CLIN. EXPL. METASTASIS, 1989, VOL. 7, NO. 2, 127–167

Review

Interactions between cancer cells and the microvasculature: a rate-regulator for metastasis

LEONARD WEISS†, F. WILLIAM ORR‡
and KENNETH V. HONN§

† Department of Experimental Pathology, Roswell Park, Memorial Institute, Buffalo, NY 14263, U.S.A.
‡ Department of Pathology, McMaster University, Hamilton, Ontario, L8N 3Z5, Canada
§ Department of Radiation Oncology, Wayne State University, and Gershenson Radiation Oncology Center, Harper/Grace Hospitals, Detroit, MI 48202, U.S.A.

(*Received 20 June 1988*)

Hematogenous metastasis is a major consideration in the staging, treatment and prognosis of patients with cancer. Key events affecting hematogeneous metastasis occur in the microvasculature. This is a brief, selective review of some interactions involving cancer cells and the microvasculature in pathologic sequence, specifically: (1) intravasation of cancer cells; (2) the arrest of circulating cancer cells in the microvasculature; (3) cancer cell trauma associated with arrest; (4) microvascular trauma; (5) the inflammatory; and (6) the hemostatic coagulative responses associated with arrest, and finally (7) angiogenesis, leading to tumor vascularization. The evidence shows that through a series of complex interactions with cancer cells, the microvasculature acts as a rate-regulator for the metastatic process, in addition to providing routes for cancer cell dissemination and arrest sites for cancer cell emboli.

Introduction

The blood-stream is the ultimate disseminative route for most metastases, and the interactions of cancer cells with blood-vessels play a pivotal role in the metastatic cascade.

The metastatic potential of cancer cells cannot be determined by any single parameter, but is probably represented by a sum of many phenotypic characteristics (e.g. surface receptors, proteolytic enzymes, growth factors, etc.) some of which may be transiently expressed during the metastatic process [222, 227]. Each of these phenotypic characteristics may not be of equal importance, as cancer cells may use several strategies to achieve the same result. For example, human lung carcinoma cells prefer thrombospondin over laminin as an attachment substrata, whereas the opposite is true for melanoma cells [214]; nevertheless, the attachment of circulating cancer cells to endothelium/subendothelial matrix is recognized as a critical event in the metastatic cascade [241].

In this review the major focus will be on interactions involving cancer cells and the microvasculature, particularly those associated with cancer cell entry, arrest, adhesion and exit; events which affect the efficiency of the metastatic process.

(1) Intravasation

Cancer cells usually enter the circulation via small veins and the microvasculature. Larger veins may also be invaded, for example the renal vein in carcinomas of the kidney. However, arterial invasion is a rare and usually terminal event [224].

Intravasation is often but not invariably associated with degradation of perivascular basement membrane, which is composed primarily of type IV collagen and lesser amounts of laminin, heparan sulfate proteoglycan, fibronectin and vitronectin. Enzymes directed against any of these substrates are expected to promote intravasation by weakening basement membrane 'barriers' to invasion. Correlations have been demonstrated between various facets of invasion and metastasis and the release of type IV collagenase [10a, 65, 116, 207, 251], cathepsin B [187] and plasminogen activators [39, 157] from cancer cells, as well as cancer cell-associated heparanase (heparan sulfate endoglycosidase) [139, 140]. Some correlations with enzyme production have also been shown for the behavior of human tumors, including survival in head and neck cancer, invasion and metastasis in colon and breast cancers [reviewed in 45]. However, such relationships do not seem to apply to all systems, possibly because cancer cell proteolytic activity is influenced by a variety of factors, e.g. cell cycle, cell density, or the presence of protease inhibitors [45, 101, 204].

Emphasis on basement membrane degradation by cancer cell-associated enzyme should not be taken to imply that these are the only, or even the most important, mechanisms. In the rat mammary tumor system loss of basement membrane, which is associated with increased invasive potential, is independent of collagenase production [218]. The steady state of basement membrane integrity may be perturbed by the malignant process itself. Thus, cell transformation by retroviruses is sometimes associated with reduced synthesis of connective tissue components [2], and decreased levels of mRNAs [188] and transcription rates [209]. However, while failure to maintain basement membrane is not invariably due to decreased synthesis [5, 6, 62], it seems prudent to consider both of the non-exclusive mechanisms of decreased synthesis and increased degradation.

Additional degradative mechanisms may include the transition from a state of confrontational coexistence between a cancer and host tissue, to one of host tissue atrophy caused by the increasing hydrostatic pressure exerted by an expanding tumor, with subsequent competition for nutrients [62, 211].

In sarcomas, blood is often carried in vascular clefts, which are lined by cancer cells as distinct from vascular endothelium. Intravasation via these clefts therefore does not require prior focal destruction of a basement membrane. The neovasculature of tumors also provides an intimate entry point for cancer cells and, by virtue of fenestrations and general leakiness, intravasation under these circumstances would appear to require little or no prior degradation. Cancer cells may also enter the blood-stream via lymphaticovenous anastomoses, and entry into the lymphatic saccules or capillaries will also require minimal degradation, since these structures contain only irregular patches of basement membrane [15]. Even in the case of the 'normal' vasculature, the thickness of the basement membranes or analogous tunica adventitia, varies considerably in different sites, from 45 to 70 μm in the larger veins (inferior vena cava, renal veins, etc.) down to the pulmonary alveolar capillaries, where the combined thickness of epithelium, basement membrane and endothelium is only 100 μm.

Finally, as discussed later, leukocytes in the region of the invasive interface of tumors and endothelial cells are also an important potential source of histiolytic

enzymes [250]. Cancer cell-associated enzymes play a role in invasion, culminating in intravasation. However, it is unlikely that they provide the only mechanism for these processes, and the extent to which these or other mechanisms are involved would appear to vary considerably with the site of intravasation and the differentiation of the invading cells. In common with other invasive processes, cell motility may also be important [192, 242].

(2) Cancer cell arrest and adhesion

In the present context it is important to discriminate between *arrest* of circulating cancer cells in the microvasculature, and their *adhesion* to the walls of the microvasculature. As discussed later, arrest or trapping can be accounted for in macroscopic terms, whereas adhesion, which follows arrest, can be accounted for in microscopic terms at the molecular and submolecular levels. For example, a non-deformable, chemically inert sphere falling down a tapered, non-deformable, chemically inert tube will be arrested and the mechanical impaction can be accounted for in the macroscopic terms of sphere and tube diameters. In contrast, a sphere falling down a tube of greater diameter than its own will not be arrested. However, if one or other is coated with glue, movement of the sphere may cease due to adhesion, which can be accounted for in microscopic terms of the properties of the adhesive and the two adherends.

Arrest

The site of arrest of cancer emboli within the vasculature depends to a large extent on their size. Thus, multicellular aggregates will not enter capillaries, but will remain confined to larger vessels, and may even pass directly through organs [254], although this is a rare event. Entry into the microcirculation appears to be confined to single cancer cells. The disparity between cancer cell and capillary diameters requires that either the cells and/or the capillaries be deformable. In fact, deformed cancer cells have been observed passing through capillaries [253].

Cancer cells are viscoelastic [232], and as a consequence, when a cell is deformed into a capillary, its elastic recoil will tend to force it against the vessel wall. When a cell moves through a fluid some of the fluid moves with the cell, constituting its hydrodynamic field. Contact between the cell and a vessel wall will depend on expulsion of the fluid film between them [44, 238].

In the case where the cell is more deformable than the vessel, the driving force for fluid expulsion is the elastic recoil of the deformed cancer cell towards the capillary wall. Contact, resulting in cancer cell arrest, will occur when the 'recoil' time is less than the time taken for the cancer cell to be driven through the capillary bed (transit time) under the action of a blood-pressure gradient. The rate of approach of the cell to the vessel wall has been calculated as a function of cell velocity, driving force, viscosity and geometry [44]. Detailed theoretical studies indicate that contact between cancer cells and capillary walls can occur by these mechanisms within realistic transit times [234, 235], in accord with the observation that most of the cancer cells delivered to the lungs of mice via the systemic circulation, are almost immediately arrested in the pulmonary circulation [236].

As cancer cells approach the vessel wall, rheologic factors limit/retard their interactions. However, in spite of hydrodynamic barriers [46] and electrostatic repulsion between their negatively charged surfaces [239], adhesion can occur through the agency of adhesive macromolecules, as discussed later.

It should be emphasized that arrest of cancer cells in the microvasculature can be accounted for in terms of their relative biomechanical properties and the fluid mechanical behavior of thin films of plasma. However, in the present absence of experimental data, specific-site arrest or non-arrest cannot be accounted for in these terms. Although the mechanical and biochemical properties of cancer cells and the microvasculature are often treated as separate and distinct entities, they are mutually dependent to some extent.

Adhesion and adhesive macromolecules

General comments on adhesion of cancer cells to the endothelium. Arrest and survival of cancer cells within the microvasculature is mandatory for hematogenous metastasis. During the hematogenous phase of the metastatic cascade, cancer cells must interact with host cells (i.e. platelets/endothelial cells) in order to be arrested and adhere to the endothelial/subendothelial matrix [92, 241]. Therefore the factors (i.e. receptors, platelets, hemodynamic factors) regulating initial cancer cell arrest and adhesion are of critical importance to the development or the prevention of metastases [97, 241].

In animal models and *in vitro*, kinetic studies on experimental metastasis have demonstrated initial attachment of cancer cells to the endothelial surface at junction points between adjacent endothelial cells, followed by endothelial retraction and by adhesion of cancer cells to the underlying basement membrane [35, 108]. Possibly because tissue culture studies have suggested that cells attach more readily to the basement membrane than to the endothelium [107, 141, 215] and because of a wide interest in the chemistry of connective tissue, the mechanisms involved in adhesion of cancer cells to the endothelium have not been examined as widely as have the interactions with extracellular matrix adhesion molecules in the basement membrane. Nonetheless, studies *in vitro* have provided evidence for the hypothesis that variations between the surface properties of endothelial cells from different target organs may account for some differences in the initial binding rates of circulating cells, leading to organ preference in metastatic localization [8, 141]. The important topic of specificity with particular reference to metastatic pattern is covered in a recent, excellent, exhaustive review by Nicolson [142], and will not be dealt with here. Others have observed correlations between cancer cell adhesiveness to endothelial monolayers and malignancy *in vivo* [200].

As discussed below, numerous adhesive proteins (e.g. laminin, fibronectin, thrombospondin, etc.) and their receptors are implicated in metastasis. Recent evidence suggests that a platelet glycoprotein receptor (GpIb) and receptors of the integrin family may play a heretofore unexpected, but a major, role in cancer cell interaction with other cancer cells, host cells, and subendothelian and/or other extracellular matrices.

Cancer cell adhesion molecules and metastasis. There is evidence that carbohydrates on the surface of cancer cells are involved in metastatic interactions with host cells and the extracellular matrix [96]. Briefly, some experiments have demonstrated positive correlations between metastatic potential and the degree of sialylation or beta 1–6 linked branching of complex-type asparagine-linked oligosaccharides [e.g. 43, 252]. In the latter case it has been reported recently by Dennis *et al.*, [43] that increased beta 1–6 linked branching can alter the expression of a cell surface glycoprotein gp130, which is associated with acquisition of metastatic potential. In

other experiments, cell lines resistant to treatment with plant lectins had altered metabolism of carbohydrates, modified surface carbohydrate structure, and were less metastatic than the parental lines from which they were derived [103]. In some experimental systems [97, 149], modification of cancer cell surface carbohydrates by drugs or enzymes can alter metastatic potential. The mechanisms possibly involve interference with adhesive interactions between cancer cells and the extracellular matrix (after treatment with tunicamycin) or some other other, as yet undefined, mechanism, possibly acting at a stage of metastasis distal to arrest [castanospermine or swainsonine treatment; 97]. Cell surface sialylation has been shown to correlate with the platelet-activating activity of human cancer cell lines [11]. Endogenous cancer cell surface lectins may also be involved in the formation of homotypic aggregates by some cancer cells, and treatment of such cells by monoclonal antibodies against the endogenous lectins can cause marked reduction in the formation of experimental metastases [129]. In addition, molecular interactions between various attachment molecules are postulated to be of potential importance when one or more of the molecules is produced endogenously by the cell, e.g. production of laminin, permitting attachment of type IV collagen, proteoglycans, nidogen [9] or thrombospondin, a high molecular weight glycoprotein released from the alpha granules of platelets during activation [214]. Injection of thrombospondin intravenously into mice prior to injection of T241 sarcoma cells has been shown to potentiate subsequent lung colony formation [208].

It is reemphasized that cancer cell adhesion to the vasculature cannot be considered exclusively in terms of cancer cells.

Platelet GpIb and integrin receptors, including the GpIIb/IIIa complex. Glycoprotein Ib is the principal sialoglycoprotein of the platelet membrane. The intact glycoprotein has an apparent molecular weight of 170 000, and it consists of two disulfide-linked subunits, containing hydrophobic sequences. GpIb is an integral membrane glycoprotein [165] and a platelet receptor for von Willebrand factor (in the presence of restocetin [164] thrombin [64] and immunoglobulin Fc fragment [131].

The integrins are a family of transmembrane glycoproteins composed of noncovalent heterodimers of two basic subunits, $\alpha(M_r = 140–200\,\text{kDa})$ and $\beta(M_r = 95–150\,\text{kDa})$. The fibronectin receptor (FnR), the vitronectin receptor

Table 1. Characteristics of Yoshida ascites hepatomas and Yoshida sarcoma (YS) in rats.

Tumor	Deforming pressure[b] (cmH_2O)	Percentage viable cells after one pulmonary passage	Post-pulmonary spread after i.v. injection	Lung metastasis from i.p. tumor	Cancer cells in blood from i.p.
AH100B	7·63 ± 0·71	1·7	−	−	10–15 days
AH130	5·40 ± 0·35	41·5	±	±	3 days
AH66F	1·95 ± 0·13	92·0	+ +	+	1 day
YS	1·62 ± 0·12	92·2	+ +	+	12–24 h

[a] Data from ref. 181.
[b] Using 6·5 μm diameter micropipettes.

Table 2. Intraperitoneal (i.p.) bioassays on myocardium and lungs removed 5 min after left ventricular injections (LVI) of cancer cells [49].

Cell type	LVI Numbers of cancer cells	Percentage dose received[a]	IP Bioassays	
			Tumor incidence	Survival of tumor-bearers
B16	5×10^5			
Heart		4·7	3/14	30 days
Lungs		2·2	11/14	27 days
EAT	10^5			
Heart		4·7	0/15	>60 days
Lungs		2·2	9/14	31 days

[a] Using [^{125}I]microbead arrest data to avoid label-distribution artifacts [48].

Table 3. Intraperitoneal (i.p.) bioassays on quadriceps muscles, 65 days after LVI of 5×10^5 b16 cells [54].

	Femoral nerve transected	Intact control	Electrically stimulated
Weight of cancer in implants (g ± SE) (*n*)	0·63 ± 0·08[a] (25)	0·03 ± 0·004 (37)	0·001 ± 0·001[a] (13)
Proportion of i.p. takes	27/31 (87%)	37/42 (88%)	4/13[a] (31%)

[a] Significantly different from controls ($p < 0{\cdot}01$).

(VnR), and the platelet membrane glycoproteins GpIIb/IIIa are representative members of the family of integrins [52, 99, 179]. GpIIb and GpIIIa exist as a Ca^{2+}-dependent heterodimer complex (GpIIb/IIIa) on inactivated human platelets [51]. Only in the presence of EDTA can the distinct proteins be found. GpIIb and GpIIIa are probably always complexed *in vivo* [123, 167]. The fibronectin receptor and the vitronectin receptor appear to exist as Ca^{2+}-independent heterodimers. This functional difference has allowed immunological discrimination between these receptors and related receptors on the cancer cell surface (see below).

