should result only if some T-cells had escaped killing by anti-ϱ. In practice, no increased killing was observed as shown in table 3. This demonstrates clearly that there are no thy-1 containing (T)-spleen cells which do not also contain ϱ.

Together these 2 experiments provide quite strong evidence that ϱ-determinants are present on the surface of mouse T-, but not B-lymphocytes. The sequential cytotoxicity experiment, in fact, suggests that ϱ is expressed by all mouse T-cells and not simply by a subpopulation of them. Both experiments, however, admit the possibility that a small but significant population of B-cells may express. Further studies will be required to clarify this point.

The demonstration of ϱ-determinants on the T-cell-surface raises the issue of whether ϱ may be identical to any of the previously described mouse T-cell specific antigens such as thy-1, TL, Ly and MTLA. So far, it appears that this is not the case. For example, if ϱ were the same as thy-1, then L-cells expressing ϱ ought to express thy-1 as well. Our experiments as shown in table 4 have demonstrated that this is not the case. Furthermore, ϱ antigen (mol.wt 100,000) and a protein (mol.wt approximately 120,000) recently precipitated specifically from solubilized mouse T-cell-membranes by anti-ϱ (S. Cancelosi and J. Brown, unpublished observations) are found to have considerably higher mol.wts than the thy-1 antigen molecule (mol.wt 27,000)[13–15]. Similar but less conclusive arguments can also be made in the case of TL-, Ly- and MTLA-antigens. Cohen et al.[16] have shown that L-cells do not express TL-antigens and Ly-antigens are expressed on the surfaces of lymphoid cells only[17,18]. MTLA has a mol.wt significantly different from ϱ[19,20]. These results are consistent with the view that ϱ is not identical to thy-1, TL, Ly or MTLA antigens.

The information we have about ϱ-antigen at the present time gives us very few clues about what its function may be. ϱ is found to be present on the surface of L-cells and of T-, but not B-lymphocytes from the mouse and rat. In addition, we have detected ϱ on the surface of Swiss mouse embryo fibroblasts, but not human or mouse erythrocytes. Since ϱ antigen is itself a con A-receptor and since con A is found to be mitogenic for T-, but not B-lymphocytes, the possibility exists that ϱ may be involved in the con A-induced mitogenesis of T-lymphocytes. Experiments employing anti-ϱ-serum are currently in progress to test this possibility.

Table 4. Resistance of mouse L-cells to anti-thy-1

Serum dilution	Dead cells (%) in NRS	anti-ϱ	anti-thy-1
N	3.0	88.7	10.3
1:10	–	–	8.4
1:20	–	–	6.2
1:40	–	–	5.7

L-cells grown as described in table 1 were washed free of serum and resuspended in Eagles Minimal Essential Medium at a concentration of 2×10^6 cells/ml. Varying concentrations of anti-thy-1, anti-ϱ or normal rabbit serum (NRS) were added to 0.1 ml samples of the cell suspension and incubated at 4 °C for 1 h. 0.1 ml guinea-pig complement (diluted 1:10) was then added to each tube and incubation was continued for a further one hour at 37 °C. Cell viability was determined by trypan blue dye exclusion.

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Early foetal thrombosis induced by Thalidomide in mouse: Possible explanation for teratogenicity

C. Petter

Laboratoire de Physiologie du Développement du Collège de France et de l'Université Pierre et Marie Curie, 4, place Jussieu, F–75230 Paris Cédex 05 (France), 30 March 1977

Summary. Mouse foetuses were treated by Thalidomide on days 11–12 in order to verify whether the drug would induce blood abnormalities leading to circulatory troubles. About 18% of the treated foetuses showed both severe limb haemorrhages on day 14, and obvious alterations of the nucleated red blood cells of vitelline origin. These blood abnormalities, occurring suddenly during the well-known 'critical stage' of foetal development, could be responsible for circulatory blocks leading to necrosis.

