The Cytokineplast: Purified, Stable, and Functional Motile Machinery from Human Blood Polymorphonuclear Leukocytes

POSSIBLE FORMATIVE ROLE OF HEAT-INDUCED CENTROSONAL DYSFUNCTION

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ABSTRACT We examined the formation of motile, chemotactically active, anucleate fragments from human blood polymorphonuclear leukocytes (PMN, granulocytes), induced by the brief application of heat. These granule-poor fragments are former protopods (leading fronts, lamellipodia) that become uncoupled from the main body of the cell and leave it, at first with a connecting filament that breaks and seals itself. The usual random orientation of such filaments can be controlled by preorientation of cells in a gradient of the chemotactic peptide, N-formylmethionylleucylphenylalanine (F-Met-Leu-Phe) (2 × 10^{-9} M-1 × 10^{-8} M). Cytochalasin B, 2.5-5 μg/ml, prevents fragment formation; colchicine, 10^{-5} M, does not. In scanning electron micrographs, fragments are ruffled and the cell body rounded up and rather smooth. In transmission electron micrographs, fragments contain microfilaments but lack centrioles and microtubules. Like intact cells, both bound and free fragments can respond chemotactically to an erythrocyte destroyed by laser microirradiation (necrotaxis); the free, anucleate fragments may do so repeatedly, even after having been held overnight at ambient temperatures. We propose the name cytokineplast for the result of this self-purification of motile apparatus.

The exodus of the motile machinery from the granulocyte requires anchoring of the bulk of the cell to glass and uncoupling, which may involve heat-induced dysfunction of the centrosome. In ultrastructural studies of the centrosomal region after heat, centriolar structure remains intact, but pericentriolar osmiophilic material appears condensed, and microtubules are sparse. These changes are found in all three blood cell types examined: PMN, eosinophil, and monocyte. Of these, the first two make fragments under our conditions; the more sluggish monocyte does not. Uncoupling is further linked to centrosomal dysfunction by the observation that colchicine-treated granulocytes (10^{-5} M, to destroy the centrosome’s efferent arm) make fragments after less heat than controls.

If motive force and orientation are specified mainly from the organelle-excluding leading front, then endoplasmic streaming in PMN is a catch-up phenomenon, and microtubules do not provide the vector of locomotion but rather stabilize and orient the “baggage” (nucleus, granuloplasm)—i.e., they prevent fishtailing. Moreover, constraints emanating from the centrosome may now be extended to include maintenance of the motile machinery as an integral part of the cell.
tion), and phagocytosis. The sensory, transducer, and effector mechanisms involved are subjects of intense current interest, and a great deal of progress has been made in the identification of various components (1-4). However, some parts of the motile apparatus are unknown, and the PMN is replete with background molecules, including hydrolytic enzymes that may, in disrupted cells, obscure or destroy critical molecular evidence. Ideally, one would like the motile machinery to concentrate itself and then to leave the rest of the cell, yet demonstrate its integrity by maintaining its functional capacity.

We suspected that Keller and Bessis (5, 6) had come upon just such a self-purification of the motile apparatus, in PMN subjected to the carefully controlled and timed application of heat. The resultant membrane-bound, anucleate, cytoplastmic fragments contained no, or few, granules and thus resembled the hyaloplasm (peripheral cytoplasm) of intact cells, which is especially prominent in the protopod (leading front, lamellipodium) of migrating leukocytes; their provenance from such protopods was indicated microscopically. Most important, the newly formed fragments were capable of membrane movement, including adherence, spreading, random locomotion, chemotaxis, and phagocytosis. Thus, at least in the short term, these functions did not depend upon the presence of a nucleus.

That work was done under direct microscopic observation, with video and cinematographic attachments to permit timed recording of the orientation and locomotion of all moving parts in the field. Chemotaxis of individual fragments was observed toward erythrocytes freshly destroyed by laser microirradiation, a process that Bessis (7) has called necrotaxis. In confirming and extending these findings, we are also interested here in the topography of the fragmenting leukocyte, in the mechanism of locomotion and functional stability of the fragments, and in the effects of heat that permit their exodus.

MATERIALS AND METHODS

Preparation of Human Blood Leukocytes

25 ml of heparinized venous blood from normal donors was allowed to sediment at room temperature for 2 h, in tubes angled at ~60°; the plasma and leukocyte-rich buffy coat were reserved. Just before use, the cells were gently centrifuged, the plasma was removed, and a few drops were added of NCTC 135 medium (Eurobio, Paris) containing 5% fetal calf serum (referred to subsequently as “medium”). The resuspended cells were used in two types of slide preparation, designated thin and thick. For thin preparations, only enough suspension was added to cover the area beneath the cover slip; the edges were sealed with paraffin. These preparations were used when cells or fragments were to be observed over extended periods of time, as for the study of locomotion or chemotaxis. Thick preparations were used when the cover slip would have to be removed, as for electron microscopy. Two spacing strips of Parafilm “M” (American Can Co., Greenwich, CT) were placed on the slide as to support the ends of a 24×60-mm cover slip. The resuspended cells were added, and the cover slip placed but not sealed; except for the brief heating to produce fragments (see below), incubations of thick preparations were done in moist petri dishes in a CO2 incubator. For both preparations, the adherent leukocytes (granulocytes and monocytes) take hold of the glass almost immediately; they were always equilibrated for several minutes at 37-38°C before further manipulation.