The GpIIb/IIIa complex has been implicated in the binding of four adhesive proteins (fibrinogen, fibronectin, von Willebrand factor, and vitronectin). The GpIIb/IIIa complex is known to play a role in platelet aggregation and platelet adhesion to subendothelial matrix. Both platelet GpIb and GpIIb/IIIa are transmembrane proteins [23, 58]. However, in the resting platelet, both glycoproteins have been localized both on the platelet surface and to internal platelet structures [249]. Woods *et al.* [249] have recently demonstrated that the reappearance of GpIIb/IIIa on the surface of antigenically denatured platelets originates from a compartment within the platelet, probably the canalicular system. GpIb is also stored intracellularly in membrane-bound vacuolar structures [246]. These proteins exist on the surface of platelets as transmembrane proteins which may bind directly to endogenous membrane actin [148, 161, 190, 249] Monoclonal antibodies

against various epitopes on GpIIb/IIIa results in time-dependent patching and capping followed by internalization of the complex [249]. However, GpIb could not be shown to cap upon exposure to specific monoclonal antibodies [180].

Evidence for the presence of immunologically related GpIb and GpIIb/IIIa on cancer and endothelial cells. Recent evidence suggests that glycoproteins related to GpIb and GpIIb/IIIa are present on a variety of cell types. Tabilio *et al.* [197] first demonstrated the presence of platelet glycoprotein Ib in a minority of cells of the human leukemic cell line (HEL), and Kieffer *et al.* [105] selected a stable HEL subclone with increased expression of glycoprotein Ib. A protein was isolated which was a single polypeptide chain of an apparent molecular weight $M_r = 60\,000$, which was precipitated under reducing and non-reducing conditions by specific polyclonal antibodies to platelet glycoprotein Ib and monoclonal antibodies to the α-complex of GpIb. There was no evidence for the β subunit of GpIb (i.e. GpIx) [105], a small ($M_r = 17\,000$–$22\,000$) membrane protein which is co-isolated with GpIb by immunoaffinity chromatography [105]. This protein also demonstrated abnormal oligosaccharide chains. Thiagarajan *et al.* [202] isolated a functionally active GpIIb/IIIa complex from HEL cells; however, Ca^{2+}-dependent binding of fibronogen to this glycoprotein was not demonstrated in intact cells.

In addition to the studies with leukemic cell line (HEL), recent evidence suggests the presence of GpIb and GpIIb/IIIa-like glycoproteins on human and rodent solid tumor cells. These glycoproteins have been termed immunologically related GpIb (i.e. IRGpIb) and immunologically related GpIIb/IIIa (i.e. IRGpIIb/IIIa) [82]. Immunological evidence has been reported for the presence of IRGpIb and IRGpIIb/IIIa on human colon carcinoma (close A), human cervical carcinoma (MS751), rat Walker 256 carcinosarcoma, murine B16 amelanotic melanoma and Lewis lung carcinoma cancer cells [27, 82, 88, 90]. Immunological identification was determined with specific polyclonal antibodies and monoclonal antibodies (mAb10E5, mAb7E3, mAbP-2) raised against the human GpIIb/IIIa, complex. The monoclonal antibodies used in these studies are complex-directed (i.e. they do not interact with the disassociated monomeric glycoproteins). One of the monoclonal antibodies (mAb7E3) cross-reacts with the vitronectin receptor which allowed for the determination of a functional relationship between cancer cell IRGpIIb/IIIa and platelet GpIIb/IIIa, in addition to discrimination from the vitronectin receptor. Endothelial cells (which possess the vitronectin receptor [52]) do not demonstrate decreased binding of complex-directed mAb7E3 in the presence of EDTA or calcium Ca^{2+}–Mg^{2+}-deficient media [27, 87], consistent with the calcium independence of the vitronectin receptor complex. In contrast, human and rodent cancer cells do demonstrate decreased binding of GpIIb/IIIa complex-directed monoclonal antibodies in the presence of EDTA [27, 87, 88], consistent with their similarity to platelet GpIIb/IIIa.

The IRGpIIb/IIIa receptor mediates cancer cell attachment to platelets, endothelial cells, subendothelial matrix, and isolated components of the subendothelial matrix. Grossi *et al.* [27] have demonstrated that human clone A and MS751 cell adhesion to fibronectin was mediated by the IRGIIb/IIIa receptor, in that adhesion was blocked by specific monoclonal and polyclonal antibodies to GpIIb/IIIa, but not by antibodies against unrelated antigens. Similar results have been reported for the B16a and 3LL cancer cell adhesion to fibronectin [88, 90]. As stated above, the integrin receptor GpIIb/IIIa is multifunctional, i.e. it is capable of

interacting with at least four known adhesive proteins [52, 99, 179]. At least one of those adhesive proteins (i.e. von Willebrand factor) is known to be expressed on the surface of endothelial cells and several of these adhesive proteins (i.e. von Willebrand factor, fibronectin and vitronectin) are components of the subendothelial matrix. Finally, another adhesive protein (fibrinogen) which binds to GpIIb/IIIa complex is known to be present on surface of platelets [166]. Therefore, cancer cell IRGIIb/IIIa may serve as a multifunctional receptor for interaction with platelets and endothelial cells, and the subendothelial matrix. In fact, cancer cell IRGpIIb/IIIa may mediate, in part, adhesion to a variety of cell types and matrices [27, 82, 88–90], including the adhesion of B16a and 3LL cells to endothelial cells [88, 90] and subendothelial matrix [88–90], on the evidence that can be inhibited by specific polyclonal and monclonal antibodies to GpIIb/IIIa. In addition, cancer cell IRGpIb may also contribute as an adhesive protein receptor, mediating cancer cell interaction with endothelial cells and subendothelial matrix, possibly via von Willebrand factor. The adhesion of several cell types to those substrata is inhibited by specific polyclonal and monoclonal antibodies to GpIb (K. V. Honn and I. M. Grossi, unpublished observation). Similarly, IRGpIIb/IIIa may serve as an attachment site for platelet adhesion to the cancer cell plasma membrane. Human and rodent cancer cells treated with polyclonal and monoclonal antibodies to GpIIb/IIIa, but not antibodies against unrelated antigens, demonstrate partial to complete inhibition of their ability to induce platelet aggregation [27, 87, 89], suggesting that IRGpIIb/IIIa may serve as an adhesion site for platelet attachment to the cancer cell plasma membrane (see below). In addition, ultrastructural examination of treated cancer cells and untreated platelets reveals a lack of platelet attachment to cancer cell surface and a lack of specific cancer cell membrane process formation in response to platelet attachment [87, 89 and see below]. These results suggest that IRGpIIb/IIIa, and possibly IRGpIb, may play a role in metastasis.

Onoda *et al.* [150] reported the separation, by centrifugal elutriation, of subpopulations of B16a cells which differed in their lung colonizing ability. Using these subpopulations it was determined that plasma membrane expression of IRGpIIb/IIIa, correlated positively with their ability to adhere to fibronectin, induce platelet aggregation, and form long colonies following tail vein injection [82, 89]. Interestingly, the plasma membrane expression of IRGpIIb/IIIa and IRGpIb correlated with the phase of the cell cycle, i.e. increased expression by cells in the G_2 phase [89]. Similarly, the ability of cancer cells to induce platelet aggregation, adhere to endothelial cells, subendothelial matrix, and its components also correlates positively with cells in the G_2 phase of the cell cycle [89], and increases in lung colonizing ability have been observed in cells in the G_2 phase of the cell cycle [76, 124, 150]. These results suggest that intrinsic factors, which may be cell-cycle-related, and external stimuli may regulate expression of these glycoprotein receptors by cancer cells. They lend support to the hypothesis that cancer cells may transiently pass through high and low metastasis states, as first discussed by Weiss [222, 227].

Immunofluorescence studies of cancer cells labeled with monoclonal antibodies against GpIb or GpIIb/IIIa have suggested a difference in the pattern of distribution of these glycoprotein receptors on the cancer cell surface. GpIb appears to demonstrate a homogeneous labeling pattern [27], in contrast, IRGpIIb/IIIa is distributed heterogenously, with areas of high and low fluorescent intensity [27, 87]. Cancer cell (IRGpIIb/IIIa)GpIIb/IIIa complex appears to be linked to cytoskeletal elements and undergoes patching and capping in response to specific monoclonal

antibodies. Cancer cell IRGpIIb/IIIa and IRGpIb may also be stored in intracellular vacuolar structures (K. V. Honn, unpublished observation).

These differences are more obvious at the ultrastructural level, and immunocytochemical studies have demonstrated that IRGpIb is confined to the cancer cell plasma membrane (in non-permeabilized cells) and demonstrates a homogeneous labeling pattern on the plasma membrane [27]. Immunocytochemical labeling of human and rodent cancer cells for IRGpIIb/IIIa reveals a punctate distribution of reaction product on their surface membranes [27, 82, 87], in addition, clusters of high-density reaction product are also evident. This heterogeneous distribution of the IRGpIIb/IIIa receptor on the cancer cell surface suggests the possibility of preexistent sites for preferential attachment to platelets, endothelial cells, and subendothelial matrix [27, 90]. In addition to the immunological and functional correlation between platelet G'IIb/IIIa and cancer cell IRGpIIb/IIIa, the presence of mRNA transcripts coding for IRGpIIb and IRGpIIIa are reported for a variety of human and rodent cancer cells [25, 87]. When Northern blot analysis was performed using cancer cell RNA and GpIIb and GpIIIa cDNA probes, the size of the transcripts hybridized with the GpIIIa cDNA probe were approximately 3·7 kb and 2·1 kb, while the size of the transcript detected with the GpIIb cDNA probe was approximately 3·7 kb with a minor band of 18S rRNA position [87]. The transcript for IRGpIIb in cells derived from solid tumors differed from the transcript size observed in a leukemic cell line [25].

Only recently has the expression of adhesion molecules by endothelial cells been studied in detail. In the past the ability of endothelium to bind fibrinogen, vitronectin, von Willebrand factor, and fibronectin has been taken as evidence for the existence of receptors for these molecules, and in most cases the specificity of this binding has been defined by the ability of monoclonal antibodies to inhibit attachment or to cause detachment of the endothelial cells from extracellular substrates. The endothelial receptors for extracellular matrix constituents generally have properties attributed to members of the integrin class of adhesion molecules, and can be isolated from affinity matrices consisting of peptides containing the sequence Arg–Gly–Asp (RGD).

Thus Fitzgerald *et al.* [50], using Western blot analysis, recently demonstrated the presence of glycoproteins related to IIb and IIIa on human umbilical vein endothelial cell (HUVE) and bovine aortic endothelial cell (BAE) [26, 53]. These proteins were immunoprecipitated with polyclonal antibodies prepared against purified human platelet GpIIb/IIIa complex. Two membrane proteins were detected on both HUVE and BAE which were similar to human platelet GpIIb and GpIIIa. However, the GpIIb/IIIa complex from human endothelial cells [50] or rat endothelial cells [87] does not dissociate following chelation of calcium. It is now known that the glycoprotein complex on endothelial cells is the vitronectin receptor and not the IIb/IIIa complex [52]. Fitzgerald *et al.* [50] have obtained the sequence of GpIIIa from translation of the cDNA for endothelial cell GpIIIa. The translated cDNA sequence is exactly matched to the amino acid sequences of platelet GpIIIa, indicating that platelet and endothelial cell GpIIIa are identical primary translation products. This has led to the realization that several intergrin receptors share common β subunits (i.e. IIIa and vitronectin β) which is the basis for the immunologically cross-reactivity of the various integrin receptors [30, 52, 71, 196].

Some endothelial surface receptors appear to be constitutively expressed, and published experiments suggest a role for them in the attachment of endothelial cells

to the surrounding matrix. The possibility that such receptors could be involved in the attachment of intravascular cancer cells to the apical surface of endothelial cells has yet to be shown. One problem relates to the observation by immunofluorescence microscopy that some of the receptors are localized at cell substratum contact points [30]. Thus, identification of endothelial adhesion molecules isolated from cells in suspension may be difficult to relate to metastasis, since the basal and apical surfaces of attached endothelial cells exhibit polarization in the distribution of their integral membrane proteins [136].

Effects of endothelial metabolism on adhesion. Studies on the interactions between the endothelium and circulating leukocyte or platelets indicate that endothelial cells carry interactive sites for stimulants such as interleukin-1, tumor necrosis factor, and interferon. Perturbation of the endothelium by these cytokines is followed by cell activation with the synthesis and expression of a second group of adhesion molecules which are either not present, or are present in only small amounts on unstimulated endothelium [168–170]. Experiments *in vitro* and *in vivo* have shown that the expression of this group of inducible molecules is associated with enhanced attachment of neutrophils, monocytes, and lymphocytes to the perturbed endothelial cell surface and that this occurs at sites of inflammation [16, 24, 33]. In addition, perturbed cells also release a variety of humoral products of endothelial metabolism including interleukin-1, tissue factor, inhibitor of tissue plasminogen activator, platelet activating factor, prostacyclin, and platelet-derived growth factor [32 a].

It is likely that the expression of such inducible surface adhesion molecules and the release of humoral mediators from perturbed endothelium are also relevant to metastatic processes. Recent experiments have demonstrated that the adhesiveness of human umbilical vein endothelial cells for A549 human lung carcinoma cells is increased following perturbation of the endothelium with interleukin-1 or peptide chemoattractants. In addition, there was enhance retention of intravenously injected A549 cells in the lungs of nude mice pretreated with interleukin-1 [112]. Other experiments have demonstrated that the motility of human A549 lung carcinoma cells is stimulated in response to endothelial-derived interleukin-1 (Orr *et al.*, unpublished results, 1988), suggesting the existence of a mechanism by which the endothelium could regulate cancer cell motility following intravascular cancer cell arrest. These two mechanisms may be involved in the localization of neoplastic cells at inflamed sites where endothelium is likely to be in an activated state.

Subendothelial attachment sites. Due to endothelial retraction, the subendothelium is exposed, and many of the major components of the subendothelial basement membranes have the ability to act directly as cellular binding sites. Among these are type IV collagen [9], the glycoproteins laminin [175] and fibronectin [179], and heparin sulfate proteoglycan [59]. In the case of laminin, specific receptors have been found and identified as integral plasma membrane proteins in malignant cells, and it has been shown that there are functional domains in the laminin molecule which variously regulate attachment and spreading, migration and growth, and metastasis [116, 176]. Fibronectin has also been shown to contain distinct domains which regulate the adhesion and motility of metastatic melanoma cells [122]. Cell surface receptors have been identified for fibronectin, type I collagen, vitronectin, and laminin [18, 41, 42, 118, 173, 174].

RGD sequences. The amino acid sequence arginine–glycine–aspartic acid (RGD) appears to be common to the cell adhesion site of many attachment molecules, having been identified in fibronectin [99, 179], vitronectin, type I collagen, fibrinogen, von Willebrand factor and osteopontin, and may also be important in other attachment molecules, e.g. laminin [179]. The relevance of this specific sequence to attachment has been shown by the ability of immobilized RGD peptides to act as attachment sites for a variety of cells, and by the ability of soluble RGD peptides to inhibit the attachment of cells to surfaces which also contain the sequences [85]. RGD-receptor expression may be modulated by environment. For example, selection of a human osteosarcoma cell line for growth in the presence of RGD peptides, results in development of a cell line with increased numbers of fibronectin receptor [42]. The possible relevance of RGD sequences to metastasis has been demonstrated by Humphries *et al.* [95, 98], who reported that co-injection of Gly–Arg–Asp–Ser with intravenous B16-F10 murine melanoma cells inhibits the subsequent development of pulmonary colonization by these cells [96] and is associated with extension of survival time in recipient animals [98]. The potential importance of the RGD peptide sequence is also indicated by the lesser ability of peptides with amino acid inversions or substitutions to block metastasis, with correlations between the relative inhibitory activity *in vivo* and the ability to disrupt adhesion *in vitro*. The diminished lung colonization was associated with an increased rate of loss of cell incorporated [^{125}I]IUdR from the lung within 6 h of intravenous injection. The effects did not appear to be due to interference with platelet or natural killer cell activity [98]. RGD-containing synthetic peptides have also been reported to inhibit the penetration of melanoma cell lines and a glioblastoma through human amniotic basement membrane [67].