Although the teratogenic effects of Thalidomide have been known for many years in man and animal, particularly in mice[1], and in rabbits[2], its mechanism still remains unknown. Among the different modes of action postulated, it has been suggested that this drug could be an antagonist of glutamic acid, considering the similarity of their formulae[3]. It has also been hypothesized that Thalidomide could be antagonistic to several vitamins of the B-group, particularly to riboflavin[4] or folic acid[5]. The fact that some antagonists of folic acid have been proved to be teratogenic supports the latter hypothesis[6]. Some hereditary limb amputations, greatly resembling the induced Thalidomide malformations, have been described in the rabbit[7]. It has been shown that these abnormalities could be induced by a blood defect appearing very early in the fetal life[8]. This trouble could lead to thrombosis which in turn induces hypoxia and then limb necrosis. However, these amputations can be prevented in utero by either hyperoxic treatment or treatments aiming at the reduction of abnormal erythro-

blasts, such as folic acid or vitamin-B_{12} injections in the pregnant mother[9]. Moreover, it has been shown that a drug, pyrimethamine, blocking the folic acid metabolism, could induce in the rat foetus both blood macrocytosis and necrosis leading to limb amputation[10].

In this research, we wanted to verify whether Thalidomide administered to pregnant mice would act in a analogous manner on the erythroblasts, which could in turn produce circulatory blocks and necrosis.

Material and methods. 20 C 57 Bl/6 J female mice, originating from the Jackson Laboratory (Bar Harbor, Maine, USA), were mated with males of the same strain placed in the cage one evening and removed the day after (considered as day 0 of gestation). Pregnant females were recognized by palpation on day 11 and isolated. A Thalidomide suspension of 1000 mg/kg in 1 ml of NaCl 0.9% was injected i.p., being certain to inject obliquely in order to avoid damaging the uterus. The females were injected twice, once on day 11 and once on day 12. The mice were sacrificed on day 14 with ether. The reason why this stage was chosen is that the limbs are developed and the nucleated red cells of vitelline origin are still numerous.

6 pregnant females used as controls received only the same volume of saline solution at the same stage. After laparotomy, the foetuses were removed with their adnexa for macroscopical examination; the limbs were examined. Blood smears were taken by section of axilliary vessels and stained with May-Grünwald-Giemsa.

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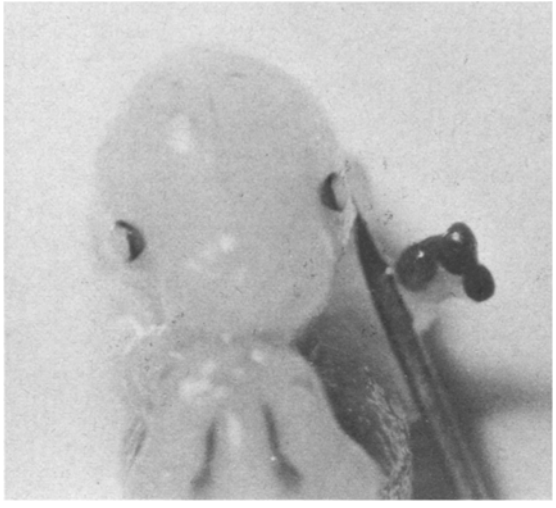


Fig. 1. Haemorrhagic blebs of the front limb in a 14-day-old mouse foetus after Thalidomide treatment.

