Acta orthop. Scandinav. 43, 157–162, 1972

Anatomy Department B, Universitetsparken 1, 2100 Copenhagen Ø, and
Orthopaedic Hospital, Department I, Hans Knudsens Plads 3, 2100 Copenhagen Ø,
Denmark.

CONGENITAL MEROMELIA OR "SPONTANEOUS AMPUTATIONS"

Experimental Studies on Mice

FREDE BRO-RASMUSSEN, BENTE JENSEN &
AUGUST HALBORG SØRENSEN

Accepted 13.iv.72

At the end of the past and beginning of the present century it was widely held among teratologists that many congenital malformations were caused mechanically by amniotic adhesions or bands. For instance, extremity malformations in which major or minor parts of the limbs were missing, the so-called spontaneous amputations, were considered to be an obvious result of constriction by amniotic bands.

On the basis of his comprehensive studies of human extremity malformations, Streeter (1930) rejected the amniotic hypothesis and demonstrated that spontaneous amputations were due to "primary" focal deficiencies or inferior quality of the limb mesenchyme". Keith (1940) went further still and concluded partly that "the tissue deficiencies are probably due to a circulatory failure, which may be placental in origin" and partly that the amniotic bands "are the results, not the cause of foetal malformations".

Inmann (1941) found hereditary brachydactyly in the rabbit to be caused by vascular injuries leading to oedema, haemorrhage, and necrosis. Bagg & Little (1924) demonstrated that certain inherited malformations in mice were preceded by subcutaneous blebs and haemorrhages.

It is common to all these investigations that they have been concerned solely with hereditary malformations.

In the course of our studies on malformations in mice induced by intraperitoneal injection of fluorodeoxyuridine (FUDR) (Bro-Rasmussen et al. 1971), we found a motley mixture of limb malformations, *inter alia* syndactyly, brachydactyly, club-foot, split-foot, and more

or less extensive "spontaneous amputations". As will appear from what will be stated below, the teratogenesis in the experimentally induced malformations seems to have features in common with that of the hereditary malformations.

MATERIAL AND METHODS

The experiments were carried out on albino mice of an inbred strain from our own laboratory. The material comprised 47 pregnant mice, 20 of which served as controls.

11–11½ days after copulation each experimental mouse was injected intraperitoneally with 2 mg (i.e. 60–70 mg/kg) 5-fluoro-2-deoxyuridine (FUDR) in 1 ml sterile water. The controls were injected with 1 ml physiological saline.

The mice showed good tolerance of the injections and went to term, around the 19th–20th day. The litter sizes were analysed on the day after birth, i.e. at a time when the less viable young had died and had been eaten. The individual litters were kept apart while they were growing up, and at 4 weeks of age the males were separated from the females.

At 10 weeks of age siblings with extremity malformations were mated. The mating was continued among the offspring for another 3 generations.

RESULTS

The control mice bore 202 young, i.e. the litter size averaged 10.1 young. None of these young exhibited malformations and developed normally with the exception of 3, which died of unknown causes.

The experimental mice bore 148 young, i.e. a litter size averaging 5.5. 147 developed normally and 1 died of an unknown cause. Twenty

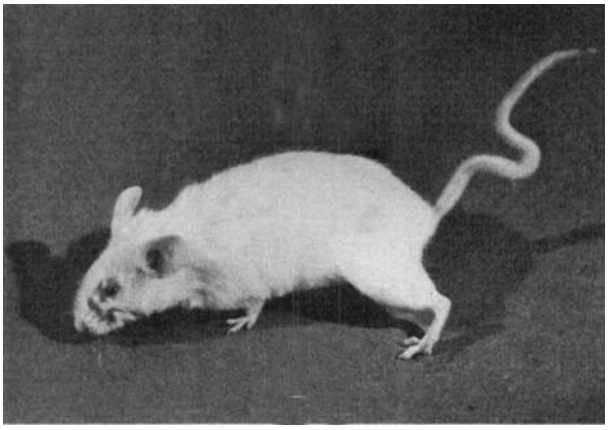

Figure 1. 8-week-old mouse with oligodactyly. 3 toes on the left foreleg and 2 on the left hind leg. Deformity of the tail.