In addition to acting as attachment points, binding of cells to some of the basement membrane adhesion molecules is followed by enhanced cell migration. This may take the form of chemotaxis, i.e. movement in a concentration gradient of a soluble stimulus, or haptotaxis, i.e. movement in the context of an adhesive gradient on the substratum. Fibronectin, laminin, and collagen-derived peptides [121–123, 137], can stimulate cell motility which may contribute to penetration of the basement membrane. The mechanisms involved in such motility responses seem to be analogous to those in leukocytes with receptor–ligand interactions, activation of intermediate metabolites (cyclic nucleotides and arachidonic acid) [13]. The response of cells to interaction with basement membrane molecules may not be restricted to cell migration. There is also evidence for increased cell adhesiveness by cancer cells and the release of degradative enzymes (Type IV collagenase and plasminogen activator) in response to chemotactic stimulation by humoral substances [204, 213]. Whether similar phenomena result from interactions with the basement membranes is not clear.

Increased metabolism of arachidonic acid via the cyclo-oxygenase enzyme pathway to generate a number of prostanoids, and via the lipoxygenase enzyme pathway to form a number of monohydroxy fatty acid metabolites [17, 94], also simulate platelets, leukocytes, and endothelial cells. Important among these products are 12-L-hydroxy-eicosatetranenoic acid (12-S-HETE or 12 HETE) from platelets, 5-HETE and the leukotienes from leukocytes, and 15-HETE from the endothelium. More recent studies indicate that, under basal conditions, leukocytes and endothelial cells also synthesize linoleic acid via the lipoxygenase pathway to a monohydroxy fatty acid 13-hydroxy-octadecadienoic acid (13-HODE) [19, 189].

The production of 13-HODE seems to contribute to the nonadhesive properties of the endothelium with platelets and leukocytes under basal conditions [19]. Following stimulation, 13-HODE production stops and HETE production commences (5-HETE in leukocytes; 12-HETE in platelets; 15-HETE in endothelial cells). Under these conditions, leukocytes adhere to the endothelium and initiate migration through it; platelets adhere to and spread on the damaged vessel wall [20, 162].

Analogous pathways of fatty acid metabolism have now been identified in cancer cells and have been shown to influence their adhesiveness. Under basal conditions several human cancer cell lines produce both 13-HODE and HETEs, the intracellular levels of which were found to have a marked effect of adhesivity. When non-metastatic and metastatic cells from the same line were compared, it was found that the non-metastatic cells synthesized predominantly 13-HODE while the metastatic cells showed predominant HETE metabolism. Stimulation of the cells with the synthetic peptide FMLP decreased 13-HODE synthesis and increased HETE synthesis. The production of HETE molecules was associated with increased adhesion of cancer cells to the endothelium and to the extracellular matrix. Conversely, inhancing 13-HODE synthesis by increasing intracellular cAMP was associated with decreased adhesion [12].

Regulation of cancer cell membrane expression of integrin receptors by lipoxygenase products of arachidonic and linoleic acids. Honn *et al.* [82] reported that exposure to a phorbol ester (TPA) increased the surface expression of IRGpIIb/IIIa on human cervical carcinoma cells. TPA is also known to increase cancer cell adhesion and metabolism of arachidonic acid via the cyclooxygenase and lipoxygenase pathways [54], suggesting that TPA enhanced adhesion is, in part, mediated via the IRGpIIb/IIIa receptor, possibly also involving cancer cell metabolism or arachidonic acid. When cancer cells are stimulated with TPA, labeled with monoclonal antibodies to GpIIb/IIIa, and subjected to flow cytometric analysis, the level of surface expression that receptor may be quantitated [82]. Cancer cells treated with TPA demonstrate an enhancement of fluorescence intensity, indicating increased surface expression and/or activation of IRGpIIb/IIIa receptors, and an increase in the percentage of cells labeling positive for IRGpIIb/IIIa [81, 82]. This effect can be blocked by an inhibition of the lipoxygenase enzyme, but not by inhibition of the cyclooxygenase enzyme [81, 82]. These results suggest that cancer cell lipoxygenation products of arachidonic acid may mediate the enhanced expression of IRGpIIb/IIIa. In fact, the effects of TPA can be 'mimicked' by 12(S)-HETE, but not by 12(R)-HETE, 5-HETE, or 15-HETE [81]. Both TPA and 12(S)-HETE enhanced expression of IRGpIIb/IIIa can be inhibited by the endothelial cell cyclooxygenase product, prostacyclin, and the endothelial cell lipoxygenase product of linoleic acid, 13-HODE [81]. In parallel with the observed effects of TPA and 12(S)-HETE on enhanced expression of IRGpIIb/IIIa, these factors also enhanced cancer cell adhesion to endothelial cells, subendothelial matrix and fibronectin [81, 90]. An increase in adhesion was not observed with 12(R)-HETE, 15-HETE, or 5-HETE, consistent with their lack of effect on IRGpIIb/IIIa expression as observed by flow cytometric analysis [81, 82, 90]. TPA and 12(S)-HETE enhanced adhesion to all of the previously mentioned substrata were inhibited by: (1) specific polyclonal antibodies and monoclonal antibodies to GpIIb/IIIa; (2) lipoxygenase, but not cyclooxygenase inhibitors in the case of TPA; (3) prostacyclin; and (4) 13-HODE [81, 82, 88, 90]. These results suggest that external stimuli, possibly by an

intracellular lipoxygenase-dependent mechanism, can increase cancer cell expression of the integrin receptor IRGpIIb/IIIa [81] and also IRGpIb (K. V. Honn and I, M. Grossi, unpublished observation) and thereby facilitate cancer cell adhesion to endothelial cells, subendothelial matrix, and its components.

Possible involvement of the cancer cell cytoskeleton and the mobility of membrane receptors in cancer cell/platelet interaction. It has been reported that agents disrupting specific components of the cancer cell cytoskeleton [177] modify parts of hematogeneous metastasis [14, 68, 83, 84].

One mechanism for this metastasis-inhibitory activity, which has only recently been considered, is an alteration in the mobility of cancer cell membrane receptors and the ability of cancer cells to induced platelet aggregation. Chopra *et al.* [27] recently reported that disruption of cancer cell microfilaments and/or intermediate filaments, but not microtubules, inhibits their ability to induce the aggregation of homologous platelets. Ultrastructural studies confirmed a lack of platelet adhesion to the cancer cell surface and an absence of specific membrane processes associated with cancer cell platelet interactions [27, 125, 126, 128, and see below]. This effect was due to an inhibition of plasma membrane receptor mobility, but not membrane fluidity because paraformaldehyde treatment inhibits the ability of cancer cells to induce platelet aggregation concurrent with an inhibition of receptor mobility, but no inhibition of membrane fluidity [27]. Monoclonal antibodies directed to GpIIb/IIIa induced the patching and capping response of cancer cell IRGpIIb/IIIa receptors. This response was inhibited by disruption of microfilaments and intermediate filaments, but not by disruption of microtubules [27, 28]. As stated above, pretreatment of cancer cells with specific antibodies to GpIIb/IIIa inhibits their ability to induce platelet aggregation and platelet attachment to the cancer cell surface [27, 28, 87]. These results were interpreted as evidence that IRGpIIb/IIIa may serve as at least one receptor for platelet adhesion to the cancer cell surface, and that the ability of this receptor to undergo patching and capping is necessary for the induction of a platelet aggregation response [27, 28, 87]. This phenomenon may explain the observed focal interaction of platelets with the cancer cell surface [125, 126, and see below]. In a follow-up study, Chopra *et al.* [28] used calcium channel blockers to disrupt elements of the cancer cell cytoskeleton; once again, disruption of cancer cell intermediate filaments and inhibition of IRGpIIb/IIIa receptor mobility correlated with an inhibition of platelet aggregation and platelet adhesion to the cancer cell surface. Disruption of microfilaments and intermediate filaments also results in decreased cancer cell adhesion to endothelial cells, subendothelial matrix, and its components (K. V. Honn and H. Chopra, unpublished observation) suggesting that mobility of the IRGpIIb/IIIa, and possibly other receptors, are necessary for cancer cells to secure stable adhesion to a variety of substrata.

Interpretation of adhesion experiments

In addition to differences between arrest and cancer cell/vessel wall adhesion, it is important to recognize differences between the various techniques used to study adhesion, since interpretations are technique-dependent. Many *direct* experiments on adhesion, particularly those designed to explore the role of adhesive macromolecules, have been made *in vitro*, when cancer cells are often allowed to sediment under the influence of gravity, on to monolayers of endothelial-derived cells or films

of subendothelial constituents. Although hydrodynamic factors are also thought to play a role in determining the rates of cancer cell adhesion in these *in vitro* systems [238], there are obvious physical differences between them and the *in vivo* situation, where the driving force is cancer cell elastic recoil.

In the *in vivo* situation, cancer cells interact with the subendothelium only after its exposure by endothelial retraction or loss [111]. This situation is mimicked in *in vitro* experiments, in which cancer cells are allowed to sediment onto endothelial monolayers which cover films of stromal constituents [144]. It is not known whether the endothelial retraction process is different *in vivo* with respect to mechanism and rate, under conditions of shear due to blood-flow and cancer cell recoil.

(3) Cancer cell trauma associated with arrest

It appears from many observations on humans, and other animals, that there is a gross disparity between the millions of cancer cells released into the blood per day from primary tumors [72, 75] and the comparatively small numbers of metastases developing [228]. This disparity has been termed *metastatic inefficiency* [225], and is mainly due to cancer cell death, much of which occurs in the microcirculation as a consequence of rapid and slow mechanisms.

Rapid killing

Bioassays of the lungs [119, 223], liver [244], myocardium [230] and skeletal muscle [231] of mice after they had received tail-vein, portal vein or left ventricular injection of cancer cells, indicate that the majority of these cells are killed in the microcirculation of these organs within the time taken to remove them for bioassay (2–3 min). The rapidity of cell killing suggested that mechanical trauma was a distinct possibility.

Cell deformability. Pioneering observations on mechanical aspects of intravascular damage to circulating cancer cells were made by Sato and Suzuki [181]. Different types of cancer cells were introduced into, and recovered from, the pulmonary circulation of rats, and the damage inflicted on them by transpulmonary passage was estimated by dye-exclusion tests. A positive correlation was obtained between the percentage viability (dye-exclusion) of recovered cells, and the ease with which they could be deformed. Deformability was measured by determination of the negative pressures required to such bulges from cancer cells into micropipettes applied to their peripheries [221].

In these experiments [181], although the percentages of dye-excluding cells leaving the pulmonary circulation increase with increasing cell deformability, the number of cells leaving or remaining in the lungs are not given, presumably because of the technical difficultues associated with this type of sampling. Therefore, although there is an increasing tendency of the different cell types to generate post-pulmonary tumors following intravenous injection, it is not apparent to what extent this is due to variation in cancer cell number or percentage viability. In addition, the invasive capabilities of the different cell types vary considerably, as assessed by the time taken for them to intravasate from intraperitoneal ascites tumors. Therefore, their metastasis-related behavior could be at least partially accounted for by differential invasiveness. Thus, as deformability and invasiveness have not been demonstrated as dependent variables, the relationship of cancer cell deformability to

metastasis is not defined by Sato and Suzuki's analysis of their experimental data. Finally, as discussed below, the relationship of (micropipette) deformability measurements to membrane properties is complex, and varies with the degree of deformation.

Shape transitions. When approximately spherical, circulating cancer cells enter a capillary from a larger vessel, they undergo a shape transition. At constant cell volume any deviation from spherical shape requires an increase in surface area, which may be apparent or true. Apparent increases in cell surface area may be achieved by unfolding a previously folded surface membrane. True increases in cell surface area may be achieved by stretching. In general, bending or unbending of membranes requires less energy than stretching [48]. Therefore, unfolding will occur before stretching and, if the increase in surface area required for shape transition can be accommodated by unfolding, then stretching is not expected to occur.

Experiments have been made to demonstrate the feasibility of the unfolding hypothesis, by suspending cancer cells in hypotonic solutions, in fixing them, and subsequently examining them by electron microscopy. The degree of surface folding was determined by computerized planimetric measurements made on electron micrographs of the cell membrane. The results show that some, but not all, types of cancer cells examined, respond to the demand for increased surface area (in response to hypotonic swelling) by unfolding of their surfaces [24]. However, with decreasing osmolarity of the suspending fluid the membranes rupture, indicating that, beyond a critical yield point, a reversible unfolding process cannot meet the demand for increase in surface area.

When parts of cancer cells were sucked into micropipettes, and the cells were then expelled by positive pressure, hemispherical bulges were rapidly 'resorbed', and the cells returned to spherical shape, indicating reversible deformation of a viscoelastic structure. However, if elongated sausage-like protrusions were sucked from cells, they remained after expulsion, indicating plastic deformation beyond an irreversible yield-point [232]. This dual type of deformability has also been documented in leukocytes [182]. The irreversible phase of membrane expansion is associated with loss of membrane integrity, since when true membrane expansion results in an increase in area of more than 2–4 per cent, the increase in membrane tension results in rupture [48, 149].

This sequential model for the cancer cell periphery (figure 1), in which transition from a spherical shape is associated first with unfolding and then possible stretching [229], is in accord with independent observations on leukocytes [182]. The model partially accounts for the mechanically induced death of cancer cells during transit through 5 μm diameter, glass pipettes [181], through 5 μm pores in Nuclepore membranes [63, 104], and in the microcirculation [229]. The differential survival of cancer cells exposed to mechanical trauma may therefore indicate differential capacities of their peripheries to unfold, and to accommodate to shape transition without membrane stretching.

As it is technically impossible, at present, to directly observe cancer cells within the microcirculation in the deeper parts of organs, most of the information is derived from indirect experiments and theoretical studies.

Attempts have been made to predict the outcome of these mechanical interactions on cancer cell viability and metastasis, by calculating the tensions at the

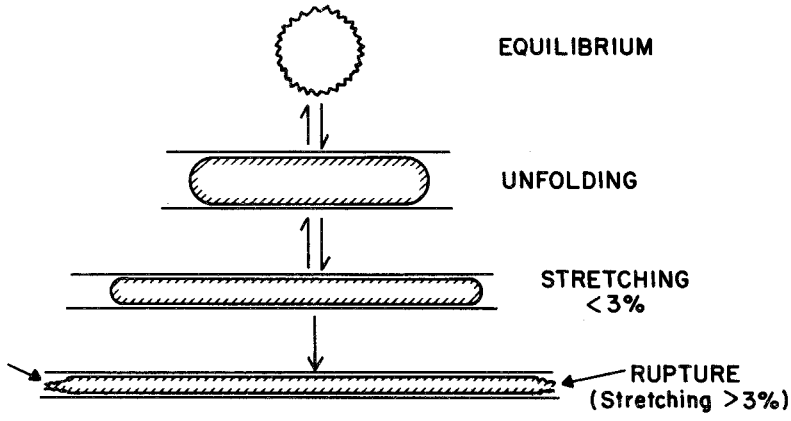

Figure 1. Shape transition occurring when a spherical cancer cell with a folded surface enters a capillary. First the surface unfolds, next it is stretched and finally, if the areal increase exceeds approximately 3 per cent, the increased tension results in membrane rupture.

surfaces of the cells and vessels; a critical value for rupture of 50 per cent of some cell populations appears to be approximately 4 dyn. cm^{-1} [233–235]. Although the calculations for the vessels appear reasonable, the abolute values obtained for the cancer cells are questionable because they were based on micropipette measurements of cell deformability [181], which depend not only on cell surface tension, but also folding and the contribution of internal structures particularly the cytoskeleton [229, 234]. However, as the same assumptions were made with respect to the cancer cells throughout, the variables associated with vessel wall properties could be quantitatively assessed, and have served to establish the feasibility of mechanically induced trauma to circulating cancer cells.