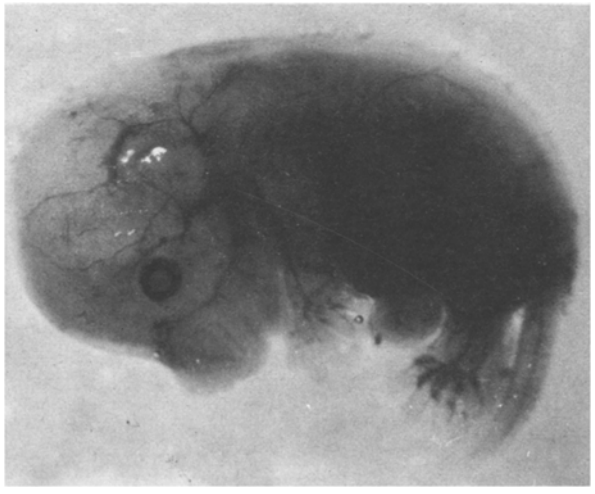
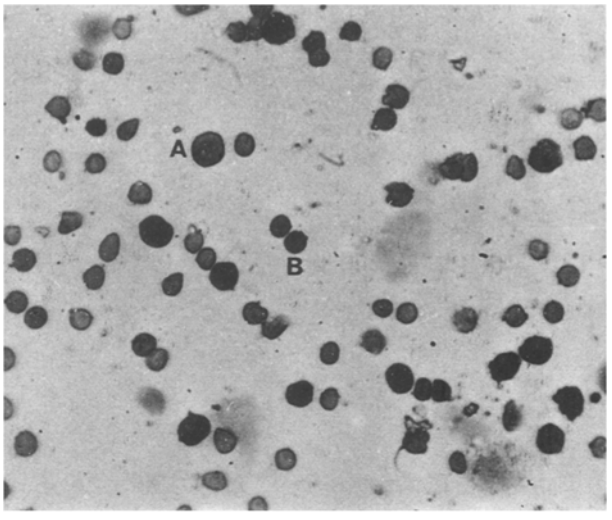


Fig. 2. Congestion of the limb vessels in a 14-day-old mouse foetus after Thalidomide treatment.



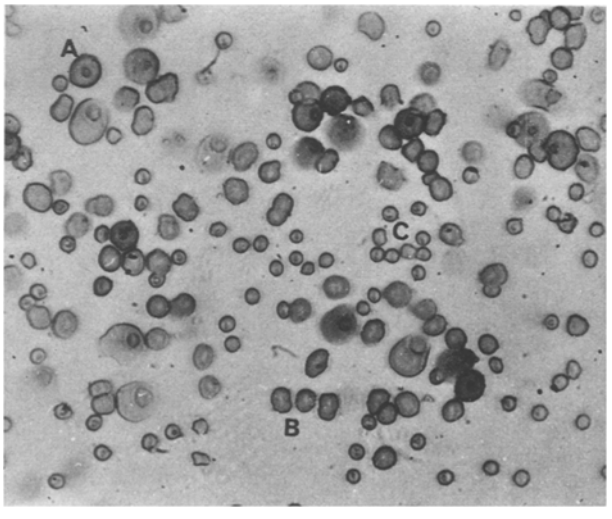


Fig. 3. *I* Blood cells of a 14-day-old control foetus. ×500. *A* Nucleated red blood cells of vitelline origin. *B* Red blood cells. *II* Blood cells of a 14-day-old foetus after Thalidomide treatment. ×500. *c* Many nuclei covered by a thin layer of cytoplasm are visible.

Results and discussion. In the controls (46 foetuses), no limb abnormality was found. 115 treated foetuses were studied and classified into 3 groups according to their macroscopical characteristics. 21 foetuses showed very severe limb haemorhages, especially in the front limb. The lesions appeared as haemorrhagic blebs of the finger tips (figure 1); they resembled the lesions of the br rabbit but generally were more extensive. 28 foetuses had no visible haemorrhage but presented a congestion of the limb vessels which appeared to be enlarged (figure 2). 66 foetuses seemed to be entirely normal when examined macroscopically.

