shown here to occur between rat and guinea-pig indicate that several mechanisms for regulating the medullary blood supply may exist. In fact, it has been argued that contraction of the medullary vein is an essential factor in determining the amount of corticosteroids reaching chromaffin cells.

In addition to a local regulation of blood flow, contractile mechanisms in the vascular wall, and especially in endothelial cells, could also serve to regulate vascular permeability by either widening intercellular gaps os or transendothelial channels[21]. In this investigation, actomyosin could not be detected in the endocrine cells of adrenal medulla or cortex. This does not exclude the presence of contractile proteins in these structures, but the amount may have been too low to be detected with the methods applied here. While this paper was in preparation Creutz[22] presented immunofluorescence pictures from bovine adrenal medulla, suggesting that chromaffin cells contain myosin. Since his approach to the problem of immunocytochemical localization of myosin was somewhat different from ours – he used myosin from bovine adrenals as an antigen – the results cannot be directly compared.

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## Induction of developmental anomalies in mice by maternal stress[1]

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*Summary.* The short-time restraint of pregnant mice on day 8 of gestation led to a significant increase of the anomaly rate in fetuses. This effect may be due to stress factors of endocrine origin.

Teratological studies using ionizing radiation at low dose rates require relatively long exposure times for the pregnant animal. When only a part of the body is to be exposed, or the field size of the radiation beam (85% isodose) just covers the animal, the movements of the animal have to be prevented either by anesthesia or by restraining in a 'snug-fitting' cage.

Since anesthesia may be an effective agent in radioprotection[2], or some narcotic drugs can themselves be teratogenic (like Epontol, in publication) we used the restraining method to avoid chemical interactions. In our earlier experiments[3], using 200 kV X-rays at high dose rates, no harmful effects could be observed in sham-irradiated animals, which were restrained only for a short period. However, in recent work with negative pions at low dose rate, or with protracted 140 kV X-irradiation, the prolonged restraint represents a stress situation which is teratogenic in mice. This so-called 'cage effect' has been seen also in experiments using Lucanthone as a possible

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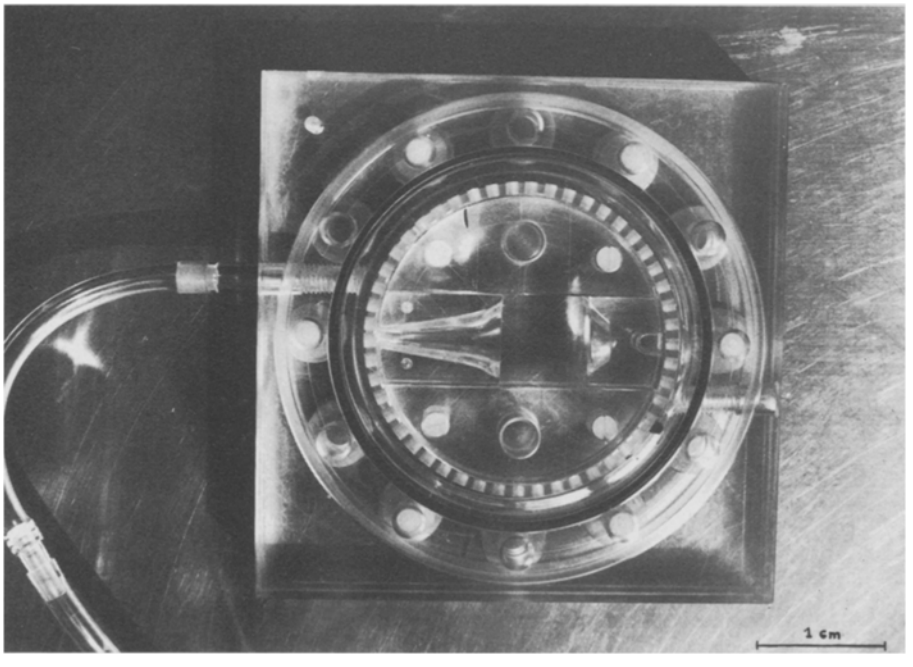


Plexiglas cage with air supply used in this study. The restraining chamber in the centre of the cage.