Factors influencing intravascular destruction: (a) Relative deformability. The true or apparent increase in cancer cell surface area depends on the degree of cellular deformation within the microcirculation which, in turn, depends on the relative deformabilities of the cancer cells and the microvessels. In the extreme case, where cancer cells are very much less deformable than the vessel, a bulge in the vessel caused by the hydrodynamic field surrounding the cancer cell could move along the vessel, preceding the cell during its transit. Under these circumstances cancer cell deformation, transit time, arrest, adhesion and destruction would be minimal. In contrast, when the vessel wall is much less deformable than the cancer cell, cell deformation transit time, arrest, adhesion and destruction would be maximal. An intermediate situation would arise, when the vessel wall and the cancer cell were equally deformable; and mechanical heterogeneity is covered by these three cases [234].

Calculations based on lung tissue elasticity and alveolar surface tension indicate that during expiration, when tension in the capillary walls is minimal, cancer cells can enter and travel along the capillaries without lethal damage, because the vessels are deformed by the cells and the hydrodynamic fields surrounding them [234]. Under these circumstances any cancer cell deformation is expected to be associated with unfolding only. During inspiration, when significant increases in capillary wall

tension occur, intravascular cancer cells are expected to be deformed to a major extent, and their surface membranes to become both unfolded and stretched, and therefore develop tensions above the critical level for rupture and cell death.

(*b*) *Tissue pressure.* The capillary beds of the myocardium and skeletal muscles are other examples of sites in which physiologic changes (contraction) may be linked to differential cancer cell-killing properties. These sites illustrate the effects of pressures developed in the surrounding tissues, on cells trapped in the microvasculature.

(*c*) *Death of cancer cells in the myocardium.* Intramyocardial pressures vary from approximately 120 mmHg near to the endocardium, to 90 mmHg near to the epicardium (in the dog), corresponding to 'hoop' tensions in the muscles of 3·3 to $2{\cdot}2 \times 10^5$ dynes respectively [7]. These tensions are orders of magnitude higher than those required to rupture cancer cell membranes. It is therefore of interest that, under specified circumstances, there is a slow incidence of myocardial metastases in patients with cancer, compared with other target organs. It has been suggested that this is in part due to mechanically induced cancer cell death in the myocardial microcirculation [230, 235].

In mice, immediately following injections of radiolabeled cancer cells into the left ventricular cavity (LVI), 4·7 and 2·2 per cent were arrested in the myocardial capillary bed and lungs respectively [243]. After receiving LVI of non-labeled B16 melanoma cells, evidence of cancer cell death was provided by many intramyocadial deposits of cell-free melanin. Immediately following LVI of non-labeled cancer cells, the lungs and heart were removed from each animal. minced and transplanted into the peritoneal cavities of two fresh mice. The incidence of tumors in the myocardial implants was significantly less than in the lung implants, in spite of twice as many cancer cells originally being arrested in the heart. That the rapid death of cancer cells was not due to inherent toxicity of the heart muscle, was shown by experiments resulting in 100 per cent of tumor takes, when similar numbers of cancer cells to those delivered by LVI were added to minced myocardium and then implanted. Thus the rapid death of cancer cells arrested in the myocardial capillaries was dependent on myocardial contraction, in accord with the mechanical trauma hypothesis. Two observations which at least partially account for the occurrence of myocardial metastases in the face of this trauma, are: (1) that not all of the cancer cells are killed, and (2) that metastases initially tend to develop at the myocardial surface, where there is pressure relief [230].

(*d*) *Death of cancer cells in skeletal muscle.* In humans, hematogenous metastasis to skeletal muscle is uncommon [217, 226, 247] and, for example, in 347 autopsies on cases with a history of metastatic colon carcinoma, only 10 had detectable metastases in skeletal muscle [237].

In order to study trauma associated with muscle contraction, anaesthetized mice were given left ventricular injections (LVI) of cancer cells; the quadriceps femoris muscles were ultimately removed within several minutes and implanted into the peritoneal cavities of separate mice, for bioassay. The muscles fell into three major experimental groups: those with intact muscle, which in the anesthetized animals did not contract, but which possessed tone; those animals which the femoral nerve on one side was transected 1 h before LVI, in which the muscle was flaccid; and animals

in which, immediately after LVI, the muscle on one side was made to contract by electrical stimulation. The results showed that cancer cell survival was greatest in the flaccid muscle, intermediate in the non-contracting intact muscle, and least in the electrically stimulated (contracting muscle [321]. These observations are in accord with the hypothesis that mechanical trauma to cancer cells, associated with muscle contraction, can act as a rate-regulator for metastasis in skeletal muscle.

(*e*) *Tissue compliance.* In 'solid' organs the deformation of the microvasculature by cancer cells will depend only only on their own properties, but also on the properties of the tissues surrounding the vessels; for finite deformations of the type discussed here, any analysis must take account of their highly complex, non-linear, stress–strain relationships [60]. At present, apart from the lungs, myocardium and skeletal muscle, no analyses have been made which are relevant to other organs, and which might indicate the contribution of site-specific, differential mechanical trauma to seed-and-soil effects [160] in metastatic pattern.

(*f*) *Blood-pressure differentials.* In theoretical studies [233–235] attempts were made to calculate the effects of blood-pressure differentials, on cancer cells deformed into 'sausage' shape following entry and arrest in capillaries. In these calculations a number of simplifying assumptions were made: first that the cancer cell surface was planar, consistent with non-lethal unfolding; second, that an arrested cancer cell completely blocks the vessel in which it is arrested, and third, as a consequence of blocking, that the blood-pressure differential which formerly acted over the whole length of a capillary, now acted between the free ends of the arrested cancer cell. It was calculated, that under these conditions, physiologic blood-pressure differentials, added on to the effects of shape-transition, would generate enough tension at the surface of the cancer cell to cause lethal rupture. Against this third assumption is the suggestion that, in the case of leukocytes trapped in a capillary network with patent and obstructed vessels, the pressure differentials between the ends of a trapped leukocyte are thought not to exceed the viscous pressure drop in a neighboring capillary [183]. However, the often incomplete capillary blockage caused by granulocytes is not analogous to the complete blockage observed with cancer cells, and it is not clear whether the short-term sequelae of cancer cell arrest affect the patency of neighboring capillaries, and whether the microvascular anatomy and pressures across capillary beds in different organs permit lethal blood-pressure gradients along cancer cells. At present the contribution of blood-pressure gradients to the mechanical destruction of cancer cells cannot be quantitatively assessed.

Slow killing

Arrest of cancer cells shares many features with microembolism in general, including elicitation of the inflammatory response. The traumatic effects of inflammatory and associated processes on arrested cancer cells are discussed below (sections 4–6).

(4) Microvascular trauma

Historical considerations

Since cancer cells attach to extracellular matrix components in preference to endothelial surfaces [107, 215], it has been postulated that endothelial damage might facilitate the metastatic process by causing endothelial retraction or denudation.

Indeed, many antineoplastic treatments are potentially cytotoxic to the endothelium, and have been reported to enhance the metastasis of intravenously injected cancer cells or spontaneously metastatic tumors. These include commonly employed antineoplastic drugs [22, 102, 130, 172, 193, 212], high concentrations of oxygen [194], and X-irradiation [38, 86, 132]. Many of the antineoplastic drugs induce retraction of confluent endothelial monolayers when added to tissue cultures in concentrations comparable to those found in plasma during chemotherapy [115, 143].

Correlation between endothelial damage and metastasis

Using established experimental models of lung injury it has been possible to obtain more direct evidence for the existence of correlations between endothelial injury, retention of arrested cancer cells, and metastasis. In one study, pulmonary endothelial damage was induced in C57BL/6 mice by intravenous bleomycin (120 mg/kg) or by exposure of 90 per cent oxygen for 2–4 days [3, 4, 155]. Vessel wall injury was detected by swelling and retraction of endothelial cells in the lung microvasculature and by finding increased content of protein or intravenously injected [^{125}I]albumin in bronchoalveolar lavage fluids. There was a 6–30-fold increase in the arrest of [^{131}I]iododeoxyuridine-labeled fibrosarcoma cells when these were injected intravenously during periods of endothelial injury. Subsequently, more metastatic tumors formed in the experimental groups with injured endothelium and there were direct correlations ($r=0{\cdot}9$) between the extent of endothelial injury and the numbers of metastatic tumors. The importance of the endothelium as a target was emphasized by the observation that no increase in tumor localization or metastasis was found after endothelial repair even if there was pulmonary fibrosis.

Enzymatic degradation of the basement membrane

Penetration and degradation of the vascular basement membrane by extravasating cancer cells has been observed by electron microscopic analysis in several experimental systems [35, 186], and generally appears to be a requirement for metastasis. As the composition of the vascular basement membrane is analogous but not identical to that of other extracellular matrices, it has been postulated that proteolytic enzymes are also involved in its penetration. This has been examined *in vitro* where production of proteases and glycosidases by cancer cells facilitates their ability to degrade endothelial basement membrane constituents [107, 140, 144, 146, 215, 216]. Some of these analysis have shown correlations between the differing metastatic potential of related cancer cell lines *in vivo* and their ability to degrade specific basement membrane components, e.g. HS proteoglycans [138] and type IV collagen [140]. Nakajima *et al.* [140] have also shown that collagenolysis could be attributed to the action of more than one proteinase, and that much of the type IV collagenolytic activity was secreted in a latent form, requiring activation by trypsin-like enzymes or the presence of serum for its expression. The relevance of enzyme production by cancer cells to the degradation of vascular basement membranes *in vivo* requires further analysis since, in at least one group of experiments, infusion of biologically effective doses of inhibitors of these enzymes failed to block experimental metastasis of the B16 murine melanoma [158].

In contrast, Ossowski [157] has demonstrated that inhibition of urokinase-type plasminogen activator with antiurokinase results in inhibition of metastasis in the chick chorioallantoic membrane model. In this study she has indirect evidence that urokinase may act at the level of cancer cell intravasation through the basement membrane into the vasculature. This may explain the discrepancy between the two studies. An alternative explanation for the failure of inhibitors to prevent metastasis, and one raised by Ostrowski *et al.* [158], is that the inhibitors are ineffective against membrane-associated enzymes. Urokinase-type [for review see 16 a] and tissue-type [83 a] plasminogen activators, metalloproteinases [255, 256] and cysteine proteinases [for review see 187 a] have all been found to be membrane-associated. More importantly, both types of plasminogen activator [16 a, 83 a] and cathepsin B [178 a] are resistant to inhibition by inhibitors, when membrane-associated.

(5) Inflammatory responses associated with arrest

Systemic reactions

The effect polymorphonuclear leukocytes (PMN) have on metastasis is controversial. Some laboratories have reported that PMNs (in particular neutrophils) can inhibit metastasis, whereas others have demonstrated that PMNs augment cancer cell metastasis. In examining the egress of intravascular VX2 carcinoma cells from the vessels on the rabbit ear, Wood observed that the cells appeared to leave the vessel through defects caused by leukocytes [248]. Indirect evidence for involvement of neutrophils in metastasis comes from studies where activation of systemic inflammatory processes has been shown to promote metastasis. For example, it has been observed that intravenous injection of inflammatory stimuli leads to increased experimental metastasis associated with a systemic reaction, characterized by intravascular activation of inflammatory mediators, leukocytosis, and pulmonary damage [71, 73, 156, 210]. Ishikawa *et al.* [99 a] reported that mice bearing the BMT-11 fibrosarcoma demonstrated increased numbers of PMNs associated with tumor growth. The i.v. injection of B16 cells into these tumor-bearing mice resulted in a pulmonary retention of cancer cells which was 3–10 times greater than pulmonary retention in non-tumor-bearing controls. These effects were attributed to the granulocytosis present in the tumor-bearing mice. Ward and Weiss [219] have also demonstrated that the i.v. injection of cancer cells into mice increases the lung colony retention of subsequent injections with the same cancer cells. Similarly, leukocytosis has been associated with a poor prognosis in human cancer. Shoenfeld *et al.* [185] reported that 30 per cent of patients with 10 different types of non-hematological malignancies demonstrated leukocytosis at the time of diagnosis. Leukocytosis in these patients was associated with the presence of metastasis, and was associated with a shorter survival time. The mechanism for this increased leukocytosis in tumor-bearing patients is unknown, but it could be due to metastasis-associated local damage to host tissues. In addition, Takahashi *et al.* [198] observed that the conditioned medium from cultured human gall bladder carcinoma cells contained a colony-stimulating factor for human leukocytes. *In vivo* the stimulation of neutrophils by non-tumor-derived agonists has also been associated with an increase in metastasis.

Leukocytes could promote metastasis by several means, Orr and Mokashi [153] examined inflammatory exudates following intratracheal injection of suspensions of carbon particles in mice. Neutrophils accounted for more than 75 per cent of the inflammatory cells in the first week following intratracheal injection. *In vitro*, cancer cells were found to undergo chemotaxis in response to lavage supernatants from animals with inflamed lungs. The magnitude of this chemotactic response was directly proportional to the number of neutrophils in the exudate. Intravenous injection of cancer cells into animals previously receiving intratracheal injection of carbon particles resulted in a 5-fold increase in pulmonary retention of these cells. Since activation of neutrophils is followed by their sequestration in the lung microvasculature, release of hydrolytic enzymes, and by the generation of activated oxygen species [133, 205], it is also possible that systemic activation of circulating neutrophils could facilitate metastasis by causing damage to the endothelium and basement membrane. To test this hypothesis directly, Sprague–Dawley rats were given intravenous injections of cobra venom factor resulting in complement activation, rapid sequestration of netrophils in the lung, and endothelial damage. The latter was demonstrated by edema of lung endothelium, retraction of endothelial cells from the lung basement membrane, and by increased protein content and leakage of intravenous [^{125}I]albumin into bronchoalveolar lavage fluids. When radiolabeled Walker 256 carcinosarcoma cells were injected intravenously during the period of endothelial injury, there was a 3-fold increase in the number of these cells retained in the lung after 24 h in treated animals, compared to controls. This enhanced localization was prevented by pretreatment of the rats with catalase or by antineutrophil antisera. When animals were examined 2 weeks after cell injection, treatment groups had significantly more metastatic tumor nodules and a greater area of lung tissue involved by metastases. In this model the formation of aggregates between cancer cells and leukocytes could also contribute to metastasis. Such heterotypic aggregates can be formed within 3 min of leukocyte activation *in vitro* and are not necessarily toxic to cancer cells [153]. Starkey *et al.* [192] have reported that the presence of polymorphonuclear leukocytes or activated macrophages in cancer cell aggregates promotes the attachment of the cancer cells to endothelial monolayers, and that intravenous injection of such multicellular aggregates increased pulmonary metastasis over that obtained by injection of cancer spheriods alone.

