

Results and discussion. In the controls (46 fetuses), no limb abnormality was found. 115 treated fetuses were studied and classified into 3 groups according to their macroscopical characteristics. 21 fetuses showed very severe limb haemorrhages, 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 fetuses had no visible haemorrhage but presented a congestion of the limb vessels which appeared to be enlarged (figure 2). 66 fetuses 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 protozoa¹¹ and on blood cells of chick

embryo¹². In addition, it has been shown that the limb lesions occurred during 'critical stages' of foetal development: days 28–42 in man¹³, days 11–12 in mouse and days 16–18 in rabbit⁷. These periods coincide partially with the intensive hematopoietic activity of the yolk sac in the 3 species: days 23–35 in man¹⁴, days 8–12 in mouse¹⁵, days 16–18 in rabbit¹⁶.

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

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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 activity². 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 mechanisms⁷ 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 response¹⁰. 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 described¹¹. 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 calculated¹¹. 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

at 37°C were taken at 30 frames/min using a Leicina super 8 camera and a Diavert microscope (Leitz, Wetzlar, W. Germany). Cell shapes and contact between neutrophils and substratum was analyzed by means of phase contrast pictures and reflexion contrast microscopy¹². Neutrophil-substratum adhesion was tested as follows: 10⁶ neutrophils suspended in 1 ml of the respective medium were incubated in tissue culture chamber/slides (Lab-tek, Naperville, Ill., USA) for 30 min at 37°C. The medium was poured off. Thereafter the slides were washed twice by addition of 1 ml of the respective medium and vigorous shaking on a Vortex-type mixer on position 30 for 10 sec (Eckli-Electronics, Zürich). Neutrophils per unit area were counted on ethanol-fixed, Giemsa-stained smears. The counts are expressed as percentage of the neutrophils applied. Viability had been tested by dye (nigrosin) exclusion¹³.

The results shown in table 1 demonstrate that 2% HSA (w/v) stimulated locomotion of neutrophils suspended in Gey's solution. The speed as well as the percentage of cells showing active locomotion was significantly higher if serum albumin was present in the medium, whereas viability as measured by dye exclusion was not changed. No preference for a particular direction of locomotion had been observed. We conclude that HSA has chemokinetic properties as determined by direct observation of moving cells and that neutrophils respond in the form of orthokinesis (for definitions, see Keller et al.⁷). The quantitative differences between the filter technique and direct observation are presumably of a technical nature. If the cells move slowly, displacement is measurable by direct observation but not necessarily by the filter technique used. Furthermore the substratum is somewhat different (glass vs mixed esters of cellulose).

Phase contrast pictures showed that neutrophils in Gey's solution alone have a more marked tendency to spread

than in Gey's solution containing 2% HSA. Examination by means of reflexion-contrast microscopy revealed that neutrophils in Gey's solution alone have larger and more intense dark areas, which are characteristic for close contact between cell and substratum. Functional studies on neutrophil adhesion substantiated these findings. The proportion of neutrophils remaining attached to the substratum was lower in presence of HSA (table 2). Taken together, the findings are interpreted to mean that HSA decreases attachment of neutrophils to the substratum. It appears to be reasonable that increased attachment correlates with decreased locomotion. The more firmly a cell is attached, the more force is presumably required for detachment which must also occur in the course of locomotion.

Chemokinesis seems to be a major regulatory process influencing neutrophil accumulation in inflamed tissues⁵. The expression of the intrinsic locomotor capacity of neutrophils in the form of random or directional locomotion in vitro depends on the presence of chemokinetic factors such as HSA. It is likely that serum albumin, as a major constituent of plasma, plays also a major role in promoting neutrophil locomotion in vivo.

Table 2. The influence of human serum albumin (HSA) on neutrophils adhesion

Culture medium	Neutrophils attached (%)
Gey's solution	51.9
2% HSA in Gey's solution	6.5

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Clinical and immunological studies on persons exposed to *Parthenium hysterophorus* L.¹

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Summary. Studies on 300 persons subjected by occupational hazard to the allergenic weed, *Parthenium hysterophorus* L. for periods ranging from 3 to 12 months revealed that 4% of them developed contact dermatitis of the exposed parts of the body, while 56% of them got sensitized to the weed without apparently exhibiting any dermatitis. None of them suffered from allergic manifestations like rhinitis or bronchial asthma during the period of study which extended for 2 years.

Although it is well established that *Parthenium hysterophorus* L. (Compositae), an alien weed growing wild in many parts of India, is a very potent contact allergen which is responsible for hundreds of cases of contact dermatitis⁵, it is not known what percentage of the population exposed to this weed become affected. Since the aggressive spread of *Parthenium* is posing both agricultural and health hazards, efforts are being made to eradicate the weed by various methods, including manual uprooting. In Bangalore (India), the city corporation has employed 300 manual labourers for removing the weed from

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