Alternatively, PMN were prepared from drops of freshly shed blood (from a finger prick) allowed to clot on glass in a moist petri dish at 37°C for 30 min. The clot was gently washed away, leaving a field of adherent granulocytes (but few monocytes) (8). Medium was added, and thin or thick preparations were made.

Preparation of Motile Fragments

After considerable preliminary work to determine the best conditions for the production of motile fragments by granulocytes, we chose an incubation time of 9 min on a (detached) heidelase microscope stage whose surface registered 44.5-45°C. As used subsequently in the text, “heating” or “heated cells” refers to these conditions, except as noted. For the further development of fragments (see Results), slides were either returned to incubators at 37-38°C or transferred to a microscope whose ×40 water-immersion phase-contrast objective was heated by a coil that kept the immersing water at 33-34°C. Motile functions were followed and recorded as described below under chemotaxis.

The heat actually delivered to the cells depends of course upon its source and upon the physical properties of the enveloping materials. In a series of cell orientation experiments with the Zigmond chamber (see below), whose base is plastic, relatively thick, and holds more medium than slide preparations, we produced fragments by heating the ensemble for 9 min in a moist petri dish within a closed water bath set at 47°C.

EFFECTS OF CYTOCHALASIN B AND COLCHICINE: Cytochalasin B (Sigma Chemical Co., St. Louis, MO) was dissolved at 3 mg/ml in dimethyl sulfoxide (DMSO; J. T. Baker Chemicals N.V., Deventer, Holland) and diluted in medium as described; colchicine (Sigma Chemical Co.) was both dissolved and diluted in medium. Each was sealed in thin preparations for examination of its effects on fragment formation and function, the former with concomitant DMSO controls.

Orientation

We used a Zigmond chamber (9), made from a plastic block containing a bridge—the area of observation—between two parallel wells; its roof was an inverted cover slip bearing the adherent cells. Cells were oriented over ~30 min at room temperature, toward a well that contained the chemotactic peptide, N-formylmethionylleucylphenylalanine (F-Met-Leu-Phe; Sigma Chemical Co.), dissolved in DMSO at 10^{-5} M and diluted in medium (the other well contained only medium).

After heating (see above), the chamber was returned to room temperature and the contents of the well that initially received peptide were replaced with fresh material at a higher concentration, 1×10^{-4} M (to insure the establishment of a new gradient at room temperature). The percentage of consecutive, mid-bridge cells that were oriented—i.e., those pointing in the general direction of the peptide-containing well (excluding 10° from the vertical)—were enumerated before heating and again after establishment of the new gradient.

Chemotaxis

A chemotactic gradient lasting several minutes was produced by destruction of an erythrocyte by a ruby laser (6,943 Å) that emits 3 Joules in 500 μs and focuses (using the microscope’s optics in reverse) to a diameter of 5 μm (10). The gradient lasted longer if two adjacent erythrocytes were destroyed, still longer if an adjacent leukocyte was killed. The response of individual PMN and fragments in thin preparations was followed in phase-contrast microscopy in a Zeiss Photomicroscope I and recorded either by time-lapse cinematography (Ariflex camera) on Kodak 16mm Plus-X TV reversal film or in serial photograms (built-in camera) on Kodak 35mm panatomic-X film.

Electron Microscopy

For scanning electron microscopy, thick preparations were immersed in 1% glutaraldehyde in NCTC 135 for 30 min at 37°C, washed with NCTC 135, and the most interesting areas of the cover slip were identified in the light microscope and cut out (~1 cm² each) for further use. These sections were washed three times in Sorenson’s phosphate buffer, 0.1 M, postfixed in 1% osmium tetroxide (30 min, room temperature), dehydrated in increasing concentrations of alcohol, and immersed in Freon 113. They were then dried by a rapid critical point method (11) with Freon 13 as transition fluid, coated with gold-palladium, examined in a Cambridge Stereoscan instrument operated at 25 kV, and photographed with Ilford FP4 120mm panoramic safety film.