In contrast to the above studies there is also evidence in the literature which suggests that neutrophils are an effective component of the cytolytic host cell response in circulating cancer cells. Glaves [73] reported decreased pulmonary retention of B16 melanoma cells in mice following i.v. injection of the vital dye, trypan blue. Macrophage function was normal or depressed in the trypan blue-treated mice; however, there was an increase in the numbers of circulating polymorphonuclear leukocytes. Treatment of mice with an anti-polymorphonuclear leukocyte antiserum resulted in a decrease in circulating PMNs and a concomitant increase in the pulmonary retention of B16 melanoma cells in leukocyte-depleted animals. In a subsequent study the pulmonary retention of Lewis lung carcinoma cells was significantly increased in animals treated with the oxygen radical scavenger, superoxide dismutase [74]. In addition, following injection of superoxide dismutase, there was a parallel increase in the number of pulmonary tumor colonies formed in mice injected with Lewis lung carcinoma cells. These results were interpreted as an indication that superoxide anion plays an important role in the destruction of

arrested cancer cells. Lichtenstein *et al.* [114] reported the destruction of a murine ovarian teratocarcinoma after the intraperitoneal injection of *Corynebacterium parvum*. The increased cytotoxicity of intraperitoneal cancer cells following i.p. *C. parvum* injection correlated with the presence of neutrophils in the peritoneal cavity. Recently Lichtenstein [113] has demonstrated that the destruction of murine ovarian teratocarcinoma cells in the peritoneal cacity is mediated by reactive oxygen intermediates released from PMNs. Although *C. parvum*-elicited neutrophils are demonstrated to have cytotoxic effects against ascites tumor cell lines, it is not clear what effect these neutrophils would have on hematogenously circulating cancer cells (i.e. increased or decreased lung colony formation). Nevertheless, the studies of Glaves [71, 73, 74] indicate that mild stimulation of neutrophils (e.g. trypan blue) elicits an antimetastatic effect, while depletion of neutrophils with specific polyclonal antiserum abrogates this effect. Glaves [72] has demonstrated that large numbers of Lewis lung carcinoma cells (3LL) are released into the circulation from primary subcutaneous tumors. In addition, large numbers of circulating cancer cells are found in animals bearing Lewis lung carcinomas. However, the actual numbers of metastases found in these animals are orders of magnitude lower than would be predicted from the number of circulating cancer cells, suggesting that metastasis is an inefficient process. Honn *et al.* [reviewed in 241] demonstrated that tail vein injection of elutriated 3LL cells into C57BL/6J mice results in the formation of few (range 0–7) lung colonies 21 days later. In contrast, tail vein injection of a similar number of B16 amelanotic melanoma (B16a) cells, results in a significantly larger number (range 50–80) of lung tumor colonies. Animals injected with 3LL cells were sacrificed, the lungs removed and processed for ultrastructural examination. The number of arrested cancer cells in close proximity (i.e. membrane–membrane interaction) with PMNs was recorded. At 10 min following injection, 20 per cent of the arrested 3LL cells were found to be in association with PMNs [100, 241]. This figure increased to a maximum of 95 per dent at 4 h. When a comparable number of B16a cells were injected into C57BL/6J mice less than 10 per cent of the arrested cancer cells were found in association with neutrophils. These results suggest a role for circulating PMNs in the destruction of arrested Lewis lung carcinoma cells, and the absence of such a role for arrested B16a cells. Therefore, C57BL/6J mice were pretreated with the IgG fraction of rabbit anti-mouse neutrophil antiserum. Injection of antiserum 1 h prior to the injection of 3LL cells resulted in a dose-dependent increase in the number of pulmonary colonies.

These results implicate PMNs in the destruction of intravascular arrested 3LL cells and demonstrate a heterogeneity in the response to neutrophils by various cancer cell types within the same strain of murine host. However, these results do not resolve the conflict which exists in the literature regarding the role of neutrophils in metastasis. In most experimental protocols involving the intravascular stimulation of neutrophils and/or an increase in their number [99 a, 156] a positive effect (i.e. increase) on metastasis is observed. However, depletion of the circulating pool of neutrophils also results in increased metastasis [100]. Therefore, a general nonspecific activation of circulating neutrophils may result in endothelial cell damage with concomitant exposure of the subendothelial matrix. As noted above, cancer cells preferentially attach to components of the subendothelial matrix compared with attachment to endothelial cells [108]. However, in untreated animals, neutrophils may exist in a 'resting' state, from which they are activated in response to arrested cancer cells. In the latter case the cytolytic effect of these neutrophils may be

specifically directed against cancer cells. It would be interesting to observe whether the intravenous injection of *C. parvum*-elicited neutrophils [113, 114] prior to the tail vein injection of cancer cells would increase or decrease their lung colony formation. Alternatively, the discrepancies which exist may be due to the differential sensitivity of various cancer cell lines to the cytolytic effect of activated neutrophils, as has been observed for the Lewis lung carcinoma and the B16 amelanotic melanoma.

Oxygen-derived free radicals

There is reasonable evidence that the endothelial damage observed after bleomycin and hyperoxia is the consequence of oxygen-derived free radical generation [40, 163]. Likewise, in the experiments on neutrophil-mediated lung injury described above, the effects on metastasis have been postulated to be the consequence of oxygen-derived free radical release by neutrophils, especially H_2O, since pretreatment of animals with catalase (to be deplete H_2O_2) blocked the enhancing effect of leukocyte activation and since the effect of complement activation was diminished in animals depleted of neutrophils [156]. It has been well established that neutrophils are capable of damaging vascular basement membranes by synergistic mechanisms dependent upon oxygen free radical generation, activation of latent proteases and inactivation of protease inhibitors [31, 184, 245]. The possibility that free radicals released from intravascular cancer cells might degrade basement membranes by a similar means is suggested by evidence that many non-neoplastic and neoplastic cell types can generate free radicals [110, 147]. In addition, like leukocytes, the cancer cell line, Walker 256 carcinosarcoma, is capable of being activated by environmental factors which generate oxygen-derived free radicals [112 a]. Taking the experiments of Glaves into account, it appears that free radicals may have an enhancing or an inhibitory effect on metastasis. On the one hand, free radicals released from cancer cells, host leukocytes, or generated in endothelial cells, could facilitate metastasis by causing damage to the endothelium or vascular basement membrane. On the other hand, as superoxide anion is toxic to some cancer cells, this radical may be involved in clearing arrested cells from the microvasculature.

Influence of localized inflammation

There are obvious analogies between the processes of metastasis and acute inflammation. Thus it has seemed reasonable to look for similar regulatory mechanisms in both. Evidence for this includes the observations that the arrest and metastasis of circulating cancer cells is often promoted at locally inflamed sites. As noted above, in models of peritonitis and experimental pneumonitis [152–154], it has been demonstrated that acute inflammatory reactions are associated with the local generation of chemoattractants for cancer cells, most likely due to digestion of the C5a component of complement by leukocyte-derived lysosomal enzymes [151]. It has also been suggested that inflammatory processes could promote metastasis through the local generation of growth factors [210], although in some experiments a search for these has been unsuccessful [152]. It has been repeatedly observed that local or systemic infusion of vasopermeability factors does not alter the colonization of cancer cells injected into the circulation [152, 159, 210]. As suggested earlier in this review, inflammatory processes may also be associated with the expression of endothelial adhesion molecules or the release of humoral mediators which could alter the properties of local cancer cells.

(6) The hemostatic response associated with cancer cell arrest

The platelet is believed to be a major factor in promoting metastasis, on account of its role in cancer cell arrest and initial adhesion to the vessel wall. In 1968 Gasic and co-workers [66] provided the first experimental evidence for the participation of platelets in metastasis, by demonstrating that the induction of thrombocytopenia, prior to tail vein injection of cancer cells, reduced subsequent lung colony formation. However, subsequent studies, using a variety of anti-platelet agents, have produced equivocal results [93].

Numerous human and animal cancer cells have been shown to induce the aggregation of platelets [11, 199, 206]. This ability has been demonstrated to correlate positively with their metastatic potential in some [11, 89, 199, 206] but not all [47, 105 a] cell lines examined. Crissman and colleagues [34, 35, 126] recently described cancer cell–platlet–endothelial cell interactions *in vivo* following the injection of either B16a, 3LL of 16C mammary adenocarcinoma cell lines in syngeneic mice. Platelet/cancer cell emboli were observed in the pulmonary vasculature immediately following tail vein injection of elutriated cancer cells of the three histological types mentioned above [34, 126]. The degree of platelet association with arrested cancer cells was time-dependent [34, 35, 126]. Initial signs of platelet aggregates were observed as early as 10 min post-cell injection, and they increased to their maximum between 4 and 8 h. Platelets were not observed interposed between the cancer cell and endothelial cell plasma membrane [34, 35]. Cancer cells arrested at early time intervals demonstrated a limited focal association with activated platelets [35]. These results suggest that *in vivo* cancer cell-induced platelet aggregation occurs after cancer cell arrest and attachment to the vascular endothelium–subendothelial matrix. Arrested cancer cells exhibit process formation similar to that reported by Honn and colleagues for studies of *in vitro* cancer cell platelet interactions [28, 87, 125, 126, 128]. *In vivo* and *in vitro*, these processes interdigitate with the platelet aggregate. These processes were found only in areas of cancer cell plasma membrane contact with activated platelets, and not in areas of cancer cell plasma membrane contact with unactivated platelets, endothelial cells, or blood elements [34, 35, 126]. Intravascularly, platelet activation and degranulation were observed primarily in platelets associated with the cancer cell processes [35] and increased in a time-dependent manner. Specifically, at early time intervals, less than 10 per cent of the cancer cell plasma membrane, exposed to the vascular lumen, was associated with activated platelets. This association with activated platelets increased with time (i.e., $\frac{1}{2}$–4 h) until the entire luminal surface of the cancer cell was surrounded by activated platelets. The associated thrombi disappeared approximately 24 h after arrest; however, cancer cells remained inside vessels for up to 3–5 days and demonstrated evidence of intravascular proliferation [34, 35]. The decrease in platelet thrombi associated with arrested cancer cells correlates with the increase in cancer cell association with the subendothelial matrix following endothelial cell retraction. It is of interest to note that, at early time intervals, up to 100 per cent of the arrested cancer cells were found associated with activated platelets, whereas at later time intervals (i.e. >24 h) intravascular cancer cells, adherent to the subendothelial matrix, were observed with unactivated platelets juxtaposed to their luminal surface [34, 35]. This effect was not due to a fibrin coat on the cancer cell surface. Honn and colleagues have previously demonstrated that platelets can enhance the level of cancer cell adhesion to endothelial cell monolayers [91] and their subendothelial matrix [127, 128] *in vitro*. This effect was dependent upon elements of the platelet

plasma membrane (i.e. GpIIb/IIIa) and intact platelet microfilaments [127, 128]. Thus it seems reasonable to suggest that platelets may participate in facilitation of cancer cell metastasis by stabilizing the initial cancer cell adhesion to endothelial cells and possibly subendothelial matrix during the early time intervals post-cancer cell arrest. Once a cancer cell establishes significant plasma membrane contact with the subendothelial matrix, the associated thrombi disappear, suggesting a lack of platelet participation in this stage of cancer cell adhesion and/or extravasation from the vasculature.

Gorelik and co-workers [77, 78] have suggested that platelets/fibrin protect intravascular cancer cells from cytolytic destruction by circulating natural killer cells. However, such a function for platelet/fibrin could be operative only for the first 24 h following cancer cell arrest, since there is an absence of platelet/fibrin association with arrested cancer cells after 24 h [34, 35]. While undergoing proliferation, cancer cells remain intravascular up to 5 days in the absence of a platelet/ fibrin barrier to circulating NK cells [34, 35].

It has also been suggested that platelets facilitate cancer cell metastasis by releasing adhesive glycoproteins stored in their alpha granules and dense granules. Several adhesive proteins (i.e. fibronectin, von Willebrand factor, and thrombospondin) are contained within platlet granules and have been shown to enhance cancer cell adhesion *in vitro* when coated on plastic or glass surfaces. Nevertheless, Menter *et al.* [128] could not demonstrate positive effect of a platelet lysate or thrombin-induced platelet releasate on adhesion of cancer cells to subendothelial matrix. Isolated components of platelet granules (i.e. fibrinogen, serotonin, thrombospondin, etc.) were also ineffective at enhancing cancer cell adhesion to subendothelial matrix [128]. However, an intact platelet cytoskeleton, platelet membrane components, and the mobility of the platelet GpIIb/IIIa receptor were necessary for platelet enhanced cancer cell adhesion [127, 128].

Ultrastructural studies have, under defined *in vitro* conditions, revealed a specific and reproducible interaction between cancer cells and platelets, which is not exhibited during cancer cell interaction with endothelial cells or blood elements [126]. These studies indicate that cancer cells are not passive participants in their interaction with platelets, but rather suggest that a bidirectional interaction occurs with each cell type responding to the presence of the other. Initial interaction begins with the adhesion of isolated platelets to distinct areas of the cancer cell surface, rather than the entire circumference [87, 125, 126]. This effect is blocked by pretreatment of cancer cells with antibodies which react with IRGpIIb/IIIa [87] suggesting that this intergrin receptor may serve as a site for initial platelet attachment. This hypothesis is supported by the fact that IRGpIIb/IIIa is not homogeneously distributed on the cancer cell plasma membrane, but rather is heterogenously distributed, with focal areas of high receptor concentration [27, 87]. Following the focal interaction of a few isolated platelets with the cancer cell surface, aggregation proceeds by recruitment of additional platelets to this initial site. Within 30 s following the addition of cancer cells to homologous PRP, individual platelets are found associated with the cancer cell plasma membrane. Samples removed during mid-phase of a normal aggregation curve typically reveal a single, small aggregate of platelets attached to the cancer cell plasma membrane, some demonstrating evidence of degranulation. Platelets are not found to envelop the entire cancer cell circumference, and multiple focal points of attachment were rarely observed [87, 125, 126]. During mid-phase, cancer cell processes extended into the

developing platelet aggregate. As stated above, the development of these processes, adhesion of platelets to the cancer cell surface and induction of aggregation are dependent upon intact cancer cell microfilaments/intermediate filaments and mobility of the IRGpIIb/IIIa receptor [87].

Ultrastructural studies of cancer cells arrested in the pulmonary microvasculature do not support the hypothesis that heterotypic cancer–platelet aggregates lodge in the microvasculature by mechanical trapping, but rather that platelet aggregation is initiated following initial cancer cell arrest and adhesion [34, 35]. Further, *in vivo* observations suggest that platelets are only transiently associated with cancer cells during the intravascular phase of hematogeneous metastasis. Finally, these studies do not support the hypothesis [77, 78] that platelets form a cocoon around intravascular cancer cells protecting them from destruction by effectors of the cellular immune system, because the maximum association of neutrophils and lymphocytes with arrested Lewis lung carcinoma cells resulting in cancer cell destruction, coincided with the maximum association of activated platelets [65]. In addition, neutrophils were not associated with intravascular cancer cells after dissolution of the platelet thrombi [100].

(7) Angiogenesis

Important interactions of cancer cells with the microvasculature include those with pericancerous venules, leading to tumor neovascularization which on the one hand is necessary for growth and, on the other, provides one route for intravasation and initial dissemination.

Angiogenesis is the subject of a number of excellent recent reviews [55, 61, 220], and is briefly covered here, mainly to illustrate its relationship to preceding interactions involving cancer cells and the microvasculature.

A key observation linking tumor growth to neovascularization was that free-floating cancer spheroids in the avascular anterior chamber of the rabbit's eye, reached a volume of 0·5 mm^3 after 14 days and then stopped growing; when spheroids were displaced onto the vascular iris they became vascularized and grew rapidly [69]. The size limitation on avascular tumors is probably related to inward diffusion of oxygen and outward diffusion of metabolites, once tumor radius exceeds 150–200 μm [21].

It should be emphasized that angiogenesis occurs in a very wide range of physiologic and pathologic processes and that, in addition to being present in tumors and other pathologic states including chronic inflammation and some immune reactions where neovascularization is overt, angiogenic peptides are also found in tissues where it is not [36]; this suggests a tight control of physiologic expression of angiogenic factors [57].

Stimuli from the region of non-vascularized tumors act on nearby venules, causing concomitant, focal activation of endothelial cells (EC) and fragmentation of the basement membrane. Although it has been suggested that EC-derived proteases are responsible for this fragmentation, as discussed in the section on intravasation, it is difficult to pinpoint this type of invasive phenomenon to any specific cell type. Next, EC migrate towards the tumor in the form of 'sprouts' which, initially at least, may not require EC proliferation. In view of the modulation of EC behavior by interactions with surrounding tissue matrices [145], it is possible that their degradation could act as a trigger for angiogenesis. Later, EC proliferation occurs

under the action of a number of mitogens and lumina are formed; this process may also be modulated by the surrounding matrix. Adjacent capillary sprouts next fuse to form new capillary loops, and a new basement membrane is formed [10, 36, 61, 220]. The relative contributions of EC and pericytes to basement membrane formation is not known, although in some situations neovascularization is characterized by the relative absence of pericytes. Although it is generally accepted that the neovasculature invades the tumor, a contrary view is that the tumor infiltrates and grows in the developing neovascular net [203].

