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CAPILLARY TUBE MIGRATION OF HUMAN PERIPHERAL BLOOD CELLS

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Abstract. The migration area of human peripheral blood cells from capillary tubes reflects the migration of the polymorphonuclears. This may partly explain the conflicting reports on inhibition of migration of peripheral blood cells in delayed hypersensitivity, since such inhibition is not strictly comparable to that of macrophages. A method was devised for demonstrating mononuclear cells adherent to glass in a migration system. This method is suitable for the study of human macrophages in delayed allergy.

Specific inhibition of migration of macrophages by antigens was shown to be an in vitro expression of delayed hypersensitivity of animals by George & Vaughan (12). Bloom & Bennett (4) and David (10) found that the inhibition of guinea-pig macrophage migration was due to the liberation, by specific antigen, of a migration-inhibitory factor (MIF) from the sensitized lymphocytes.

The method of George & Vaughan has been modified and used for studying human delayed allergy in vitro. Thor (30) developed a method for studying inhibition of human macrophage migration in delayed allergy to microbial antigens: he used cells obtained from human lymph nodes. Thor et al. (32) also found that migration of guinea-pig macrophages can be inhibited by a factor released from human lymphocytes in the presence of allergen. This system has been used in investigations on PPD, histoplasmin and coccioidin allergy. Søborg & Bendixen (28) investigated the effect of *Brucella* antigen on the migration of human leucocytes from patients with delayed allergy to this antigen. Their modification has also been used in studies of delayed hypersensitivity in ulcerative colitis, Crohn's disease, glomerulonephritis and Hashimoto's disease (1, 2, 29). Clausen & Søborg (6) and Mookereje et al. (20) used the test successfully in studies on human tuberculin hypersensitivity. But Kaltreider et al. (13), Lockshin (17) and Nordqvist & Rorsman (23) could not demonstrate any relationship between the migration of peripheral white cells and tuberculin hypersensitivity in man. In severe contact allergy to neomycin, migration of human leucocytes was inhibited (22), but, so far, inhibition by other haptens has not been observed (Nordqvist & Rorsman, unpublished).

That the correlation found between inhibition of human leucocyte migration and delayed hypersensitivity is less good is not surprising, since the methods used demonstrate not only the migration of the macrophage-type of blood cells, but also the migration of polymorphonuclears and lymphocytes.

This paper describes a method for observing human macrophages formed from peripheral blood in a migration system.

MATERIAL AND METHODS

The blood donors were healthy volunteers and patients with various dermatoses. 40 ml of venous blood was obtained in heparinized glass tubes, where it was allowed 1 hour at 37°C to sediment. The supernatant was afterwards transferred to centrifuge tubes. The white cells were separated by centrifugation at 350 *g* for 5 min and washed three times in Parker 199. A suspension containing 12×10^6 cells per ml was prepared in Parker 199, to which autologous serum was added to a final concentration of 30%. The suspension was transferred to capillary tubes. After centrifugation at 1 500 *g* for 5 min the tubes were cut at the cell–fluid interface and the portion containing the cells was placed in 1 ml chambers. The chambers, prepared by fixing a glass ring on a slide with the aid of a small amount of petrolatum and filled with Parker 199 and autologous serum to a final concentration of 30%, were closed with small coverslips and maintained at 37°C. Within a few hours the cells migrated in a fanshape fashion on the bottom of the glass chamber.

Table I. *Migration area (mm²) of different blood cells in 24 hours*

Subject no.	Compact cell layer	Lympho-cytes	Mono-cytes	Granulo-cytes
1	7	18	24	54
2	6	23	43	48
3	2	12	32	69
4	8	18	39	81
5	5	17	36	95
6	3	20	35	79
7	7	16	28	93
8	17	32	57	86

In one series of experiments about 1/10 of the incubation fluid was pipetted off after 24 hours and replaced by concentrated formalin. Fifteen minutes later all fluid was decanted, the chamber was gently loosened from the slide and the specimens were stained in hematoxylin-eosin. These slides were examined under high magnification and differential cell counts were made proceeding from the open end of the capillary tube.

In another series of experiments capillary tubes prepared in the way described above were incubated at 37°C for 48 hours, whereafter most of the incubation fluid was exchanged. After 96 hours the slides and the chambers were turned upside down. After a further 6 hours in this position the coverglass was carefully loosened and the chamber fluid, which contained cells not adhering to the slide, was decanted. Any slightly adherent cells were washed off with Ringer solution, after which the cells still adhering to the glass were fixed in 96% ethanol for 30 min. Staining was then performed in hematoxylin-eosin.