For transmission electron microscopy, fixation was done at room temperature (~20°C), but otherwise the preparations were the same as for scanning. Slides were washed twice in cacodylate buffer, 0.2 M, postfixed in osmium tetroxide, dehydrated in increasing concentrations of alcohol, and immersed twice in propylene oxide and then for 30 min in a 1:1 mixture of propylene oxide and Epon. Capsules containing Epon were applied to previously marked areas of the slide, and the amorphous was incubated at 37°C overnight and at 60°C to the point of semipolymerization. The slides were placed on dry ice to separate the glass from the Epon capsules in which the cells were now embedded; the latter were then returned to 60°C for an additional 24-48 h. Sections of 500-1,000 Å were made (Diatome, Reichert Optical Co., Vienna), stained with uranyl acetate and lead citrate, and examined and photographed in an Elmiskop 101 electron microscope (Siemens, Berlin).

RESULTS

Formation and Functional Characteristics of Cytoplasmic Fragments from Human PMN

The fragments are derived from protopods that become uncoupled and begin to leave the main body of the PMN

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during the 9-min heating period, taking with them variable numbers of cytoplasmic granules—usually few, sometimes none (5). Just after heating, the typical fragment is attached to its cell of origin by a narrow bridge that becomes a thin filament. There is usually one attached fragment per cell, sometimes two. Their orientation appears random but can be controlled. For example, we preoriented PMN (74 of 75 consecutive cells) at room temperature toward a well containing the chemotactic peptide, F-Met-Leu-Phe (see Materials and Methods). Just after heating, the new and still-connected fragments were in general similarly oriented with respect to their cells of origin. An hour after a new gradient had been initiated (and filaments had had more time to extend themselves), that orientation persisted (91 of 100 consecutive bound fragments). It is clear that the large majority of adherent PMN are capable of producing fragments. The eosinophil leukocyte can also produce

![Image of various stages of fragment development in human blood PMN](image)

**Figure 1.** Various stages of fragment development in human blood PMN are seen in the scanning electron microscope. (a–c) Normal PMN. Ruffled membrane is integrated over the leading front and cell body, whether the PMN is in the classic position of orientation (a; protopod to right, uropod to left) and locomotion, oriented and spreading (b; see Fig. 2 d); or apparently pulling against an adherent uropod (c; see Fig. 2 f). (d–l) Heated cells. Ruffled membrane is uncoupled from the cell body, whose surface is smooth or tufted. With mutual distancing, the connection between cell body and fragment is attenuated. Free fragments (j–k) may exhibit “tails” (upper left and bottom, respectively; see Fig. 4). In l, a family of heated cells is seen. Bars, 5 μm.
them under these conditions; the relatively sluggish monocyte does not.

In contrast to the motile, still-attached fragment, the cell body is generally rounded-up, refractile, and motionless. Should part of the motile material remain with, or retreat to, the cell body, the former may move about the surface of this otherwise stationary hulk and perhaps take leave again.

Surface characteristics of control and heated PMN, and of fragments in various stages of their development, can be seen in the series of scanning electron micrographs shown in Fig. 1. In intact PMN a veil of ruffled membrane is integrated over the leading front and cell body (Figure 1a–c). In heated cells the ruffled membrane is uncoupled from the cell body, whose surface is smooth or tufted (Fig. 1d–i). With mutual distancing, the connection between cell body and fragment is attenuated.

**FREE FRAGMENTS:** If the heated cells are maintained at 37–38°C for 15–60 min, maximal numbers of free fragments are produced. This process includes further attenuation of the

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*Figure 2.* Chemotactic behavior of an intact human blood PMN is seen in time-lapse cinematography. (a) The last image before laser destruction of the central erythrocyte (zero time) is shown. (b and c) Local granulocytes approach from above and from two corners. When one strikes (d), and begins contact circling (e and f), the chemotactic gradient is apparently disrupted, and others turn aside (alternatively, several may arrive and vie for contact). As the target loses its chemoattraction, contact circling lessens (g), and the cell begins to leave (h; its protopod faces the uropod of a passing granulocyte). The exhausted target, dislodged, sticks to the tip of the cell's own uropod. The PMN changes direction (i) and heads above and to the left of the erythrocyte seen at upper right (the same red cell is in the lower central part of the new field seen in j). The last three frames (j, k, and l) show the PMN pulling against the temporarily re-anchored uropod. Phase contrast, approximately x 750.
connecting filament—which may (occasionally) reach 20 cell diameters in length—its rupture, and the sealing and retraction of its ends. If the resultant anucleate fragments contain trapped granules, the more centrally located ones exhibit rapid, apparently Brownian movement. The free fragments are randomly motile and chemotactically active for at least as long as intact unheated PMN. Held at ambient temperature and re-warmed before use, they may retain their functional capacities for over 24 h, longer than we could show for the intact cells.