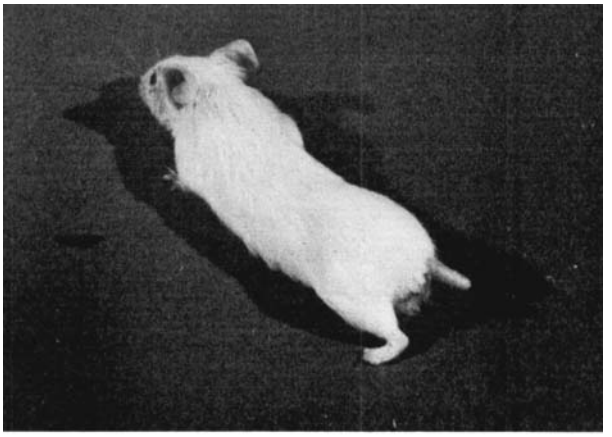

Figure 2. 8-week-old mouse with adactyly on the left hind leg. Toes normal on the forelegs. Malformation of the tail.

Figure 3. 8-week-old mouse with "spontaneous amputation" of the right thigh. Left hind leg and both forelegs normal. Tail misshapen.

Figure 4. 8-week-old mouse with "spontaneous amputation" proximally on the right thigh. The stump of the femur is covered with normal skin. Traces of 3 digits on the left hind leg. Forelegs normal.

of the young showed no malformations, whereas all the remaining ones had malformations of one or more limbs of an extremely diverse nature, there being club-foot, split-foot, syndactyly, polydactyly, brachydactyly, radial and/or ulnar, fibular and/or tibial defects, oligodactyly (Figure 1), adactyly (Figure 2), apodia, and "amputations" of major or minor parts of the lower leg, or in one case of the thigh (Figures 3 and 4). The defects varied from forelegs to hind legs, being on the whole more pronounced on the hind legs. Frequently, there were symmetrical defects on the forelegs or the hind legs. A total of 332 "spontaneous amputations" were recorded. This includes total absence of toes and feet as well as partial absence of the lower leg or thigh. Thus, on the average, the mice had "spontaneous amputations" of 2–3 limbs or parts of limbs. The absence of one or more toes was usually symmetrical, whereas the more severe "amputations" were frequently unsymmetrical, both on the forelegs and on the hind legs, or else they affected only one limb. Tail defects were common.

The offspring of the malformed mice and the later inbred generations had an average litter size of 10.3 and showed no malformations.

DISCUSSION

Investigations of early teratogenesis (Bro-Rasmussen et al. 1971) following injection of 2 mg FUDR/mouse 11–11½ days after copulation, removing the foetuses at varying times after the injection up to the 18th–19th day of gestation, had shown an increased number of foetal resorptions (40 per cent as compared with 8 per cent in the control material). Investigations of foetuses 24 hours after the injection revealed large dilated vessels and fresh haemorrhages in the limbs which otherwise had developed normally during the period after the injection. Two or more days after the injections there were characteristic saccular haematomas of varying size in the limbs (Figure 5) and tail. With increasing intervals after the injections the number of haematomas decreased. They were rarely observed in foetuses immediately before the normal time of birth. As the number of haematomas decreased, the number of malformations increased. Injection at an earlier stage of gestation than the 11th day induced spinal and cranial defects. Injection after 12–12½ days of gestation did not induce any defects. Investigations of the uteri showed no amniotic bands.