Appearance of the blood cells on day 14. All of the blood smears observed showed numerous nuclei covered by a thin layer of cytoplasm (figure 3), the diameter of these cells being much more reduced than those of mature red blood cells. Moreover, many nucleated red cells seemed to be on the verge of expelling their nucleus. Our preliminary observations on rat foetus had shown a very important macrocytosis accompanied by expulsion of numerous nuclei. These altered young erythroblasts could induce some circulatory disturbances in the limb vessels, leading to thrombosis produced either by nuclei accumulation or by cytoplasmic fragments suddenly released in the small arteries. The abnormalities of the erythroblasts could be induced by mitotic perturbations provoked by Thalidomide. Similar effects of the drug have been observed on protozoa[11] and on blood cells of chick embryo[12]. In addition, it has been shown that the limb lesions occurred during 'critical stages' of foetal development: days 28–42 in man[13], days 11–12 in mouse and days 16–18 in rabbit[7]. These periods coincide partially with the intensive hematopoietic activity of the yolk sac in the 3 species: days 23–35 in man[14], days 8–12 in mouse[15], days 16–18 in rabbit[16].

Thus Thalidomide, similar to other teratogenic drugs taking effect on the same foetal stage, could hinder the physiological evolution of vitelline erythropoiesis, which in turn induces accumulation of abnormal cells in the vessels, and finally necrosis of the extremities. The blood abnormalities following Thalidomide administration have yet to be studied. These abnormalities, those observed after several treatments, for example pyrimethamine, and those seen in the br rabbit foetuses, support the hypothesis of a possible blood origin of certain foetal limb malformations.

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The chemokinetic effect of serum albumin

H. U. Keller, M. W. Hess and H. Cottier[1]

Department of Pathology, University of Bern, Freiburgstrasse 30, CH-3010 Bern (Switzerland), 11 July 1977

Summary. Experiments performed by means of time lapse cinematography or the filter technique show that human serum albumin has marked chemokinetic effects on neutrophil cultured in Gey's solution. The average speed of the cells, as well as the proportion of neutrophils showing locomotion, is increased. Enhanced locomotion correlates with decreased attachment to the substratum as determined by morphological and functional criteria.

Serum albumin is a major constituent of plasma with a variety of essential functions. It regulates the plasma volume by colloid osmotic pressure, binds fatty acids and other substances and represents a storage form of proteins and amino acids. Furthermore, we have reported that serum albumin promotes directional locomotion of leucocytes but lacks chemotactic activity[2]. These findings have since been confirmed and extended[3–6]. In addition, recent experiments have shown that human serum albumin (HSA) at physiological concentrations stimulated not only directional locomotion but also random locomotion of neutrophils suspended in Gey's solution. These results suggested that serum albumin has marked chemokinetic properties[5,6]. Chemokinetic effects can be induced through a variety of mechanisms[7] including changes in cell-substrate adhesivity[8,9]. The filter technique, which had been used in the past to analyze the phenomenon, provides only for indirect evidence on the locomotor behaviour of cells. We therefore studied the locomotor response of neutrophils to HSA also by means of time lapse cinematography, which allows for a direct evaluation of the response[10]. Furthermore we investigated whether the chemokinetic effect of human serum albumin (HSA) is related to neutrophil adhesion and spreading. Solutions of human serum albumin (Behringwerke Marburg, W. Germany) and Gey's solution were prepared as previously described[11]. Neutrophil locomotion was assessed with the filter technique using modified chambers and a two-filter system (8 μm and 0.45 μm pore size respectively)[11]. The culture media in the upper and lower compartment of the chamber were identical. The proportion of the neutrophils that had moved through the entire thickness of the filter has been calculated[11]. Phase-contrast pictures of cells kept in Sykes-Moore chambers

Table 1. The influence of human serum albumin (HSA) on neutrophil viability and locomotion

Culture medium	Viability (% nigrosin-positive cells)	Locomotion Filter technique (% cells migrated)	Time-lapse cinematography Average speed (μm/min)	Neutrophils showing locomotion (%)
Gey's solution	1	< 0.01	3.8	47.4
2% HSA in Gey's solution	1	4.6 ± 1	13.2	100