The chemotactic response of fragments is similar to that of unheated PMN (Figs. 2 and 3). For both, ingestion of a necrotactic red cell remnant is rare, presumably because they are responding not to the remnant itself but to components of its transient effluent. Thus, on arrival they tend either to run around it, which seems to entail a leading edge that rolls forward along the surface and lets go farther back (contact circling), or to swarm over it. Alternatively, several cells or fragments may vie for contact. As the remnant loses its che-

![Figure 3](https://example.com/figure3.jpg)

**Figure 3** Chemotactic behavior of an anucleate cytoplasmic fragment made 20 h earlier is seen in time-lapse cinematography. (a) The fragment is moving to the right. In the last image before laser destruction of the central erythrocyte (b, zero time), a random process has begun to protrude toward lower left. That process enlarges, withdraws, and another appears that carries the fragment to the left (not shown), whence it closes on the target (c–f). On striking (g) and increasing its area of contact (h), the fragment dislodges the target, so that, while circling (i), it drags the target about. As the target loses its chemoattraction, the fragment envelops it less (j), and finally takes leave (k and l). (Fragments with “tails” [see text and Fig 4] may remain bound by them to the target [compare Figure 2 h–l].) This fragment responded to three more laser targets over the ensuing 46 min. Phase contrast, approximately x 750.
moattraction, the cell or fragment wanders away. It may, however, respond to subsequent stimuli; the day-old fragment shown in Fig. 3 did so three more times over the ensuing 46 min.

Depending perhaps upon the point of rupture of the connecting filament, the free fragment may bear a retracted remnant, or “tail” (5) (Figure 1/ and k), which is found thereafter at the rear of the moving fragment. Unlike the fragment proper, tails are nonmotile but often “sticky;” they may impede the fragment’s progress by anchoring to glass, to a laser target that has ceased to be chemotactic, or to the tail of another fragment heading in the opposite direction (Fig. 4). In this respect they resemble the hindmost point (uropod tip) of an intact migrating PMN (Fig. 1 c, Fig. 2 b–f).

Figments without tails locomote by a repeating pair of actions, a protrusion of the surface in the direction of locomotion, followed by a contraction that brings forward the remainder of the fragment (Fig. 3 c–f). Should that contraction not occur, there is no overall displacement, and the protrusion may be withdrawn, perhaps as a new one appears in another direction. In a chemotactic gradient, even in the same cell at different times, the two phases may overlap somewhat, resulting in a rather smooth progression, or occur discretely, seriatim. The initial reaction to a fresh, laser-induced chemotactic stimulus appears to be the protrusion, which in the extreme, in time-lapse cinematography, may simulate a startle reaction.

BOUND FRAGMENTS: If the heated cells are maintained at 33–34°C (the approximate temperature in the immersing water of the objective lens) instead of 37–38°C, the connecting filament between the fragment and its cell of origin is more likely to remain intact. Bound fragments have the same functional capacities as free ones, except for the physical constraint imposed by the connecting filament. They can be freed artificially by laser destruction of an erythrocyte that is adjacent to the connecting filament (Fig. 5). The filament breaks, seals itself, and retracts as in spontaneous rupture (or sometimes fails to seal itself; the fragment then swells and moves more). The freed fragment may turn and approach the target whose irradiation freed it (the actively motile one in Fig. 5, bound to glass at the juncture with its new tail, did not; it eventually pulled free after the chemotactic stimulus had abated, and wandered off).

Finally, if the heated cells are left at ambient temperatures for 2 or 3 min, fragments may begin to let go of the glass and to retract to or toward the cells to which they are bound. On being re-warmed, they may adhere to the glass and move away again.

Effects of Heat

ANCHORING: The conditions of heating are such that motile activity is preserved — indeed, it increases as the temperature rises — while the bulk of the cell is becoming unusually adherent to substrate. Without this anchoring, the motile part has nothing to pull against, and separation does not occur. Thus, cells heated in suspension and then put between slide and cover slip at 37°C put out fragments, if at all, only after they have had time to settle and adhere to the glass.

Anchoring is especially well demonstrated in the chemotactic response of fragments still bound to their cells of origin (Fig. 6). At 33–34°C the motile part heads for the target, the bulk of the cell tends to stay put, and the connecting filament stretches (Fig. 6). Eventually, the bound fragment may pull its cell of origin loose. The latter jerks forward as the filament contracts toward its receding, adherent, motile part. (Tails of free fragments may behave in a similar fashion.) When reanchoring ceases, the bulk of the cell becomes a float, pulled along behind the advancing fragment, by a shorter filament no longer under tension. (Sometimes the cell body lets go before a filament has formed; in that case, one sees a wide-necked protopod with a rounded-up refractile cell body riding just behind it. Until they move and thereby demonstrate their lack of anchoring, such cells resemble those seen in Fig. 6 a). These methods of progression lead to the result seen in Fig. 7, which involved laser destruction of an erythrocyte and an adjacent leukocyte, to provide a stronger and more prolonged chemotactic stimulus than usual. Fragments having arrived from all directions are clustered about the target. Surrounding them are their cells of
origin, dragged or floated into place behind the motile parts. The fragments are not necrotic but intact and may wander away as chemoattraction subsides.

