linked 'second phase aggregation' in principle are reversible phenomena. This means that the platelet is able to remove again cytoplasmic Ca^{2+}. Platelet activation is prevented and reversal reactions are accelerated by all measures which increase the intracellular level of cyclic AMP. Some of the most powerful known blockers of platelet activity, such as PGE_1, PGI_2 (prostacyclin, synthesized by endothelial cells from PG-endoperoxides) and adenosine exert their action via the activation of adenylate cyclase. We have shown [7, 8] that the effect of cAMP on platelet activity consists in the stimulation, via a protein kinase, of a 'calcium pump'. This is responsible for the uptake into a vesicular system, most likely the DTS, of the cation from the cytoplasm and perhaps also for the extrusion of Ca^{2+} ions to the outside of the cell. This finding probably also explains earlier observations partly made on tumor cells on the effect of cAMP on cell motility [9].

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The Locomotory Machinery of Fibroblasts

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All substratum-based cell locomotion may be considered as the co-operative result of three essential processes: the cell must make adhesions to the substratum; must move its bulk in relation to these adhesions, must protrude new material forward in order to make new adhesions. If the cell is to remain in a 'steady-state', it must also de-adhere from the substratum at the rear and retract the rear end into the cell bulk. One conclusion to be drawn is that a portion of the locomotory machinery must cycle in position with respect to the moving cell. This portion includes all

the adhesive material and probably includes much of the material responsible for generating and transmitting the necessary force between the cell bulk and the adhesions to the substratum. Heath and the author are studying this cycling of the locomotory components in cultured fibroblasts. By combining the use of time-lapse filming in interference reflection and high voltage electron microscopy of the same cells after fixation, much can be inferred about the dynamics of the locomotory machinery of these cells [1]. The broad leading lamella of the fibroblast is scattered with small discrete adhesions to the substratum called focal contacts. Another cluster of such adhesions is found at the tail of the fibroblast. In the central region adhesions are usually sparse or absent. As the fibroblast moves forward each focal contact is stationary relative to the substratum and each is associated with a bundle of microfilaments which passes straight from the focal contact, and obliquely away from the substratum, towards the central cytoplasm surrounding the nucleus. As the leading lamella was found to be most important for locomotive activity, in the following, the emphasis will be placed on this region, Each new focal contact/microfilament bundle complex of the leading lamella is completely assembled within a few seconds and again rapidly disassembled, as the nuclear region of the cell approaches the focal contact. There are three reasons for concluding that these oblique microfilament bundles are important for the generation and transmission of tensile forces between the main cell mass and the substratum. First, they are ideally situated—each stretched between an adhesion and the main cell mass. Secondly, they shorten as the cell moves forward. Thirdly, Isenberg *et al.*[2] have shown that isolated bundles are capable of contraction.

However, some primary fibroblasts are able to move actively in the apparent absence of focal contacts but these cells are seen invariably to possess large amorphous regions of close approach to the substratum called close contacts which, therefore, can also function as adhesions. In addition, Isenberg *et al.* [2] have shown that the bulk cytoplasm of the lamella will also contract under the same conditions as the microfilament bundles. Since most fibroblasts have both close contacts and focal contacts, they may, therefore, have two separate parallel machineries for exerting force on the substratum: if the bundles contract during fibroblast locomotion then the surrounding cytoplasm may also be contracting continuously and exerting a force on the substratum via the close contacts. In fact, phase-contrast films of moving fibroblasts do show that large inhomogeneities within the leading lamella move backward smoothly and continuously during locomotion—which is very suggestive of a continuous contraction of the cytoplasm towards the nuclear region. Rather than being two complementary mechanisms for forward traction of the cell bulk, the contracting bundles and the contracting cytoplasm may be different phases of the same mechanism. Thus, the organisation of the whole contractile machinery may depend on the mechanical loads on it by adhesions to the substratum. This hypothesis is suggested by Fleischer and Wohlfarth-Bottermann [3] with regard to *Physarum.*

As the focal contact/bundle complexes disassemble when they have contracted near the nuclear region of the fibroblast, so might the contractile cytoplasm disassemble. All this material must then be transported forward and re-assembled into the contractile meshwork at the leading margin of the fibroblast. These processes are probably responsible for protrusion of the margin. However, very little is known, except that the forward transport may be controlled and directed by the cytoplasmic microtubule system.

We have started to study the relationship between the contraction and protrusion by detaching the tail of moving fibroblasts from their substratum and thus causing a large fluctuation in the locomotory processes. After detachment, the tail of the fibroblast starts to retract due to active contraction of the main bulk of the cell. A large part of this retraction is complete within the first 10 sec and the rate of retraction returns to a normal level after 30 sec. Detachment also causes an increased rate of protrusion at the leading margin of the fibroblast, which reaches a peak after 10 sec and persists at an elevated level for about 5 min. This wave of increased protrusion is accompanied by a wave of increased density of the rearward-moving inhomogeneities in the cytoplasm of the leading lamella. These experiments, which resolve the cycle into phases, allow certain conclusions with regard to the time course of the cycle during normal locomotion and will enable a study of the mechanisms of action of drugs known to affect locomotion. Ultrastructural and immunofluorescent study of the material which passes through the various phases of the cycle during this fluctuation of the locomotory processes, are planned.

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The Cytoskeleton of Mouse Neuroblastoma Cells

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The oriented cell shape of cultured neuroblastoma cells with their long dendritic processes, each ending in a highly motile growth cone, requires mechanisms for the maintenance of stability and for control of motility. The filamentous proteins are believed to serve these functions by providing a cytoskeleton and the structural basis for force-generating phenomena.

Immunofluorescent techniques show that the distribution of tubulin and actin in neuroblastoma cells is strinkingly different [1]. Whereas microtubules are found to arise near the cytocenter and to radiate over long distances to the cell periphery, actin is localized beneath the plasma membrane and, especially accumulated, in the outer tips of the neurites. Improved methods for the visualization of the cytoskeleton of whole cells, using Triton-extracted, negatively stained cell monolayers [2, 3], have shown that actin filaments are the only filamentous components in the leading edge and at the tips of the neurites. Actin filaments have at least two supramolecular aggregation states: a planar filament meshwork and paracristalline-like filament bundles, partly corresponding to microspikes. In addition, decoration with myosin subfragment-1 (S-1) revealed that the actin filaments in this area are singly polarized towards the cell body. This unipolar organization of actin is a general feature of advancing lamellae of cultured cells, and is not only found in microspikes but also in the interconnecting filament meshwork [3, 4].

Since the polymerization of F-actin occurs in a direction opposite to that of the arrowhead complex, it is suggested that unidirectional polymerization of actin may constitute the primary force producing mechanism for the advancement of growth cones and leading edges. The direct correlation between actin polymerization and arising leading edges is especially prominent when neuroblastoma cells are treated with concanavalin A. This induces the formation of broad and extensive lamellae regions which are formed nearly around the whole perimeter and contain a filamentous actin meshwork as well as abundant paracrystalline-like actin bundles [5]. Membrane ruffling may also be explained by the same polymerization and depolymerization process. It occurs when the terminal filament meshwork locally binds to and detaches from the substrate via the plasma membrane. Depolymerization of actin results in the disappearance of the ruffles into the cell body.

While microtubules and 100 Å filaments (neurofilaments) are found in the core of cell extensions, actin filaments obviously are lacking in this region. The interaction of microtubules and 10nm filaments and their possible involvement in intracellular transport phenomena and locomotion remains to be clarified.

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