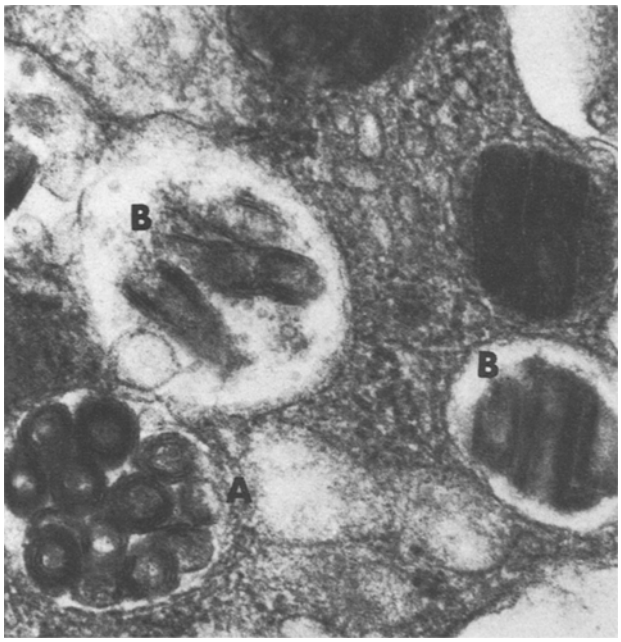

Fig. 1. Electronmicrograph of cytoplasm of a mast cell showing unusual forms of degranulation with scroll (A) and tubular (B) configurations. Tubular configurations may represent longitudinal sections through scrolls. Uranyl acetate and lead citrate. × 36,500.

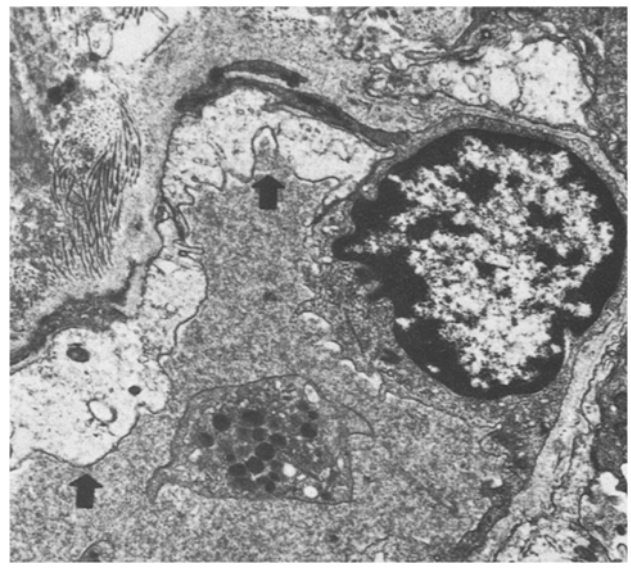

Fig. 2. Electronmicrograph of a pulmonary interstitial venule showing gross hydropic degeneration (arrows). Uranyl acetate and lead citrate. × 8,000.

viruses[3]. Endothelial changes in venules have been reported previously in secreting carcinoid tumours of the stomach[4]. Localized endothelial changes might result from local release of vasoactive factors from mast cells and argentaffin cells.

The association of atypical mast cell degranulation and hydropic endothelial degeneration in patients with marked hyperplasia and desquamation of type 2 pneumocytes leads to speculation that these reactions might have a pathogenic role in some forms of diffuse fibrosing alveolitis. The present findings are reported to focus attention on an unusual morphological association in this enigmatic pulmonary disorder in the hope that it will be confirmed by other diagnostic electron microscopists.

1 This work was supported by a grant from the National Health and Medical Research Council of Australia.
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DPH-induced macrocytosis in the 14-day rat foetus

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Summary. Phenytoin injected in the pregnant rat induces in the 14-day-old foetus macrocytosis of the primitive red blood cells which is sometimes linked with limb haemorrhages. The action of the drug is possibly the result of a blood circulation disturbance.

It is well known that epileptic patients can develop megaloblastic anaemia during long-term treatment with diphenylhydantoin (DPH)[1]; moreover, folate deficiency has sometimes been shown in these patients[2]. Nevertheless, the action of anticonvulsant drugs on folate metabolism is not yet clearly understood. Several explanations have been proposed, including inhibition of intestinal folate conjugase[1], increased hepatic degradation of folic acid during enzyme induction by anticonvulsants[3], and intestinal malabsorption of pteroylmonoglutamates[4].

Teratogenic effects of DPH in humans have been reported and reviewed by several authors[5]. The drug is also teratogenic in rodents[6-10]. The primary defect seems to be cleft palate, but many other skeletal defects have been observed. Several authors obtained the same types of abnormalities in foetal mice and rats by using a folic acid deficient diet[11,12], or by administration of folate analogs and antagonists[13,14]. More recently, several authors obtained similar results by using aminopterin[15] and methotrexate[16]. Although folic acid deficiency is certainly responsible for the teratogenicity of DPH, the processes leading to congenital malformations still remain unknown. Pyrimethamine, another antifolic drug, injected in pregnant rats, induced in foetuses both macrocytosis of the nucleated red blood cells and severe haemorrhages resulting in necrosis of the limbs during the following days[17]. This experiment shows for the first time the existence of a relationship between the presence of foetal blood macrocytosis and the induction of

congenital malformation. Such a phenomenon occurs spontaneously in one rabbit stock (brachydactylia stock) where it is genetically induced[18].

