing. Following the quantitative histological evaluation of the frontal sections (enumeration of the rosettes and listing of other anomalies), their sex was determined by inspection of the carcasses.

In order to exclude position effects in utero we examined another 55 irradiated (3 × 1.05 Gy) dams and 37 pregnant controls for the distribution of the 2 sexes within the uterus on day 18 p.c. At the same time we determined the fetal sex ratio and also the effects of sex and position on fetal weights.

Results. The distribution of the males and females in utero showed a random pattern, irrespective of whether X-irradiation was applied or not (table 1). Fetal and placental weights differed to a similar degree as a function of sex ($p < 0.01$) both in the irradiated and the control animals. While in the control animals no position effect for the fetal and placental weights was found, there was a larger decrease of these weights at the ovarian end of the uterus after X-irradiation. In the latter group of animals there was generally a less pronounced reduction of the placental (22%) than of the fetal weights (34%), as compared with the controls. The fetal sex ratio (♂ : ♀) was 1.09 in the controls and 1.12 in the irradiated animals.

The histological results are listed in table 2. The number of rosettes per brain is recorded as far as possible; if the lesions were too marked to permit a clear enumeration, this is specified by a short descriptional diagnosis. This table indicates a clear predominance of the neocortical lesions in the female fetuses. This was evident both from the number of affected males or females and from the number of the

rosettes per brain, as well as from the frequency of the alterations otherwise described. There was also a clear correlation between an increasing number of rosettes and a decrease of the cortical diameter, as reported previously. In general, there was a definite increase of the response at the histological level as doses became larger. Additionally, the variability of the magnitude of the effects also increased.

Discussion. This report on the occurrence of sexual dimorphic lesions after fetal X-irradiation is complementary to the previous findings of Ward et al.[4] on a significantly higher occurrence of lethal injuries in female rat fetuses than in male ones after gamma irradiation from days 3 to 10 of gestation. Unfortunately, those authors' findings were not followed up by pathological examinations. A similar preferential vulnerability of female fetuses was reported for chemical teratogens like trypan blue, acetazolamide, and to cadmium chloride[5]. Our findings cannot be explained by a preferential lethal effect of the X-rays to male fetuses, leaving an excess of malformed females, because the intrauterine sex ratio proved to be balanced. Similary, there is no evidence for an unequal distribution of female fetuses at the ovarian or cervical ends of the uterus, which can be responsible for different growth[6], low viability[7], and increased radiation sensitivity[8]. Clusters of ependym-resembling cells, so-called rosettes, dispersed in the cortical hemisphere, arise near the ventricular surface as a direct response to X-irradiation within 12–20 h[9,10]. It is an important fact that these rosettes remain completely unchanged until the end of gestation with no signs of repair[10]. The glial repair response starts as late as day 18 of gestation and is pronounced in the early postnatal period. Thus any developmental influence of the late fetal period, modifying the rosettes' pathogenesis, has to be ruled out, e.g. all differentiation processes shortly before term, which are linked to fetal steroid hormone production[11] and the sexual dimorphic imprinting events of the brain by these steroid hormones[12].

It was shown[13] that X-irradiation of rat fetuses on gestational day 13 with doses between 2.0 and 4.0 Gy results in chromosome damage very frequently. This was specified in greater detail by Albertini et al.[14], who described the X-chromosome damage pattern of cultured fibroblasts after X-irradiation. The mutation rate was determined by the X-linked enzyme hypoxanthine-guanine-phosphoribosyltransferase (HGPRT). The mutation rate found reached its maximum after a single X-irradiation within the 1.25–1.50 Gy dose range, thus indicating a non-linear, cumulative-type dose-response relationship between mutation and X-ray exposure, especially for the X-chromosomes.

We suggest that the sex-specific pattern of brain lesions observed in our studies may be linked to X-chromosome damage. This speculation is developed on the basis of the observations by Scott et al.[15], who showed that as early as day 12 of gestation in the rat the weight of male fetuses differs significantly from that of female fetuses. This is long before gonadal differentiation starts and was explained only by a sex-specific difference in gene expression. This opinion, however, stands in sharp contrast to the dosage compensation theory[16] which states that males with only 1 X-chromosome and females with 2 are functionally equivalent with regard to the gene products of the X-chromosome. This effect is brought about by the inactivation of one female X-chromosome early in the embryonic period[17]. But if the data of Scott et al.[15] indeed 'suggest that sex-linked genes exist, which influence embryonic growth', we may also assume that these responsible parts of the genome – in our opinion the real genosomes – will be more radiosensitive than the other chromosomes (autosomes), according to the general opinion that there is a strict correlation between gene activity and radiosensitivity[18]. This may in principle result in a defect in X-linked functions. With respect to recent reports[19], however, a reactivation of an inactive X-chromosome or a least of some of its loci by X-rays seems possible. These biochemical effects of X-irradiation in utero with respect to X-linked enzymes are presently under investigation.

The high frequency of chromosome aberrations and abnormal mitoses will be largely eliminated within 24 h after irradiation[13]. In the ventricular layer of the forebrain these necrotic processes are directly connected with telencephalic roof invaginations and rosette formation within the same time interval[9]. To explain the dimorphic outcome of the radiation lesion, we assume that the number of abnormal mitoses and thus the rate of cell necrosis is more pronounced in female fetuses due to the damage to the X-chromosomes. This will lead to a higher incidence of telencephalic abnormalities. Although this assumption of an increased cytolethal effect in female fetuses has to be put forward for any highproliferating fetal tissue, the morphological outcome is only marked in the forebrain because of the anatomical pecularities, which are important for rosette formation from the cells of the innermost ventricular cell layer.

1 We are greatly indebted for the technical assistance of Mrs E. Senft, Mrs I. Hempfling and Mrs K. Pfaff.

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