**Uncoupling:** In preliminary studies to determine the optimal conditions for fragment formation, we confirmed the earlier observation (5) that decreases in temperature of only 1°C, or in time of a minute or so, usually led to dramatic decreases in the number of fragments. Instead, at suboptimal temperatures or with heating times of 5 to 8 min, just after heating we often saw PMN like those in Fig. 8a. The cells were actively motile, but the end opposite the protopod was anchored to glass. Instead of the protopod becoming uncoupled and going off on its own, it remained an integral part of a highly attenuated but intact cell, pulling against its fixed end. After further incubation at 37–38°C, if the PMN was unable to pull free, one sometimes saw a refractile nubbin of anchored uropod connected by a filament to the rear end of a PMN whose motile leading edge (protopod) had remained integrated with an otherwise normal-appearing cell (Fig. 8b). These observations suggested that anchoring alone was not sufficient for fragment formation; at the critical time and temperature, something else seemed to happen that allowed uncoupling of the protopod.

A possible clue to the mechanism of uncoupling came from immunofluorescence studies of the monocytes in these preparations (PMN being poor specimens for immunofluorescence), with antibodies directed against pericentriolar osmiophilic material and against microtubules (12). After heat, the demonstration of both structures by immunofluorescence was severely compromised, even though putative paired centrioles were still visible in phase contrast. These findings in monocytes suggested that uncoupling in PMN might be related to heat-induced centrosomal damage mediated through resultant interference with the apparent constraints upon the membrane imposed by the assembly-disassembly of microtubules (1, 13).

Ultrastructural studies provided more direct evidence for the underlying heat-induced cellular changes that had been inferred from the examination of function and shape, and from indirect immunofluorescence. In thin sections of spread PMN, granuloplasm is generally pervasive (Fig. 9a), except for the centrosome, the region just below the plasma membrane, and, in oriented cells, the relatively narrow leading front (see Fig. 2). After heat (Fig. 9b) there is a rather discrete boundary between a relatively large area that lacks organelles (corresponding to the uncoupled ruffled membrane [e.g., Fig. 1d]) and the rounded, nucleated, granule-rich cell body. Like the leading front of intact PMN (1), fragments at greater magnification contain filaments best seen subjacent to the cell mem-

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**Figure 5** A bound fragment is freed by laser destruction of two erythrocytes (white arrows) near the connecting filament. (a) Before microirradiation. The fragment (F) is at lower left, its cell body (CB) at upper right. (b, c, and d) Over a few minutes, the cut filament at left (black arrow) retracts toward the motile fragment. Such fragments may then turn and approach the target whose irradiation freed them; this one, bound to the glass at the juncture with its new tail, did not. The proximal retracted end of the cut filament (upper right) becomes fixed to a filament from lower right, whose fragment crossed it on the way to the target. This preparation happened to have been pretreated with colchicine, which does not interfere with fragment formation or chemotactic responsiveness. Phase contrast, approximately x 400.
Shortly after laser microirradiation of erythrocytes (center), motile filament. Phase contrast, approximately x 400.

We examined the centrosomal region in thin sections from several control and heated cells. Compared to the centrosomal regions of control cells (Fig. 10), those of heated cells have two consistent abnormalities: (a) microtubules are sparse; and (b) the pericentriolar osmiophilic material from which microtubules ordinarily radiate appears condensed and either fragmented or clotted (Fig. 11). These changes are found in all three cell types examined: PMN, eosinophil, and monocyte. Structure of the centriole itself appears preserved, and its paranuclear location is maintained.

We sought further evidence of our interpretation of uncoupling, in PMN treated with colchicine; in appropriate doses this drug reliably disassembles microtubules in these cells (14, 15). If our hypothesis were right, then colchicine-treated cells should make fragments under conditions of heating that were suboptimal for fragment formation in drug-free controls but sufficient to produce some degree of anchoring. We kept the usual heating temperature but, after some exploratory work, reduced the time of incubation to between 2.5 and 3 min instead of the usual 9 min. This is about the time required for the glass slide to go from 37°C to 45°C. Under these conditions, virtually no normal PMN made fragments. We then made eight successive pairs of thin preparations with or without the usual heating temperature but, after some exploratory work, reduced the time of incubation to between 2.5 and 3 min instead of the usual 9 min. This is about the time required for the glass slide to go from 37°C to 45°C. Under these conditions, virtually no normal PMN made fragments. We then made eight successive pairs of thin preparations with or without colchicine, 10−5 M, pre-warmed them at 37°C for 30 min, and then subjected each pair to the brief heating described above. Directly after heating, each pair was given to one of us, who was asked to identify the colchicine-treated preparation. The differences were qualitative; fragments were rare or not present in drug-free controls but easily found after colchicine. On that basis, the colchicine-treated preparation was identified correctly eight out of eight times, and again after subsequent incubation of the pairs for one-half to 1.5 h (P for each reading: 1/8, < 0.004).