In this study, we tried to verify whether a short treatment with DPH in pregnant rats has the same consequences in rat foetuses.

Material and methods. Wistar albino rats were used. 19 females aged 4 months were mated with 6-month-old males. Gestation was confirmed by palpation of the foetuses on day 12. Throughout the experiment the females were kept 2 to a cage at a temperature of 22 °C. The animals were fed and given tap water ad libitum. The general condition of the pregnant rats was checked daily throughout the treatment.

The rats were injected with diphenylhydantoin i.p. 100 mg/kg in a volume of 1 ml on days 12–13. A saline suspension of DPH was used; the 17 controls received i.p. the same volume of saline. The pregnant females were sacrificed on day 14. The foetuses were then removed by Caesarean section and carefully inspected with the aid of a dissection lens. In order to study the drug's influence on the blood, day 14 was chosen since nucleated red cells are still numerous at that stage.

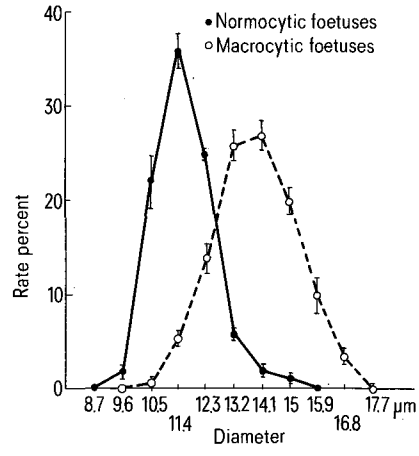
Blood study in 14-day-old foetuses. After removing the foetuses, foetal blood was drawn by puncture from the vitelline artery after the foetal membranes had been dried, and blood smears were prepared and stained by the panoptic method; the microscopic image of the blood cells was projected onto paper with a projection microscope. For each foetus the outlines of 200 nucleated cells were drawn and the diameters directly measured. The distribution curve of the diameters of the nucleated red blood cells was compared with that for controls.

Observations in 14-day-old foetuses. All the foetuses were carefully examined in order to discover limb abnormalities.

Results. Blood study in 14-day-old foetuses. In all of the living foetuses (27 treated foetuses from 19 mothers) a very obvious macrocytosis of the nucleated red cells was observed (figure): the distribution curve of the nucleated cells was shifted toward larger diameters compared with control foetuses (24 from 7 mothers).

External observations on 14-day-old foetuses. 5 foetuses out of the 27 living foetuses presented very severe haemorrhages at the extremities of the fore limbs; 2 presented only haemorrhages at the extremities of the hind limbs; 20 were free of external abnormalities, and 104 foetuses died as a result of the treatment.

Discussion and conclusions. After DPH treatment, an obvious macrocytosis of the foetal blood cells is visible in 14-day-old foetuses, as we observed in rat foetuses treated with an antifolic, pyrimethamine. This could confirm the existence of a common mechanism for the action of the 2 drugs consisting of a folic acid metabolism deficiency. However, after pyrimethamine treatment, all the foetuses presented limb or snout haemorrhages as well as red blood cell



Distribution of nucleated primordial cell diameters in rat foetuses treated with diphenylhydantoin (day 14 of gestation). Mean ± SEM.

macrocytosis, and the newborn presented limb amputations, shortening of the mandible and often microphtalmia.

It has been recognized for some time that DPH therapy is often associated with low serum folate levels and megaloblastic anaemia. While in adults the abnormalities occur after a very long period of treatment, in foetuses the blood abnormalities and limb haemorrhages can develop extremely quickly. Moreover, the limb haemorrhages, rather rare in the rat, probably because of the high degree of embryotoxicity of the drug, were found to be common in mouse foetuses after DPH treatment. We found a clear relationship between limb haemorrhages in the foetal mouse and necrosis of the haemorrhagic areas at birth; in some cases, the amputation of the limbs occurs as late as 1 or 2 days after birth. Furthermore, the importance of the amputations in the newborn depends on the size of the haemorrhagic areas during the foetal period. On the other hand, it seems difficult to find a link between cleft palate formation and haematological disorder. It is conceivable that a haemorrhagic area of the palate, similar to that observed in the rat snout after pyrimethamine treatment, occurs and is the cause of it.

Whatever the mechanism, there seems no doubt that haemorrhages can occur in rat foetuses after DPH treatment, at least in a few cases; it is probably the cause of the high mortality rate observed in our experiment. In newborn children, several cases of blood disorders and foetal haemorrhages have recently been reported[19].

This study emphazises the extreme sensitivity of the foetus towards drugs which induce haematological troubles in the adult only after long-term treatment.

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