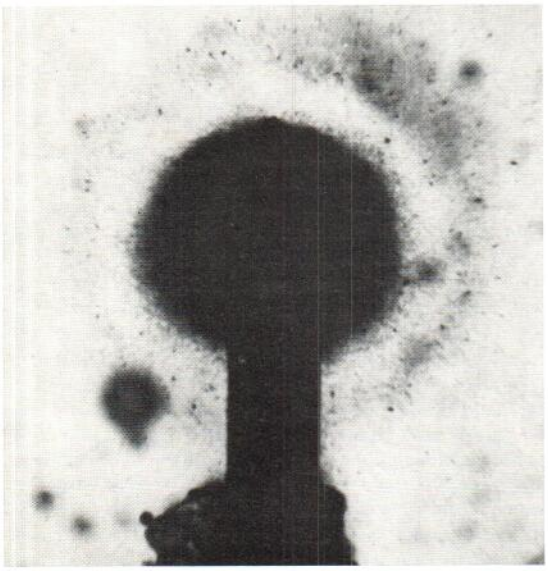

Fig. 1. Migration picture of living cells in culture after 24 hours. × 10.

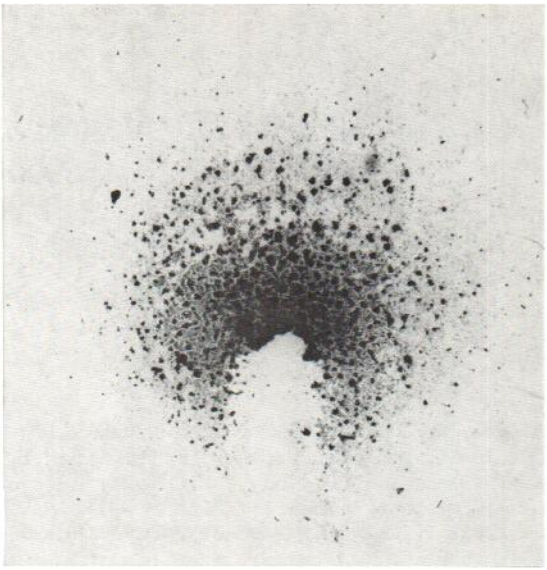

Fig. 2. Migration picture of fixed and stained cells after 24 hours. The specimen is reproduced in unstained condition in Fig. 1. × 10.

Microphotographs of the migration area were taken with a × 1 objective after 24 hours, and after 102 hours when only glass adherent cells were recorded. Cytologic examination was performed under a high power microscope.

RESULTS

Results of cytologic examinations of specimens fixed in formalin after 24 hours migration are shown in Table I. Areas of migration were calculated using the migration distance as radius without correction for the area occupied by the capillary tube. The area close to the open end of the capillary tube consisted of a compact mass of cells, and no details could be discerned. Further out, all types of white cells were present; in the next zone, only monocytes and polymorphonuclear leucocytes; and most distant from the end of the tube, only polymorphonuclears. When photomicrographs of unstained specimens were taken after 24 hours (Fig. 1) the area visualized corresponded to that calculated for polymorphonuclears. Fig. 2 shows a photomicrograph of the fixed and stained specimen, reproduced in unstained condition in Fig. 1.

Fig. 3 shows the distribution of cells adherent to the bottom of the glass chamber after 4 days' migration.

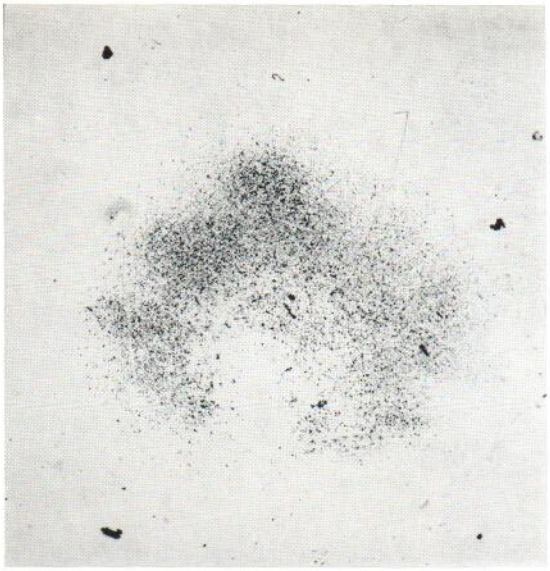

Fig. 3. Migration picture of glass adherent cells after 102 hours. × 10.