**DISCUSSION**

We have examined the heat-induced formation and function of motile anucleate fragments from human blood PMN. We knew from initial work that the fragments are derived from protopods that leave their cells of origin (5); we have now documented various stages of that exodus in the scanning electron microscope (Fig. 1). The delicate veil that ornaments the surface of normal PMN (16) is uncoupled from the bulk of the cell; the latter is rounded up and rather smooth. The initial direction taken by a parting fragment presumably follows the orientation of the protopod that it was. When we preoriented granulocytes toward a well containing the chemotactic peptide F-Met-Leu-Phe, the still-connected fragments after heating were similarly oriented with respect to their cells of origin.

The method of locomotion of free fragments—protrusion of the surface in the direction of locomotion followed by a catch-up contraction of the hind portion—is much like that described for the intact PMN (17). The lack of a motive role for endoplasmic streaming is even more clear here, where endoplasmic granules are virtually or actually absent.

**Functional Longevity of Fragments**

We also knew these anucleate fragments could continue to function just after their separation (5); we wondered how ephemeral that functional capacity was. We found that fragments could be left at ambient temperatures overnight in sealed preparations and, on re-warming a day later, reduced the time of incubation to between 2.5 and 3 min instead of the usual 9 min. This is about the time required for the glass slide to go from 37°C to 45°C. Under these conditions, virtually no normal PMN made fragments. We then made eight successive pairs of thin preparations with or without colchicine, 10−5 M, pre-warmed them at 37°C for 30 min, and then subjected each pair to the brief heating described above. Directly after heating, each pair was given to one of us, who was asked to identify the colchicine-treated preparation. The differences were qualitative; fragments were rare or not present in drug-free controls but easily found after colchicine. On that basis, the colchicine-treated preparation was identified correctly eight out of eight times, and again after subsequent incubation of the pairs for one-half to 1.5 h (P for each reading: 1/8, < 0.004).

**Anchoring and Uncoupling**

As noted earlier, the conditions of heating are such that motile activity is preserved—indeed, it increases as the temperature rises (maximum at 42°C; reference 19)—while the bulk of the cell is becoming unusually adherent to substrate. We have used the word “anchoring” (Fig. 6) to differentiate this high-heat-induced stickiness from the normal process of adherence that allows locomotion of PMN and their fragments on surfaces. Other heated structures that may become anchored include the connecting filaments of bound fragments, the filament-derived tails of free fragments (Fig. 4), and the uropod tips of suboptimally heated cells (Fig. 8). In unheated PMN the stickiness of the uropod tip (17, 20) (Fig. 2j–l) may be a similar phenomenon, in which superadherent material is transported to the rear as the cell moves away from it.
Given the above, one might ask whether fragment formation is simply an extreme example of a normal process in which superadherent, perhaps denatured membrane is left at the rear of the moving cell and torn away, as may also occur, for example, in locomoting fibroblasts (21). There is reason to doubt this simplest of formulations. First of all, the cell's motility during heating should tend to maximize anchoring of whatever portions of the membrane are susceptible to it. This flypaper effect might be expected to result in a cell body that is spread out on the glass. Instead, the bulk of the cell is rounded up; relatively little of its surface is anchored (Fig. 1). Moreover, the topographic appearance of cells with less separated ruffled parts (Figure 1 d and e) resembles that of certain PMN after 20 min of equilibration at 45°C in suspension (22), where opportunities for anchoring were presumably minimized. Thus, what we call uncoupling may not require a substratum, although progressive distancting probably does.

Second, if fragment formation were merely the result of having created increasing amounts of superadherent membrane, one would expect to see a continuum in the number of fragments formed, depending upon the degree and duration of heating. This does not occur. Under optimal conditions the large majority of granulocytes make fragments; when the target temperature is decreased by about 1°C, or the heating time by a minute or two, very few of them do (5), and one may see long uropods instead (Fig. 8). At higher temperatures or longer times, fragments quickly lose their function or do not appear. The strictness of the conditions for fragment formation supports the contention that some discrete event has occurred which permits uncoupling of the still healthy protopod from the bulk of the cell. Thus, the differential effects of heat appear to include, first of all, progressive anchoring without uncoupling, then uncoupling, and finally inactivation of the motile apparatus; tolerance between the last two events is small.