It has been postulated that a common stimulus for angiogenesis is tissue injury or death associated with ischemia, which results in the release of mitogens, which are then sequestered in endothelial cells and/or the extracellular matrix. The various endothelial growth factors may be discriminated from factors stimulating migration, and may be classified into heparin-binding and non-heparin-binding growth factors. The former are common peptide endothelial cell mitogens, and fall into two groups; class 1 containing anionic, and class 2 containing cationic peptides. At least some of the non-heparin-binding endothelial growth factors are antigenically cross-reactive with material in tumors and normal and pathologic non-cancerous tissues [10]. The cells involved in inflammatory responses also provide angiogenic stimuli; including lymphocytes (and lymphokines), neutrophils, macrophages, platelets and mast cells; in addition, angiogenesis can occur in the apparent absence of inflammation, and there may be an autocrine control of angiogenesis by the EC themselves [61].

Angiogenic factors including acidic and basic fibroblast growth factors and transforming growth factor-α, appear to act directly on endothelial cells; indirect factors act on other cells including macrophages, and extracellular matrix, which then release angiogenic factors which act on EC [57].

As discussed, enzymatic degradation of basement membrane appears to be a central mechanism in angiogenesis. It was therefore proposed that angiogenic factors cause an increased production and secretion of plasminogen activator (PA) and procollagenase by endothelial cells. The PA converts tissue plasminogen into the protease plasmin, which causes local fibrinolysis and also activates collagenase; the combined activities of these two enzymes are thought to promote the penetration of tissues by EC [79, 80], presumably in much the same way as proteolysis favors penetration (invasion) by cancer cells. In accord with this hypothesis, preparations of angiogenesis factor produced *in vitro* stimulation of both PA and collagenase, specific to capillary EC [134]. Using purified, human placental angiogenic factor, it was shown that activity measured in terms of elevated enzyme production, paralleled the ability to stimulate endothelial cell multiplication and motility [135]. Although this angiogenic factor specifically targeted *capillary* endothelial cells, its amino-acid sequence is homologous with basic fibroblast growth factor [191], and the two factors may well be identical [1]. In this context it should be noted that the degradation products of basement membrane and fibrin are also angiogenic, the latter possibly indirectly to activate macrophages, which then release angiogenic factors [171].

In connection with possible coupling between angiogenesis and other interactions, the prostaglandins PGE_1 and PGE_2 are angiogenic, whereas those of the A and F series are not. Mast cells and mast cell-derived heparin also play an ill-defined role in angiogenesis by stimulating EC migration and by augmenting the angiogenic activity of acidic fibroblast growth factor; however, in the presence of some corticosteroids heparin, or fragments lacking anticoagulant activity, inhibit angiogenesis [56].

The realization that angiogenesis may represent a general response to certain types of tissue injury, mitigates against the development of angiostatic cancer-specific agents for anti-metastatic therapy as discussed by D'Amore [37] and others. Although angiogenesis is associated with cancer and, if achievable, generalized angiostasis might induce dormancy and prevent the growth of micrometastases into vascularized metastases [69], such non-cancer-specific therapy would also block microvascular repair mechanisms in normal tissues.

Some of the complex interactions stimulating and modulating angiogenesis are summarized in figure 2.

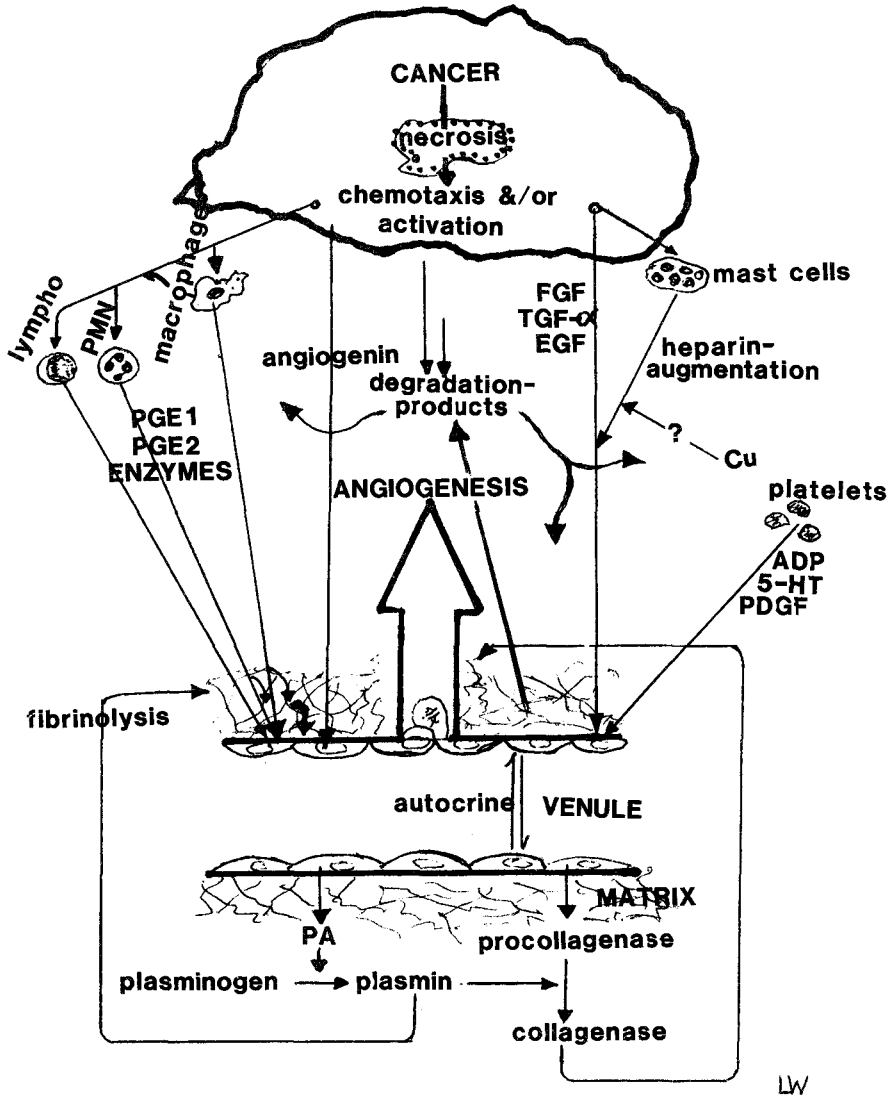

Figure 2. An oversimplified summary of interactions between a tumor, endothelial and other cells leading to angiogenesis and tumor neovascularization.

References

[1] ABRAHAM, J. A., MERGIA, A., WHANG, J. L., TUMOLO, A., FRIEDMAN, J., HJERRILD, K. A., GOSPODAROWICZ, D., and FIDDES, J. C., 1986, Nucleotide sequence of a bovine clone encoding the angiogenic protein, basic fibroblast growth factor. *Science*, **233**, 545–548.

[2] ADAMS, S. L., BOETTIGER, D., FOCT, R. J., HOLTZER, H., and PACIFICI, M., 1982, Regulation of the synthesis of extracellular matrix components in chondroblasts transformed by a temperature-sensitive mutant of Rous sarcoma virus. *Cell*, **30**, 373–384.

[3] ADAMSON, I. Y. R., ORR, F. W., and YOUNG, L., 1986, Effects of injury and repair of the pulmonary endothelium on lung metastasis after bleomycin. *J. Pathol.*, **150**, 279–287.

[4] ADAMSON, I. Y. R., YOUNG, L., and ORR, F. W., 1987, Tumor metastasis after hyperoxic injury and repair of the pulmonary enothelium. *Lab. Invest.*, **57**, 71–77.

[5] ALBRECHTSEN, R., NIELSEN, M., WEWER, U., ENGVALL, E., and RUOSLAHTI, E., 1981, Basement membrane changes in breast cancer detected by immunohistochemical staining for laminin. *Cancer Res.*, **41**, 5076–5081.

[6] ALITALO, K., KESKI-OJA, J., HEDMAN, K., and VAHERI, A., 1982, Loss of different pericellular matrix components of rat cells transformed by a T-class ts mutant of Rouse sarcoma virus. *Virology*, **119**, 347–357.

[7] ARMOUR, J. A., and RANDALL, W. C., 1971, Canine left ventricular intramyocardial pressures. *Am. J. Physiol.*, **220**, 1833–1839.

[8] AUERBACH, R., WEI CHENG LU, PARDON, E., GUMKOWSKI, F., KAMINSKA, G., and KAMINSKI, M., 1987, Specificity of adhesion between tumor cells and capillary endothelium: an *in vitro* correlate of preferential metastasis *in vivo*. *Cancer Res.*, **47**, 1492–1496.

[9] AUMAILLEY, M., and TIMPL, R., 1986, Attachment of cells to basement membrane collagen Type IV. *J. Cell Biol.*, **103**, 1569–1575.

[10] AUSPRUNK, D. H., and FOLKMAN, J., 1977, Migration and proliferation of endothelial cells in preformed and newly formed blood vessels during tumor angiogenesis. *Microvasc. Res.*, **14**, 53–65.

[10a] BARSKY, S. M., SIEGAL, G. P., JANNOTTA, F., and LIOTTA, L. A., 1983, Loss of basement membrane components by invasive tumors but not by their benign counterparts. *Lab Invest.*, **49**, 140–147.

[11] BASTIDA, E., ALMIRAL, L., JAMIESIN, G. A., and ORDINAS, A., 1987, Cell surface sialylation of two human tumor cell lines and its correlation with their platelet-activating activity. *Cancer Res.*, **47**, 1767–1770.

[12] BASTIDA, E., HASS, T. A., ALMIRALL, L., LAURI, D., ORR, F. W., and BUCHANAN, M. R., 1988, Lipoxygenase-derived fatty acid metabolites influence tumor cell adhesion to endothelial cells and their extracellular matrix. *J. Clin. Invest.* (in the press).

[13] BATCHEV, A. C., RISER, B. L., HELLNER, E. G., FLIGIEL, S. E. G., and VARANI, J., 1986, Phorbol ester binding and phorbol ester-induced arachidonic acid metabolism in a highly responsive murine fibrosarcoma cell line and in a less-responsive variant. *Clin. Expl. Metastasis*, **4**, 51–61.

[14] BEN-ZE'EV, A., and RAZ, A., 1985, Relationship between the organization and synthesis of vimentin and the metastatic capability of B16 melanoma cells. *Cancer Res.*, **45**, 2232–2641.

[15] BETTELHEIM, R., MITCHELL, D., and GUSTERSON, B. A., 1984, Immunocytochemistry in the identification of vascular invasion in breast cancer. *J. Clin. Pathol.*, **37**, 364–000.

[16] BEVILACQUA, M. P., POBER, J. S., WHEELER, M. E., COTRAN, R. S., and GIMBRONE, M. A., JR, 1985, Interleukin-1 acts on cultured human vascular endothelium to increase the adhesion of polymorphonuclear leukocytes, monocytes, and related leukocyte cell lines. *J. Clin. Invest.*, **76**, 2003–2011.

[16a] BLASI, F., VASSALLI, J. D., and DANO, K., 1987, Urokinase-type plasminogen activator: proenzyme, receptor, and inhibitors. *J. Cell Biol.*, **104**, 801–804.

[17] BORGEAT, P., and SAMUELSSON, B., 1979, Transformation of arachidonic acid by rabbit polymorphonuclear leukocytes: formation of a novel dihydroxyeicosatetranenoic acid. *J. Biol. Chem.*, **254**, 2643–2646.

[18] Brown, P. J., and Juliano, R. L., 1986, Expression and function of a putative cell surface receptor for fibronectin in hamster and human cell lines. *J. Cell Biol.*, **1103,** 1595–1603.

[19] Buchanan, M. R., Hass, T. A., Lagarde, M., and Guichardant, M., 1985, 13-hydroxy-octadecadienoic acid is the vessel wall chemorepellent factor, LOX. *J. Biol. Chem.*, **260,** 16056–16059.

[20] Buchanan, M. R., Butt, R. W., Hirsh, J., Markham, B. A., and Nazir, D. J., 1986, Role of lipoxygenase metabolism in platelet function: effect of aspirin and salicylate. *Prost. Leuk. Med.*, **21,** 157–168.

[21] Carlsson, J., Stalnacke, G. G., Acker, H. *et al.*, 1979, The influence of oxygen on viability and proliferation in cellular spheroids. *Int. J. Rad. Oncol. Bio. Phys.*, **5,** 2011–2020.

[22] Carmel, R. J., and Brown, J. M., 1977, The effect of cyclophosphamide and other drugs on the incidence of pulmonary metastasis in mice. *Cancer Res.*, **37,** 145–151.

[23] Carrell, N., Fitzgerald, L. A., Steiner, B., Erickson, H. P., and Phillips, D. R., 1985, Structure of human platlet membrane glycoproteins IIb and IIIa as determined by electron microscopy. *J. Biol. Chem.*, **260,** 1743.

[24] Cavender, D., Saegusa, Y., and Ziff, M., 1987, Stimulation of endothelial cell binding of lymphocytes by tumor necrosis factor. *J. Immunol.*, **139,** 1885–1860.

[25] Chang, Y. S., Fitzgerald, L. A., Grossi, I. M., Sundram, U., Murray, J. A., and Honn, K. V., 1988, Tumor cell expression of MRNA's coding for the integrin receptors. *FASEB J.*, **2,** A1406.

[26] Charo, I. F., Bekeart, L. S., and Phillips, D. R., 1987, Platelet glycoprotein IIb–IIIa-like proteins mediate endothelial cell attachment to adhesive proteins and the extracellular matrix. *J. Biol. Chem.*, **262,** 9935–9938.

[27] Chopra, H., Hatfield, J. S., Chang, Y. S., Grossi, I. M., Fitzgerald, L. A., O'Gara, C. Y., Marnett, L. J., Diglio, G. A., Taylor, J. D., and Honn, K. V., 1988, Role of tumor cell cytoskeleton and membrane glycoprotein IRGIIb/IIIa in platelet adhesion to tumor cell membrane and tumor cell induced platelet aggregation. *Cancer Res.*, **48,** 3787–3800.

[28] Chopra, H., Onoda, J. M., Hatfield, J. S., Fligiel, S. E. G., Taylor, J. D., and Honn, K. V., 1988, Effect of calcium channel blockers of the phenylalkylamine, benzothiazepine, and dihydropyridine classes on integrity or tumor cell cytoskeleton, mobility of the IRGIIb/IIIa receptor and tumor cell induced platelet aggregation (Submitted).

[29] Chung, D. C., Zetter, B. R., and Brodt, D., 1988, Lewis lung carcinoma variants with differing metastatic specificities adhere preferentially to different defined extracellular matrix molecules. *Invasion Metastasis*, **8,** 103–117.

[30] Cheresh, D. A., 1987, Human endothelial cells synthesize and express an Arg-Gly-Asp-directed adhesion receptor involved in attachment to fibrinogen and von Willebrand factor. *Proc. Natl. Acad. Sci., USA*, **84,** 6471–6475.

[31] Clark, R. A., Stone, P. J., El Hag, A., Calore, J. D., and Franzblau, C., 1981, Myeloperoxidase-catalyzed inactivation of alpha 1-protease inhibitor by human neutrophils. *J. Biol. Chem.*, **256,** 3348–3353.

[32] Collins, T., Lapierre, L. A., Fiers, W., Strominger, J. L., and Pober, J. S., 1986, Recombinant human tumor necrosis factor increase mRNA levels and surface expression of HLA-A, B antigens in vascular endothelial cells and dermal fibroblasts *in vitro*. *Proc. Natl. Acad. Sci., USA*, **83,** 446–450.

[32a] Cotran, R. S., 1987, New roles for the endothelium in inflammation and immunity. *Am. J. Pathol.*, **129,** 407–413.

[33] Cotran, R. S., Gimbrone, M. A. Jr, Bevilacqua, M. P., Mendrick, D. L., and Pober, J. S., 1986, Induction and detection of a human endothelial activation antigen *in vivo*. *J. Exp. Med.*, **164,** 661–666.