Examination of the migrated and adherent cells at higher magnification showed that the majority of the cells were mononuclear and most of them of macrophage appearance (Figs. 4 and 5). The cytoplasm was often abundant and vacuolized. Many cells had pseudopods. The nuclei differed from one another in shape, density and size. The preparations sometimes contained a small number of polymorphonuclear leucocytes. A few multinucleated "giant cells" were seen.

DISCUSSION

Inhibition of the migration of unseparated human peripheral leucocytes has been used to detect various types of delayed hypersensitivity (1, 2, 6, 20, 22, 24, 27, 28, 29). The present study shows that polymorphonuclear leucocytes form the outline of the migration area of peripheral blood cells that migrate from capillary tubes. The results of migration experiments using blood cells are thus not comparable with those using peritoneal or lymph node macrophages.

Both monocytes and polymorphonuclear leucocytes adhere to glass, but when the experiment was continued for 4 days the polymorphonuclears died. Therefore, when the chambers were inverted, it was mainly the macrophages that were still adherent to the slide.

The origin and the nature of mononuclear blood cells that adhere to glass have been discussed extensively since the earliest days of tissue culture (3, 5, 7, 8, 9, 11, 14, 15, 16, 18, 19, 21, 25, 26, 33). In their migration studies Thor & Dray observed that a large proportion of preincubated lymphocytes from lymph nodes developed the ability to phagocytize small iron particles, and they thought that some of their macrophages were derived from lymphocytes (31). This may have occurred also in our experiments.

The method described above for demonstrating the migration of human mononuclear blood cells from capillary tubes is at present utilized in the investigation of delayed hypersensitivity reactions

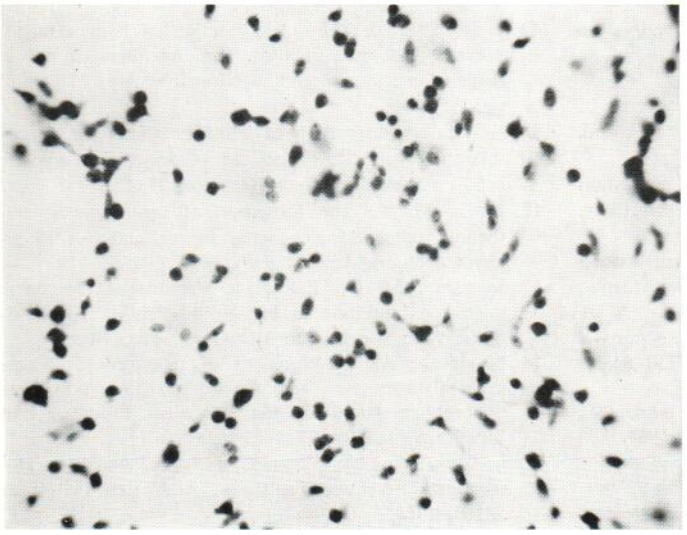

Fig. 4. Glass adherent cells after 102 hours. × 78.

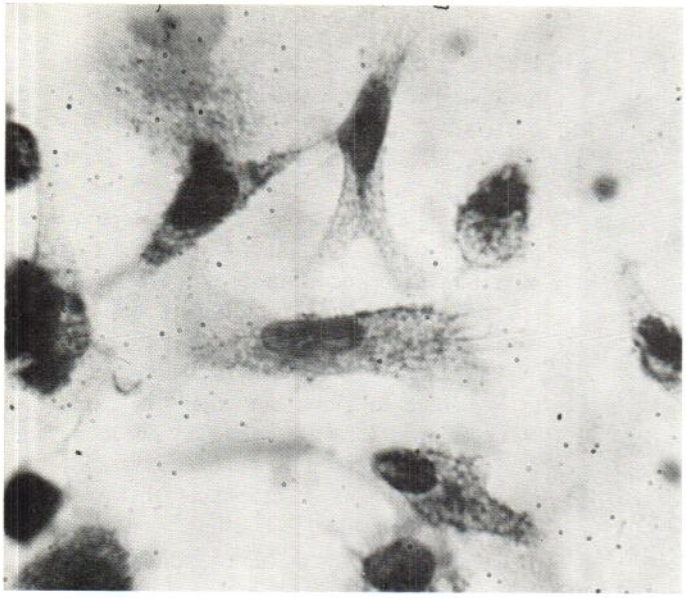

Fig. 5. Glass adherent cells after 102 hours. × 940.

in humans, and aggregation of macrophages has proved to be an in vitro sign of tuberculin allergy (23).

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