**Mechanism of Uncoupling**

We propose that uncoupling of the motile machinery from a rounded-up cell body results from heat-induced dysfunction of the centrosome. In immunofluorescence studies with anticentriole antibody, paired centrioles were not demonstrable in the heavily granular PMN, but nearby monocytes had them (12). We found corresponding paired structures in phase contrast. After heat, monocyte granules were retracted about a less indented nucleus, and centriole immunofluorescence was generally gone (as was microtubule immunofluorescence with appropriate antibody), but putative paired centrioles were at least as phase-dense as controls. That these prominent paired structures indeed marked the position of centrioles was established by their location, by their occasional residual weak fluorescence, and by the rare cell containing them that had “resisted” the usual effects of heat.

In other cells, the anticentriole antibody that we used appears to be directed against antigenic determinants on osmiophilc pericentriolar material which probably serves as microtubule organizing centers (MTOCs). Accordingly, the apparent diameter of immunoperoxidase staining increases (23, 24) up to 10 times (R. Maunoury, personal communication) in human fibroblasts and tumor cells as they begin dividing; this is a period of proliferation of that material (25). Heat damage to pericentriolar material would explain why “centrioles” disappear when viewed by indirect immunofluorescence but persist—and may appear enhanced—in phase contrast.

When the ultrastructure became available, this interpretation...
was supported not only in the relatively sluggish monocyte, which does not fragment under these conditions, but also in PMN and eosinophils (Figs. 10 and 11). After heat the pericentriolar osmiophilic material appears condensed and may be collapsed onto the centriole (e.g., Fig. 11b); microtubules are sparse. Similar aggregation of this material has been described in Chinese hamster ovary (CHO) cells fixed after 15 min at 45.5°C (26); in CHO cells, that temperature produces a time-dependent (and cell-cycle-dependent) mitotic delay (26). Thus, we suggest that the specific lesion that allows uncoupling, is heat-induced damage to the pericentriolar osmiophilic material. We have not ruled out additional effects of heat, including a direct inactivation of microtubule protein; on the other hand, the process seems too rapid for the generation of heat-shock proteins to be involved.

In the PMN we are concerned with interphase (actually, postmitotic) functions of the centrosome, also presumably mediated by microtubule assembly-disassembly (27). If one of those functions is the maintenance of cellular integrity (prevention of fragment formation), one might ask why colchicine-treated PMN, which lack the putative efferent arm (i.e., microtubules) (14, 15), do not become uncoupled. We reasoned that they do, but that without anchoring—that is, without something to pull against—separation does not occur. We therefore heated PMN suboptimally with or without colchicine pretreatment, such that anchoring of a few cells might occur, but virtually none of the controls would make fragments. Under those conditions functional fragments were easily found among the colchicine-treated preparations, which on that basis were systematically distinguishable from paired controls.

**Figure 8** Cells heated suboptimally for fragment formation are seen. (a) PMN uropods are anchored to glass. Instead of the protopod becoming uncoupled, an elongate but intact cell pulls against the fixed end. Monocytes (grouped at right) do not assume these shapes. (b) A PMN that has failed to pull free has developed an attenuated connection with the anchored nubbin, which may break and seal itself. However, the protopod (arrow) remains integrated with the bulk of the cell. Phase contrast, approximately ×350.

**Figure 9** Control and heated human blood PMN are seen in the transmission electron microscope. (a) In this thin section of a spread control cell, cut parallel to the substrate, granuloplasm is generally pervasive; its exclusion from the relatively narrow leading front is best seen in phase contrast (see Fig. 2). (b) After heat there is a rather discreet boundary between a relatively large area that lacks organelles, and the rounded, nucleated, granule-rich cell body. (c) Fragments at greater magnification contain filaments best seen subjacent to the cell membrane, but not microtubules. The numerous round dense bodies are glycogen. Approximately (a) × 4,300, (b) × 4,300, and (c) × 54,000, respectively.

MALAWISTA AND DE BOISEFURY CHEVANCE The Cytokineplast: Purified, Functional Motile Machinery
Purification of the Motile Apparatus: the Cytokineplast

These motile, anucleate, granule-poor fragments represent a significant purification of the motile apparatus. That much is clear from the light microscopic studies of function, and even without ultrastructural information the origin of the fragments allows us to make certain assumptions about their contents. As shown by immunofluorescent labeling of rabbit PMN with antiactin antibody, the nonstimulated cell maintains a uniform

![FIGURE 10](image)