[34] Crissman, J., Hatfield, J. S., Menter, D. G., Sloane, B. F., and Honn, K. V., 1988, Morphological study of interaction of intravascular tumor cells with endothelial cells and subendothelial matrix. *Cancer Res.*, **48,** 4065–4072.

[35] Crissman, J., Hatfield, J., Schaldenbrand, M., Sloane, B., and Honn, K. V., 1985, Arrest and extravasation of B16 amelanotic melanoma in murine lungs. A light and electron microscopic study. *Lab. Invest.*, **53,** 470–478.

[36] D'AMORE, P. A., and THOMPSON, R. W., 1987, Mechanisms of angiogenesis. *Ann. Rev. Physiol.*, **49,** 453–464.

[37] D'AMORE, P. A., 1988, Antiangiogenesis as a strategy for antimetastasis. *Sem. Thromb. Hemostasis*, **14,** 73–78.

[38] DAO, T. L., and YOGO, H., 1967, Enhancement of pulmonary metastases by X-irradiation in rats bearing mammary cancer. *Cancer*, **20,** 2020–2025.

[39] DANO, K., ANDREASON, P. A., GRONDAHL-HANSEN, J., KRISTENSEN, P., NIELSEN, L. S., and SKRIVER, L., 1985, Plasminogen activators, tissue degradation, and cancer. *Adv. Cancer Res.*, **44,** 139–266.

[40] DAVIS, W. B., RENNARD, S. J., BITTERMAN, P. B., and CRYSTAL, R., 1983, Pulmonary oxygen toxicity. *N. Engl. J. Med.*, **309,** 878–883.

[41] DEDHAR, S., RUOSLAHTI, E., and PIERSCHBACHER, M. D., 1987, A cell surface receptor complex for collagen type I recognizes the Arg–Gly–Asp sequence. *J. Cell Biol.*, **104,** 585–593.

[42] DEDHAR, S., ARGRAVES, W. S., SUZUKI, S., RUOSLAHTI, E., and PIERSCHBACHER, M. D., 1987, Human osteosarcoma cells resistant to detachment by an Arg–Gly–Asp-containing peptide overproduces the fibronectin receptor. *J. Cell Biol.*, **105,** 1175–1182.

[43] DENNIS, J. W., LAFERTE, S., WAGHORNE, C., BREITMAN, J. L., and KERBEL, R. S., 1987, Beta 1-6 branching of ASN-linked oligosaccharides is directly associated with metastasis. *Science*, **236,** 582–585.

[44] DIMITROV, D. S., 1983, Dynamic interactions between approaching surfaces of biological interest. *Progr. Surface Sci.*, **14,** 295–423.

[45] DUFFY, M. J., 1987, Do proteases play a role in cancer invasion and metastasis? *Eur. J. Cancer Clin. Oncol.*, **23,** 583–589.

[46] DUSZYK, M., and DOROSZEWSKI, J., 1984, Hydrodynamics of interaction of particles (including cells) with surfaces. *Progr. Surface Sci.*, **15,** 369–399.

[47] ESTRADA, J., and NICOLSON, F. L., 1984, Tumor cell-platelet aggregation does not correlate with metastatic potential of rat 13762 NF mammary adenocarcinoma tumor cell clones. *Int. J. Cancer*, **34,** 101–105.

[48] EVANS, E. A., and PARSEGIAN, V. A., 1983, Energetics of membrane deformation and adhesion in cell and vesicle aggregation. *Ann. NY Acad. Sci.*, **416,** 13–33.

[49] EVANS, E. A., WAUGH, R., and MELNIK, L., 1976, Elastic area compressibility modulus of red cell membrane. *Biophys. J.*, **16,** 585–592.

[50] FITZGERALD, L. A., CHARO, I. F., and PHILLIPS, D. R., 1985, Human and bovine endothelial cells synthesize membrane proteins similar to human platelet glycoproteins IIb and IIIa. *J. Biol. Chem.*, **260,** 10893–10896.

[51] FITZGERALD, L. A., and PHILLIPS, D. R., 1985, Calcium requirements of the platelet membrane glycoprotein IIb/IIIa complex. *J. Biol. Chem.*, **260,** 11366–11374.

[52] FITZGERALD, L. A., and PHILLIPS, D. R., 1988, Structure of platelet membrane glycoproteins. *Platelet Immunology*, edited by T. J. Kumicki and J. George (Philadelphia, J. B. Lippincott).

[53] FITZGERALD, L. A., STIENER, B., RALL, S. C. JR, LO, S.-S., and PHILLIPS, D. R., 1987, Protein sequence of endothelial glycoprotein IIIa derived from a cDNA clone. *Biol. Chem.*, **262,** 3936–3939.

[54] FLIGIEL, S. E. G., PERONE, P., and VARANI, J., 1985, Arachidonic acid metabolism in murine fibrosarcoma cells with different *in vivo* and *in vitro* characteristics. *Int. J. Cancer*, **36,** 383–388.

[55] FOLKMAN, J., 1985, Toward an understanding of angiogenesis: Search and discovery. *Perspect. Biol. Med.*, **29,** 10–36.

[56] FOLKMAN, J., and INGBER, D. E., 1987, Angiostatic steroids. *Ann. Surg.*, **206,** 374–383.

[57] FOLKMAN, J., and KLAGSBRUN, M., 1987, Angiogenic factors. *Science*, **235,** 442–447.

[58] FOX, E. B., 1985, Linkage of a membrane skeleton to integral membrane glycoproteins in human platelets. *J. Clin. Invest.*, **76,** 1673–1683.

[59] FUJIWARA, S., WIEDMANN, H., TIMPL, R., LUSTIG, A., and ENGEL, J., 1984, Structure and interactions of heparan sulfate proteoglycans from a mouse tumor basement membrane. *Eur. J. Biochem.*, **143,** 145–157.

[60] FUNG, Y. C., 1984, *Biomechanics. Mechanical Properties of Living Tissues* (New York: Springer), p. 226.

[61] FURCHT, L. T., 1986, Critical factors controlling angiogenesis: cell products, cell matrix and growth factors. *Lab. Invest.*, **55,** 505–509.

[62] GABBERT, H., GERHARZ, C. D., RAMP, U., and BOHL, J., 1987, The nature of host tissue destruction in tumor invasion. *Virch. Arch. (Cell Pathol.)*, **52,** 513–517.

[63] GABOR, H., and WEISS, L., 1985, Mechanically induced trauma suffered by cancer cells in passing through pores in polycarbonate membranes. *Invasion Metastasis*, **5,** 71–83.

[64] GANGULY, P., and GOULD, M. L., 1979, Thrombin receptor of human platelets: thrombin binding and antithrombin properties of glycoprotein I. *Br. J. Haematol.*, **42,** 137.

[65] GARBISA, S., POZZATTI, R., MUSCHEL, R. J., SATTIOTTI, U., BALLIN, M., GOLDFARB, G. H., KHOURY, G., and LIOTTA, L. A., 1987, Secretion of type IV collagenolytic protease and metastatic phenotype: induction by transfection with c-Ha-ras but not c-Ha-ras plus AD2-E1a. *Cancer Res.*, **47,** 1523–1528.

[66] GASIC, G. J., GASIC, T. B., and STEWART, C. C., 1968, Antimetastatic effect associated with platelet reduction. *Proc. Natl. Acad. Sci., USA*, **61,** 46–52.

[67] GEHLSEN, K. R., ARGRAVES, W. S., PIERSBACHER, M. D., and RUOSLAHTI, E., 1988, Inhibition of *in vitro* tumor cell invasion by Arg–Gly–Asp-containing synthetic peptides (Manuscript submitted).

[68] GEIGER, B., TOKUYASU, K. T., DUTTON, A. H., and SINGER, S. J., 1981, Vinculin and intracellular protein localized at specialized sites where microfilaments terminate at cell membranes. *Proc. Natl. Acad. Sci., USA*, **77,** 4127–4131.

[69] GIMBRONE, M. A., LEAPMAN, S. B., COTRAN, R. S., and FOLKMAN, J., 1972, Tumor dormancy *in vivo* be prevention of neovascularization. *J. Exp. Med.*, **136,** 261–276.

[70] GINSBERG, M. H., LOFTUS, J., RYCKWAERT, J. J., PEIRSCHBACHER, M., PYTELA, R., ROUSLAHTI, E., and PLOW, E. F., 1987, Immunochemical and amino-terminal sequence comparison of two cytoadhesins indicates they contain similar or identical beta subunits and distinct alpha subunits. *J. Biol. Chem.*, **262,** 5437–5440.

[71] GLAVES, D., 1980, Metastasis: reticuloendothelial system and organ retention of disseminated malignant cells. *Int. J. Cancer*, **26,** 115–122.

[72] GLAVES, D., 1983, Correlation between circulating cancer cells and incidence of metastasis. *Br. J. Cancer*, **48,** 665–673.

[73] GLAVES, D., 1983, Role of polymorphonuclear leukocytes in the pulmonary clearance of arrested cancer cells. *Invasion Metastasis*, **3,** 160–173.

[74] GLAVES, D., 1986, Intravascular death of disseminated cancer cells mediated by superoxide anion. *Invasion Metastasis*, **6,** 101–111.

[75] GLAVES, D., HUBEN, R. P., and WEISS, L., 1988, Hematogeneous dissemination of cells from human renal adenocarcinomas. *Br. J. Cancer*, **57,** 32–35.

[76] GOPALAKRISHNA, R., and BARSKY, S. H., 1988, Tumor promoter-induced membrane-bound protein kinase C regulates hematogenous metastasis. *Proc. Natl. Acad. Sci., USA*, **85,** 612–616.

[77] GORELIK, E., 1987, Augmentation of the antimetastatic effect of anticoagulant drugs by immunostimulation in mice. *Cancer Res.*, **47,** 809–815.

[78] GORELIK, E., BERE, W., and HERBERMAN, R., 1984, Role of NK cells in the antimetastatic effect of anticoagulant drugs. *Int. J. Cancer*, **33,** 87–94.

[79] GROSS, J. L., MOSCATELLI, D., JAFFE, E. A., and REFKIN, D. B., 1982, Plasminogen activator and collagenase production by cultured capillary endothelial cells. *J. Cell Biol.*, **95,** 974–981.

[80] GROSS, J. L., MOSCATELLI, D., and RIFKIN, D. B., 1983, Increased capillary endothelial cell protease activity in response to angiogenic stimuli *in vitro*. *Proc. Natl. Acad. Sci. USA*, **80,** 2623–2627.

[81] GROSSI, I. M., FITZGERALD, J. A., UMBARGER, L. A., NELSON, K. K., DIGLIO, C. A., TAYLOR, J. D., and HONN, K. V., 1988, Bidirectional control of membrane expression and/or activation of the tumor cell IRGIIb/IIIa receptor and tumor cell adhesion by lipoxygenase products or arachidonic acid and linoleic acid. *Cancer Res.* (In press).

[82] GROSSI, I. M., HATFIELD, J. S. FITZGERALD, L. H., NEWCOMBE, M., TAYLOR, J. D., and HONN, K. V., 1988, Role of tumor cell glycoproteins immunologically related to glycoproteins Ib and IIb/IIIa in tumor cell-platlet and tumor cell-matrix interactions. *FASEB J.*, **2,** 2385–2395.

[83] HAGMAR, B., and RYD, W., 1977, Tumor cell locomotion: a factor in metastasis formation? Influence of cytochalasin B on a tumor dissemination pattern. *Int. J. Cancer*, **19,** 576–580.

[83a] HAJJAR, K. A., HAMEL, N. M., HARPEL, P. C., and NACHMAN, R. L., 1987, Binding of tissue plasminogen activator to cultured human endothelial cells. *J. Clin. Invest.*, **80,** 1712–1719.

[84] HART, I. R., RAZ, A., and FIDLER, I. J., 1980, Effect of cytoskeleton disrupting agents on the metastatic behavior of melanoma cells. *J. Natl. Cancer Inst.*, **64,** 891–899.

[85] HAYMAN, E. G., PIERSCHBACHER, M. D., and RUOSLAHTI, E., 1985, Detachment of cells from culture substrate by soluble fibronectin peptides. *J. Cell Biol.*, **100,** 1948–1954.

[86] HIRATA, H., and TANAKA, K., 1984, Artificial metastases and decrease of fibrinolysis in the nude mouse lung after hemithrocic irradiation. *Clio. Exp. Metastasis*, **2,** 311–319.

[87] HONN, K. V., GROSSI, I. M., CHOPRA, H., STEINERT, B. W., ONODA, J. M., NELSON, K. K., and TAYLOR, J. D., 1988, Role of tumor cell eicosanoids and membrane glycoproteins IRGpIb and IRGpIIb/IIIa in metastasis. *Eicosanoid, Lipid Peroxidation and Cancer*, edited by S. Nigam, D. L. H. McBrien and T. G. Slater (Berlin: Springer Verlag)(In press).

[88] HONN, K. V., GROSSI, I. M., FITZGERALD, L. A., UMBARGER, L. A., DIGLIO, C. A., and TAYLOR, J. D., 1988, Lipoxygenase products regulate IRGpIIb/IIIa receptor mediated adhesion of tumor cells to endothelial cells, subendothelial matrix and fibronectin. *Proc. Soc. exp. Biol. Med.*, **189,** 130–135.

[89] HONN, K. V., GROSSI, I. M., NELSON, K. K., UMBARGER, L. A., FITZGERALD, L. A., HATFIELD, J. S., FLIGIEL, S. E. G., STEINERT, B. W., TAYLOR, J. D., and ONODA, J. M., 1988, Correlation among expression of glycoprotein IRGpIIb/IIIa, tumor cell induced platelet aggregation, tumor cell adhesion and lung colonization in elutriated subpopulations of the B16 amelanotic melanoma and Lewis lung carcinoma *Cancer Res.* (In press).

[90] HONN, K. V., GROSSI, I. M., STEINERT, B. W., CHOPRA, H., ONODA. J. M., NELSON, K. K., and TAYLOR, J. D., 1988, Lipoxygenase regulation of membrane expression of tumor cell glycoproteins and subsequent metastasis. In: *Advances in Prostaglandins, Thromboxane, and Leukotriene Research*, Vol. 18, edited by P. Wong, B. Samuelsson and F. Sun (New York: Raven Press) (In press).

[91] HONN, K. V., ONODA, J. M., PAMPALONA, K., BATTAGLIA, M., NEAGOS, G., TAYLOR, J. D., DIGLIO, C. A., and SLOANE, B. F., 1985, Inhibition by dihydropyridine class calcium channel blockers of tumor cell-platelet-endothelial cell interactions *in vitro* metastasis *in vivo*. *Biochem. Pharmacol.*, **34,** 235–141.

[92] HONN, K. V., POWERS, W. E., and SLOANE, B. F. (eds), 1986, *Mechanisms of Cancer Metastasis. Potential Therapeutic Implications* (Boston, MA: Martinus Nijhoff).

[93] HONN, K. V., STEINERT, B. W., ONODA, J. M., and SLOANE, B. F., 1987, The role of platelets in metastasis. *Biorheology*, **24,** 127–137.

[94] HOPKINS, N. K., OGGLESBY, T. D., BUNDY, F. L., and GORMAN, R. R., 1984, Biosynthesis and metabolism of 15-hydroxy-5,8,11,13-eicosatetranenoic acid by human umbilical vein endothelial cells. *J. Bio. Chem.*, **259,** 14048–14053.

[95] HUMPHRIES, M. J., MATSUMOTO, K., WHITE, S. L., and OLDEN, K., 1986, Oligosaccharide modifications by swainsonine treatment inhibits pulmonary colonization by B16-F10 murine melanoma cells. *Proc. Natl. Acad. Sci., USA*, **83,** 1752–1756.

[96] HUMPHRIES, M. J., OLDEN, K., and YAMADA, K. M., 1986, A synthetic peptide from fibronectin inhibits experimental metastasis of murine melanoma cells. *Science*, **233,** 467–470.

