lated by internal and external stimuli and, accordingly, we have a corresponding divergence in the modulation mechanisms of different cell types.

Whether the assumption of a conformance during primary motive force generation and a divergence at the level of translation to locomotion may be of heuristic value or not, we must clearly differentiate between two phenomena during cell locomotion: the generation of the linear motive force by chemomechanical energy transformation on the one hand, and the spatial transformation on these forces to complicated cytoplasmic streaming and locomotory phenomena on the other hand.

REFERENCE

1. K. E. WOHLFARTH-BOTTERMANN, Ursachen von Zellbewegungen. *Leopoldina* **21,** 85 (1975).

If we now consider motility as a factor of invasion, we must first evaluate the sources of our information. There is indirect morphological evidence of various kinds. Due to their small dimensions, many *in vitro*-models of tumor penetration can be completely surveyed by histological techniques. They thus provide convincing—although static—indication of the advance of cancer cells in cultured tissues. A cautionary interpretation, however, is necessary, as pointed out in the comparison of two- and three-dimensional models. Static morphological evidence for the contribution of cell motility to invasion can also be obtained by analyzing histological preparations from the invasion zone *in vivo*. This evidence is based on the shape of the cancer cells and on the condition of their contractile proteins, in particular of actin. On the other hand, direct evidence must be dynamic and can only be provided by microcinematography. Although the applicability of this technique to the *in vivo*-situation is restricted, enough basic observations are available to justify some reflections on cancer cell locomotion within living tissues.

Comparison of Invasion in Two-Dimensional Versus Three-Dimensional Systems

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LOCOMOTION, destruction and phagocytosis are cellular functions that presumably are involved in invasion. For the past 2 yr we have investigated the influence of potentially anti-invasive agents on these cellular activities [1, 2]. During our studies we have used various *in vitro* techniques, the choice of which was frequently determined by a need for direct observation of these cellular activities.

These techniques fall into two categories: (1) Two-dimensional systems, where the cells are maintained as a monolayer on artificial substrates; (2) Three-dimensional systems, where cells or tissues form nodules or aggregates, maintained either on a non-adhesive semi-solid substrate, or in fluid medium without contact with a solid substrate.

Our results will be discussed with regard to locomotion, destruction and phagocytosis.

Locomotion

Our model for locomotion of epithelial cells is the wounded lower layer of the chick blastoderm [3]. In the whole blastoderm, as well as in the lower layer isolated on an artificial substrate (glass or plastic), the cells at the edges of a wound migrate to close the wound. However, the histology and the ultrastructure of the edge cells in the blastoderm is so different from that of the same kind of cells on the artificial substrate that we doubt if locomotion occurs in the same way in both situations. In the blastoderm, the edge cells show multifarious extensions, most of which are filopodia, attached to the basement membrane of the ectoblast. We have not been able to identify microfilaments in these lower layer cells. In contrast, on the artificial substrate, the cells of the lower layer flatten and cells at the edge of the wound show large lamellipodia, some of which end in short filopodia. In addition, lower layer cells cultured on artificial substrates are filled with microfilaments, which are organized in bundles at the level of the lamellipodia.

This example raises the general question if locomotion of a cell on an artificial substrate,

like glass or plastic, is relevant for its behavior in its natural environment.

Destruction

When malignant mouse fibroblasts (C3H/MO_4 cells) invade an embryonic chick heart fragment, cultured in a three-dimensional system, they completely destroy the heart tissue. In contrast, if heart cells grow as a monolayer on glass, and are then confronted with the MO_4 cells, no destruction is observed. This example raises the question if the interaction of malignant cells and normal cells in monolayer culture is relevant for research on invasion properties.

Phagocytosis

TE micrographs have shown that invading HeLa cells internalize debris from heart tissue into large vacuoles. These observations suggest that phagocytosis of material from heart tissue accounts for its replacement by invading malignant cells, in three-dimensional cultures. We tried to directly observe this process by feeding debris from heart tissue to HeLa cells in monolayer culture. The debris was attached to the surface of the HeLa cells through filopodia, but internalization of particles into large vacuoles was not observed. Although it is not excluded that lack of internalization is due to the nature of the debris (prepared mechanically), this example also questions the value of two-dimensional cultures.

These comparative studies using two- and three-dimensional systems raise some doubts that the behavior of cells in monolayer cultures is relevant for their activity *in vivo*. Our policy for the future will be to use two-dimensional cultures for the study of cellular functions involved in invasion only under the condition that (a) the function is preserved in monolayer culture and (b) that it occurs in the same way as in three-dimensional sytems.

REFERENCES

1. M. Mareel and M. de Brabander, Methyl (5-(2-thienylcarbonyl)-2H-benzimidazol-2yl) carbamate, (R 17934), a synthetic microtubule inhibitor, prevents malignant invasion *in vitro*. *Oncology* **5,** 35 (1978).
2. M. Mareel and M. de Brabander, Effect of microtubule inhibitors on malignant invasion *in vitro*. *J. nat. Cancer Inst.* **61,** 787 (1978).
3. M. Mareel and L. Vakaet, Wound healing in the primitive deep layer of the young chick blastoderm. *Virchows. Arch. B. Cells Path.* **26,** 147 (1977).

Human Epidermal and Mammary Carcinoma Cells: Actin Distribution

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Spreading in the surrounding tissue is one of the most prominent features of cancer cells. Although the mechanisms which lead to invasion are little understood, it seems likely that cell movement is one of the phenomena involved in tumor invasion [1, 2, 3]. Evidence has accumulated indicating that several aspects of locomotion of nonmuscle cells are related to the presence of contractile proteins, in particular actin, in human carcinoma cells. Normal skin, basal cell carcinomas, squamous (skin, oral cavity, larynx) cell carcinoma as well as nonlactating mammary glands and infiltrating ductal carcinomas were compared by means of electron microscopy and indirect immunofluorescence using anti-actin antibodies. Anti-actin auto-antibodies were obtained from patients with chronic aggressive hepatitis [4].

Cancer cells stain more strongly for actin when compared with the normal cells. In particular, pronounced staining is observed in cells invading the surrounding tissue as is the case in invasive squamous cell carcinomas. The same holds true for mammary carcinoma, where the cells of the growing edge of the tumor, around small lymph and blood vessels, as well as the cells in isolated metastatic islets stain most intensely. The increase in immunofluorescent labelling for actin in cancer cells is correlated with an increase in microfilaments as revealed by electron microscopy [5–7]. The marginal cytoplasm contains a thick meshwork of microfilaments which extend into fingerlike cytoplasmic protrusions. These projections are in direct contact with the surrounding tissue. Concomittantly, the large tumor cells appear to be more detached from one another: the intercellular space is enlarged, hemidesmosomes and tonofilaments are less abundant in cancer tissue than in