The present studies differ from those mentioned above in having

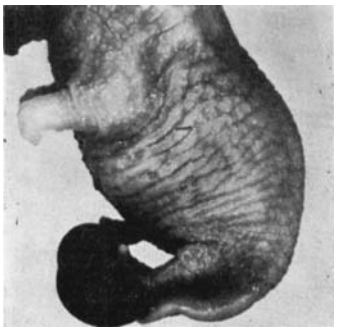

Figure 5. Haematoma in the left hind leg of a 17-day-old mouse foetus removed from a pregnant mouse injected 5 ¾ days previously with 2 mg FUDR. No outlining of digits on the forelegs and the right hind leg. Tail deformed.

been concentrated on extremity malformations and in the mice having been permitted to go to term and bear their young. This gave us a chance to form an impression of the viability of the young, to follow the defects, and to carry out generation experiments.

Many malformations of internal organs are being explained to-day as malformations of arrest, i.e. arising because development has been arrested for some reason or other (genetic or exogenous). Extremity malformations, e.g. amelia, are also usually explained as malformations of arrest.

In normal mouse foetuses aged 11–11½ days (calculated from the time of copulation) the limbs have been laid down and the outlines of the digits are more or less distinct. In 12–12½ days foetuses (normal foetuses and foetuses removed 24 hours after the injection of FUDR) the limbs have on the whole assumed their permanent outer configuration.

When previous and the present results following injection of FUDR are compared with normal development of the limbs, the FUDR-induced malformations can be explained only as primary vascular damage (confirmed by electron microscopic studies now in progress), resulting in the formation of haematomas, destruction and necrosis of extremity parts which have already been laid down, but which are then shed. In other words, there does not seem to be a question of malformations of arrest.

Pilot experiments on malformations induced by galactoflavin (riboflavin antagonist) indicate that these malformations may have the same teratogenesis as FUDR-induced malformations.

Since FUDR is a cytotoxic with thymine-antagonistic actions, it is worth noting that the young which survived the gestation and were born with more or less localized extremity defects developed entirely normally without any signs of internal malformations or retarded

development. Their fertility was normal, and in spite of the action upon the DNA synthesis, genetic defects do not appear to arise.

As stated in the introduction, vascular changes have been reported in the limb primordia of experimental animals with hereditary extremity malformations, malformations which incidentally are very similar to those we have seen following exogenous application of a teratogenic agent.

To draw parallels, if possible, to clinical findings, we have analysed 18 patients with varying degrees of "spontaneous amputations" seen in the Orthopaedic Hospital, Copenhagen, during the period 1967–1968. Definite heredity could not be demonstrated in any case. On the other hand, it was also not possible, in retrospect, to demonstrate exogenous factors as the cause of the "spontaneous amputations".

SUMMARY

Injection of fluorodeoxyuridine (FUDR) into pregnant mice 11–11½ days after copulation resulted in a motley mixture of malformations of the limbs, including a considerable number of cases with meromelia, the so-called spontaneous amputations. FUDR is a cytotoxic which acts upon the DNA synthesis (thymine antagonist).

The explanation of these malformations was found to be that FUDR damages the vascular endothelium, resulting in the formation of haematoma and necrosis, destroying parts of the limbs which have already been laid down. Similar teratogenesis has been observed in galactoflavin (riboflavin antagonist) induced malformations.

REFERENCES

Bagg, H. J. & Little, C. C. (1924) Hereditary structural defects in the descendants of mice exposed to roentgen ray irradiation. *Amer. J. Anat.* **33**, 119–145.

Bro-Rasmussen, F., Jensen, B., Hansen, O. M. & Østergaard, A. Høgsbro (1971) Fluorodeoxyuridine-induced malformations in mice. *Acta path. microbiol. scand.* **79**-A, 55–60.

Inmann, O. R. (1941) Embryology of hereditary brachydactyly in the rabbit. *Anat. Rec.* **79**, 483–505.

Keith, A. (1940) Concerning the origin and nature of certain malformations of the face, head and foot. *Brit. J. Surg.* **28**, 173–192.

Streeter, G. L. (1930) Focal deficiencies in fetal tissues and their relation to intrauterine amputation. *Contr. Embryol. Carneg. Instn* **22**, no. 126, 1–44.

Correspondence to:

Frede Bro-Rasmussen
Anatomy Department B Universitetsparken 1 2100 Copenhagen Ø Denmark