Type and frequency of anomalies following different restraining times. Treatment at day 8 of gestation; macroscopic examination on day 13 of gestation

| | Nonrestrained control | Restraint 1 min 50 sec | Restraint 10 min | Restraint 20 min | Restraint 36 min |
|---|---|---|---|---|---|
| No. of mothers | 36 | 31 | 14 | 15 | 12 |
| No. of implantations | 487 | 411 | 183 | 194 | 175 |
| Resorptions | 28 (5.75%) | 29 (7.06%) | 3 (1.64%) | 21 (10.82%) | 10 (5.71%) |
| No. of normal fetuses | 410 (89.32%) | 336 (87.96%) | 138 (76.67%) | 136 (78.61%) | 133 (80.61%) |
| No. of abnormal fetuses | 49 (10.68%) | 46 (12.04%) | 42 (23.33%)* | 37 (21.39%)* | 32 (19.39%)* |
| Growth retardation | 41 (8.93%) | 41 (10.73%) | 38 (21.11%) | 26 (15.03%) | 22 (13.33%) |
| Microphthalmia | 1 (0.22%) | 5 (1.31%) | 4 (2.22%) | 10 (5.78%) | 7 (4.24%) |
| Exencephaly | 1 (0.22%) | – | – | 1 (0.58%) | 3 (1.82%) |
| Microcephaly | 2 (0.44%) | – | – | – | – |
| Tail anomaly | 4 (0.87%) | – | – | – | – |

* Significantly greater than in control group (Wilcoxon-test, $p < 0.05$).

radiosensitizing drug[4]. The application of Lucanthone to restrained pregnant females induced more anomalies than Lucanthone without restraint.

In this paper we investigate the role of the duration of the restraining time in causing developmental disturbances. *Materials and methods.* NMRI mice were maintained in our laboratory under a 12-h light-dark cycle, at a temperature of 20 ± 1 °C and with food (NAFAG: Nr. 890) and water ad libitum. Virgin females, $2^1/_2$–3 months old, were mated overnight and impregnated females identified by the presence of copulatory plug. This day was taken as day 0 of gestation. On day 8 individual females were restrained in a well ventilated Plexiglas irradiation cage, measuring 70 × 35 × 25 mm, allowing no free movements within this box (figure). The following 4 restraining times were selected: 1 min 50 sec, 10 min, 20 min and 36 min, corresponding to the duration of exposure in irradiation experiments. A group of 36 females were used as nonrestrained controls. On day 13 of gestation the animals were killed and the uteri dissected. The number and position of resorptions as well as the number of abnormal fetuses (malformed or growth retarded) were recorded and compared with controls. The data were analysed statistically with Student and Wilcoxon tests.

*Results:* The results of restraining on mouse embryos on day 8 of gestation are presented in the table. Only a small difference in the number of affected fetuses was observed between the control and the shortly restrained animals (1 min 50 sec). Significant differences were seen between untreated and the 10-min ($p < 0.01$), 20-min and 36-min ($p < 0.05$) restrained groups. A restraint of 20 min or 36 min did not increase the frequency of abnormal fetuses compared to the 10-min group. As the restraining time increases, the incidence of affected fetuses decreases slightly, indicating the existence of a possible saturation effect.

Concerning the resorption rates, a very low frequency is apparent in the 10-min group, being significantly less compared to the 20-min ($p < 0.01$) and also to the 1 min-50 sec group ($p \leq 0.05$). Differences in the resorption rates among the remaining groups were statistically not significant.

In qualitative respect, it is obvious that growth retardation is the most frequent result of restraining. Growth-retarded fetuses were defined as those that are 2 standard deviations below the mean of control weight. Even in nonrestrained fetuses, at day 13 growth reduction was observed together with some rather rare developmental anomalies like microphthalmia, exencephaly, microcephaly and tail bifurcation. It is of interest that the 36-min exposure to the stress situation induced the highest frequency of exencephaly, although the total rate of developmental anomalies was relatively low in this group. *Discussion.* It has been reported that restraint for 48 h on days 10–15 can be teratogenic in mice[5,6]. The present data show that already short-time exposures to physical stress may have distinct deleterious effects on mouse embryos at day 8 of gestation. Since the shortest restraining period (1 min 50 sec) did not produce a significant higher incidence of malformations compared to control, it seems that some kind of threshold reaction exists. It is also interesting that no increase in the incidence of developmental anomalies was observed when the duration of restraining exceeds 10 min. On the contrary, 20-min or 36-min restraint resulted in a slighter decrease in the number of the affected fetuses examined at day 13 of gestation. These results suggest that a saturation effect may exist which apparently becomes evident after a restraint of 10 or even less minutes. The percentage of abnormal fetuses was highest in the 10-min group, which on the other hand surprisingly had the lowest resorption rate of all groups.

It seems noteworthy that some developmental defects appeared in nonrestrained animals only, even if their frequencies were very low. The most striking finding in this respect was the presence of 1 fetus with exencephaly, a severe malformation seen mainly following Lucanthone (Miracil-D) application but never observed before in more than 1400 untreated fetuses. 1 of the 3 fetuses with exencephaly in the 36-min group had an additional severe facial fissure, indicating that at least qualitatively this treatment was the most effective one.

These results demonstrate that the duration of restraint may play a decisive role and has to be taken into account in evaluating radiation-induced teratological effects. In connection with the hypophysis-adrenocortical mechanism[7], which probably is involved in the present exposure to stress, further extended investigations were started. It is also intended to investigate the existence of hypoxic conditions which may be also teratogenic.

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