FIGURE 10 The centrosomal regions of three human blood cell types—PMN, eosinophil, and monocyte—are seen in the transmission electron microscope. a, c, and e are for orientation, b, d, and f for detail, of PMN, eosinophil, and monocyte, respectively. Microtubules radiate especially from pericentriolar osmiophilic material (arrows). Approximately (a, c, and e) × 20,000 and (b, d, and f) × 50,000.
peripheral distribution of actin-containing structures (presumably microfilaments) (28). In contrast, PMN moving up a chemotactic gradient show a specific recruitment of actin filaments toward the advancing edge, which is the region of ligand-membrane interaction (28). Microtubules, like granules, are excluded from this hyaline region (29). Thus, protopods, and hence fragments, will contain the redistributed bulk of the cell's contractile protein. The fact of function dictates that the

FIGURE 11 The effect of heat is seen on the centrosomal regions of human blood PMN, eosinophil and monocyte. The design and order of cell types is as in Fig. 10. Centriolar structure remains intact (see, for example, the near cross section from a PMN, inserted in b) and its paranuclear location is maintained, but pericentriolar osmiophilic material (arrows) appears condensed and either fragmented or clotted, and microtubules are sparse. Approximately (a, c, and e) $\times 20,000$ and (b, d, and f) $\times 50,000$. 
rather circumscribed portion of the cell represented by the fragment will also bear all the other contractile proteins—known and as yet unknown—that act in concert with actin, as well as the sensing and transducing apparatus necessary for directed locomotion. In addition, they contain glycogen as a potential source of energy (Fig. 9c). Otherwise, the motile granule content, and small compared to what is left behind—and by the nonmotile tails sometimes present (Figs. 4 and 5), derived from broken and retracted connecting filaments. We propose to name these fragments cytokineplasts. They differ from cytoplasts (30) and microplasts (31) by the selective character of their contents (also, microplasts do not translocate [31]) and from peripheral hyaline blebs such as podosomes (32) by their ability to adhere, spread, and move.

**Cytokineplasts In Vivo**

The brief, harsh treatment of PMN employed in this study allowed both of the apparent requirements for cytokineplast formation—anchoring and uncoupling—to take place in concert, but each of these precondition can be achieved separately by mechanisms that might obtain in vivo. Alteration of the pericentriolar osmiophilic material in CHO cells (see above) also follows treatment at a lower temperature—42°C—for a longer period of time—1 h (26); fever in certain settings might be expected to produce uncoupling in susceptible cells. In patients treated with metaphase-arresting drugs such as colchicine, which is known to concentrate in leukocytes (33, 34), binding to tubulin might produce a similar result. Moreover, some PMN may be uncoupled without special treatment. Bessis has noted rare fragment formation in unheated PMN. In a sequence caught on film that he showed us, an opsonized erythrocyte escapes envelopment by an advancing protopod by floating (being pushed) away. The protopod follows and, unable to corner its prey, outdistances its own cell body and continues the chase as a fragment. The few cells that make fragments under suboptimal conditions of heating may be additional examples.

Anchoring of PMN in vivo might occur in a number of situations, including heat (fever, burns), the aggregation of PMN that follows activation of serum complement (35), and by the nonmotile tails sometimes present (Figs. 4 and 5), derived from broken and retracted connecting filaments. We propose to name these fragments cytokineplasts. They differ from cytoplasts (30) and microplasts (31) by the selective character of their contents (also, microplasts do not translocate [31]) and from peripheral hyaline blebs such as podosomes (32) by their ability to adhere, spread, and move.

**Role of Microtubules in Chemotaxis**

Finally, cytokineplasts provide a way of looking at the role of microtubules in chemotaxis that avoids the ambiguities attendant upon the use of exogenous drugs such as colchicine. In assays that depend upon migration of PMN across a micropore filter, colchicine seems to interfere with chemotaxis (36, 37). The general interpretation of such data has been that arrays of microtubules, organized from the centrosome, provide direction to cell movement (1, 38, 39). However, colchicine-treated PMN viewed microscopically still point in the right direction (40) and, although they show wider angles of turn than untreated cells, they continue to move toward the chemotactic source (41). Allan and Wilkinson (41) concluded that just as microtubules are not essential for phagocytosis (42–44), they are also not important for the spatial detection of gradients, nor for locomotion towards their sources; they are important for accurate turning by leukocytes and for maintaining the shape and polarity of the moving and phagocytosing cell. The earlier and current work on fragments supports that view: cytokineplasts are capable of directed locomotion and phagocytosis even though they are derived from microtubule-free hyaloplasm that is concentrated at the leading front and have no centrosome. The demonstration that this material is by itself sufficient for directed locomotion allows us to interpret the role of microtubules in chemotaxis more precisely: we suggest that they serve to stabilize and orient the “baggage” (nucleus, granuloplasm) carried by the potentially autonomous motile apparatus; in short, they prevent fishtailing. Moreover, the constraints emanating from the centrosome may now be extended to include maintenance of the motile machinery as an integral part of the cell.

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