[97] HUMPHRIES, M. J., MATSUMOTO, K., WHITE, S. L., and OLDEN, K., 1986, Inhibition of experimental metastasis by castanospermine in mice: blockage of two distinct stages of tumor colonization by liogosaccharide processing inhibitors. *Cancer Res.*, **46,** 5215–5222.

[98] HUMPHRIES, M. J., YAMADA, K. M., and OLDEN, K., 1988, Investigation of the biological effects of anti-cell adhesive synthetic peptides that inhibit experimental metastasis of B16-F10 murine melanoma cells. *J. Clin. Invest.*, **81,** 782–790.

[99] HYNES, R. O., 1986, Integrins: a family of cell surface receptors. *Cell*, **48,** 549–554.

[99a] Ishikawa, M., Koga, Y., Hosokawa, M., and Kobayashi, H., 1986, Augmentation of B16 melanoma lung colony formation in C57BL/6 mice having marked granulocytosis. *Int. J. Cancer*, **37**, 919–924.

[100] Jones, C. L., Nelson, K. K., Hatfield, J., Honn, K. V., and Onoda, J. M., 1988, Regulation of metastasis by polymorphonuclear neutrophils. *Proc. Am. Assoc. Cancer Res.*, **27**, 62.

[101] Jones, P. A., and De Clerk, Y. A., 1982, Extracellular matrix destruction by invasive tumor cells. *Cancer Metastasis Rev.*, **1**, 289–318.

[102] Kanclerz, A., and Shapman, J. D., 1987, The effectiveness of *cis*-platinum, cyclophosphamide and melphalan in treating disseminated tumor cells in mice. *Clin. Expl. Metastasis*, **5**, 199–212.

[103] Kerbel, R. S., Dennis, J. W., Lagarde, A. E., and Frost, P., 1982, Tumor progression in metastasis: an experimental approach using lectin resistant tumor variants. *Metastasis Rev.*, **1**, 99–140.

[104] Khato, J., Sati, H., and Suzuki, M., 1979, Filterability and flow characteristics of leukemic and non-leukemic tumor cell suspensions through polycarbonate filters in relation to hematogeneous spread of cancer. *Tohoku J. Exp. Med.*, **128**, 273–284.

[105] Kieffer, N., Najet, D., Wicki, A., Titeux, M., Heuri, A., Mishal, Z., Breton-Gorius, J., Vainchenker, W., and Clemetson, K. J., 1986, Expression of platlet glycoprotein Ibα in HEL cells. *J. Biol. Chem.*, **261**, 15854–15862.

[105a] Kimura, A. K., Mehta, P., Xiang, J., Lawson, D., Dugger, D., Kao, K. J., and Ambrose, L. L., 1987, The lack of correlation between experimental metastatic potential and platelet aggregating activity of B16 melanoma clones viewed in relation to tumor cell heterogeneity. *Clin Expl. Metastasis*, **5**, 125–133.

[106] Kinjo, M., 1978, Lodgement and extravasation of tumor cells in blood-borne metastases: an electron microscopic observation. *Br. J. Cancer*, **38**, 293–301.

[107] Kramer, R. H., Gonzales, R., and Nicolson, G. L., 1980, Metastatic tumor cells adhere preferentially to the extracellular matrix underlying vascular endothelial cells. *Int. J. Cancer*, **26**, 639–645.

[108] Kramer, R. H., Vogel, K. G., and Nicolson, G. L., 1982, Solubilization and degradation of subendothelial matrix glycoproteins and proteoglycans by metastatic tumor cells. *J. Biol. Chem.*, **257**, 2678–2686.

[109] Kramer, R. H., Vogel, K. G., and Nicolson, G. L., 1982, Tumor cell interactions with vascular endothelial cells and their extracellular matrix. *Prog. Clin. Biol. Res.*, **89**, 333–351.

[110] Konstantinov, A. A., Peskin, A. V., Popova, E. Y., Khomutou, G. B., and Ruuge, E. K., 1987, Superoxide generation by the respiratory chain of tumor mitochondria. *Biochem. Biophys. Acta*, **894**, 1–10.

[111] Lapis, K., Paku, S., and Liotta, L. A., 1988, Endothelialization of embolized tumor cells during metastasis formation. *Clin. Exp. Metastasis*, **6**, 73–89.

[112] Lauri, D., Orr, F. W., Bastida, E., Sauder, D., and Buchanan, M. R., 1988, Differential effects of interleukin-1 and fMLP on chemotaxis and human endothelium adhesivity for A549 tumor cells (Manuscript submitted).

[112a] Leroyer, V., Werner, L., Shaughnessy, S., Goddard, G. J., and Orr, F. W., 1987, Chemiluminescence and oxygen radical generation by Walker carcinosarcoma cells following chemotactic stimulation. *Cancer Res.*, **47**, 4771–4775.

[113] Lichtenstein, A., 1987, Stimulation of respiratory burst of muriperitoneal inflammatory neutrophils by conjugation with tumor cells. *Cancer Res.*, **47**, 2211–2217.

[114] Lichtenstein, A. K., Kahle, J., Berek, J., and Zighelboim, J., 1984, Successful immunotherapy with intraperitoneal Corynebacterium parvum in a murine ovarian cancer model is associated with recruitment of tumor-lytic neutrophils into the peritoneal cavity. *J. Immunol.*, **133**, 519–526.

[115] Lichtner, R. B., and Nicolson, G. L., 1987, Effects of the pyrimido-pyrimidine derivative RX-RA 85 on metastatic tumor cell-vascular endothelial cell interactions. *Clin. Expl. Metastasis*, **5** (3), 219–231.

[116] Liotta, L. A., 1986, Tumor invasion and membranes. Role of the extracellular matrix. *Cancer Res.*, **46**, 1–7.

[117] Liotta, L. A., Thorgeirsson, U. P., and Garbisa, S., 1982, Role of collagenases in tumor cell invasion. *Cancer Metastasis Rev.*, **1**, 277–288.

[118] LIOTTA, L. A., HORAN HAND, P., RAO, C. N., BRYANT, G., BARSKY, S. H., and SCHLOM, J., 1985, Monoclonal antibodies to the human laminin receptor recognize structurally distinct sites. *Expl. Cell Res.*, **156,** 117–126.

[119] MAYHEW, E., and GLAVES, D., 1984, Quantitation of tumorigenic disseminating and arrested cancer cells. *Br. J. Cancer*, **50,** 159–166.

[120] MCCARTHY, J., PALMARD, S., and FURCHT, L., 1983, Migration by hapotaxis of a Schwann cell tumor line to the basement membrane glycoprotein laminin. *J. Cell Bio.*, **97,** 772–779.

[121] MCCARTHY, J. B., BASARA, M. L., PALM, S. L., SAS, D. F., and FURCHT, L. T., 1985, The role of cell adhesion proteins-laminin and fibronectin—in the movement of malignant and metastatic cells. *Cancer Metastasis Rev.*, **4,** 125–152.

[122] MCCARTHY, J., HAGEN, S. T., and FURCHT, L. T., 1986, Fibronectin contains distinct adhesion and motility-promoting domains for metastatic melanoma cells. *J. Cell Biol.*, **102,** 179–188.

[123] MCKEVER, R. P., BENNETT, E. M., and MARTIN, M. N., 1983, Identification of two structurally and functionally distinct sites on human platelet membrane glycoprotein IIb-IIIa using monoclonal antibodies. *J. Biol. Chem.*, **258,** 5269.

[124] MCMILLAN, T. J., RAO, J., and HART, I. R., 1986, Enhancement of experimental metastasis by pretreatment of tumor cells with hydroxyusea. *Int. J. Cancer*, **38,** 61–65.

[125] MENTER, D. G., HARKINS, C., ONODA, J. M., RIORDEN, W., SLOANE, B. F., TAYLOR, J. D., and HONN, K. V., 1987, Inhibition of tumor cell induced platelet aggregation by prostacyclin and carbacyclin: an ultrastructural study. *Invasion Metastasis*, **7,** 109–128.

[126] MENTER, D. G., HATFIELD, J. S., HARKINS, C. J., SLOANE, B. F., TAYLOR, J. D., CRISSMAN, J. D., and HONN, K. V., 1987, Tumor cell-platelet interactions *in vitro* and their relationship to *in vivo* arrest of hematogenously circulating tumor cells. *Clin. Expl. Metastasis*, **5,** 65–78.

[127] MENTER, D. G., SLOANE, B. F., STEINERT, B. W., ONODA, J. M., CRAIG, R., HARKINS, C., TAYLOR, J. D., and HONN, K. V., 1987, Platelet enhancement of tumor cell adhesion to subendothelial matrix. Role of platelet cytoskeleton and platelet membrane. *J. Natl. Cancer Inst.*, **79,** 1077–1098.

[128] MENTER, D. G., STEINERT, B. W., SLOANE, B. F., GUNDLACH, N., O'GARA, C. Y., MARNETT, L. J., DIGLIO, C., WALZ, D., TAYLOR, J. D., and HONN, K. V., 1987, Role of platelet membrane in enhancement of tumor cell adhesion to endothelial cell extracellular matrix. *Cancer Res.*, **47,** 6751–6762.

[129] MEROMSKY, L., LOTAN, R., and RAZ, A., 1986, Implications of endogeneous tumor cell surface lectings as mediators of cellular interactions and lung colonization. *Cancer Res.*, **46,** 5270–5275.

[130] MILAS, L., MALENICA, B., and ALLERGETTI, N., 1979, Enhancement of artificial lung metastases in mice caused by cyclophosphamide. I. Participation of impairment of host antitumor resistance. *Cancer Immunol. Immunother'*. **6,** 191–196.

[131] MOORE, A., ROSS, G. D., and MACHMAN, E., 1978, Interaction of platelet membrane receptors with von Willebrand factor, ristocetin, and the Fc region of immunoglobulin G. *J. Clin. Invest.*, **62,** 1053.

[132] MOORE, J. V., and DIXON, B., 1977, Metastasis of a transplantable mammary tumour in rats treated with cyclophosphamide and/or irradiation. *Br. J. Cancer*, **36,** 221–226.

[133] MORGANROTH, M. L., TILL, G. O., KUNKEL, R. G., and WARD, P. A., 1986, Complement and neutrophil-mediated injury of perfused rat lungs. *Lab. Invest.*, **54,** 507–514.

[134] MOSCATELLI, D., 1985, Collagenase and plasminogen activator production by blood-vessel associated cells in response to angiogenic preparations. *Intracellular Protein Catabolism* edited by E. A. Khairallah, J. S. Bond and J. W. C. Bird (New York: Liss), pp. 669–671.

[135] MOSCATELLI, D., PRESTA, M., and RIFKIN, D. B., 1986, Purification of a factor from human placenta that stimulated capillary endothelial cell protease production, DNA synthesis and migration. *Proc. Natl. Acad. Sci., USA*, **83,** 2091–2095.

[136] MULLER, W. A., and GIMBRONE, M. A. JR, 1986, Plasmalemmal proteins of cultured vascular endothelial cells exhibit apical-basal polarity: analysis by surface-selective iodination. *J. Cell Biol.*, **103,** 2389–2402.

[137] MUNDY, G. R., DEMARTINO, S., and ROWE, D. W., 1981, Collagen and collagen-derived fragments and chemotactic for tumor cells. *J. Clin. Invest.*, **68,** 1102–1105.

[138] NAKAJIMA, M., IRIMURA, T., DIFERRANTE, D., DIFERRANTE, N., and NICOLSON, G. L., 1983, Heparan sulfate degradation: relation to tumor invasive and metastatic properties of mouse B16 melanoma sublines. *Science*, **220,** 611–613.

[139] NAKAJIMA, M., IRIMURA, T., and NICOLSON, G. L., 1986, Tumor metastasis-associated heparanase (heparan sulfate endoglycosidase) activity in human melanoma cells. *Cancer Lett.*, **31,** 277–283.

[140] NAKAJIMA, M., WELCH, D. R., BELLONI, P. N., and NICOLSON, G. L., 1987, Degradation of basement membrane type IV collagen and lung subendothelial matrix by rat mammary adenocarcinoma cell clones of differing metastatic potentials. *Cancer Res.*, **47,** 4869–4876.

[141] NICOLSON, G. L., 1982, Metastatic tumor cell attachment and invasion assay utilizing vascular endothelial cell monolayers. *J. Histochem. Cytochem.*, **30,** 214–220.

[142] NICOLSON, G. L., 1988, Cancer metastasis: Tumor cell and host organ properties important in metastasis to specific secondary sites. *Biochim. Biophys. Acta* (*Cancer Rev.*) (In press).

[143] NICOLSON, G. L., and CUSTEAD, S. E., 1985, Effects of chemotherapeutic drugs on platelet and metastatic tumor cell-endothelial cell interactions as a model for assessing vascular endothelial integrity. *Cancer Res.*, **45,** 331–336.

[144] NICOLSON, G. L., IRIMURA, T., NAKAJIMA, M., UPDYKE, T. V., and POSTE, G., 1984, The cellular interactions of metastatic tumor cells with special reference to endothelial cells and their basal lamina-like matrix. *Hemostatic Mechanisms and Metastasis*, edited by K. V. Honn and B. F. Sloane (Boston: Martinus Nijhoff), pp. 295–318.

[145] NICOSIA, R. F., and MADRI, J. A., 1987, The microvascular extracellular matrix. *Am. J. Pathol.*, **128,** 78–90.

[146] NIEDBALA, M. J., MADIYALAKAN, R., MATTA, K., CRICKARD, K., SHARMA, M., and BERNACKI, R. J., 1987, Role of glycosidases in human ovarian carcinoma cell mediated degradation of subendothelial extracellular matrix. *Cancer Res.*, **47,** 4634–4641.

[147] OBERLY, L. W., and BUETTNER, G. R., 1979, Role of superoxide dismutase in cancer: a review. *Cancer Res.*, **39,** 1141–1149.

[148] OKITO, J. R., PIDARD, D., NEWMAN, P. J., MONTGOMERY, R. R., and KUNICKI, T. J., 1985, On the association of glycoprotein Ib and actin-binding protein in human platelets. *J. Cell Biol.*, **100,** 317.

[149] OLDEN, K., HUMPHRIES, M. J., and WHITE, S. L., 1985, Biochemical effects and cancer therapeutic potential of tunicamycin. *Monoclonal Antibodies and Cancer Therapy*, edired by R. Riesfeld and S. Sell (New York: Alan R. Liss), pp. 443–472.

[150] ONODA, J. M., NELSON, K. K., GROSSI, I. M., UMBARGER, L. A., TAYLOR, J. D., and HONN, K. V., 1988, Separation of high and low metastatic subpopulations from solid tumors by centrifugal elutriation. *Proc. Soc. Exp. Biol. Med.*, **187,** 250–255.

[151] ORR, F. W., PHAN, S. H., VARANI, J., WARD, P. A., KREUTZER, D. L., WEBSTER, R. D., and HENSON, P. M., 1979, Chemotactic factor for tumor cells derived from the C5a fragment of complement component C5. *Proc. Natl. Acad. Sci., USA*, **76,** 1986–1989.

[152] ORR, F. W., MOKASHI, S., and DELIKATNY, J., 1982, Generation of a complement-derived chemotactic factor for tumor cells in experimentally induced peritoneal exudates and its effect on the local metastasis of circulating tumor cells. *Am. J. Pathol.*, **108,** 112–118.

[153] ORR, F. W., and MOKASHI, S., 1985, Effect of leukocyte activation on the formation of heterotypic tumor-cell aggregates *in vitro*. *Int. J. Cancer*, **35,** 101–106.

[154] ORR, F. W., ADAMSON, I. Y. R., and YOUNG, L., 1985, Pulmonary inflammation generates chemotactic activity for tumor cells and promotes lung metastasis. *Am. Rev. Respir. Dis.*, **131,** 607–611.

[155] ORR, F. W., ADAMSON, I. Y. R., and YOUNG, L., 1986, Promotion of pulmonary metastasis in mice by bleomycin-induced endothelial injury. *Cancer Res.*, **46,** 891–897.

[156] ORR, F. W., and WARNER, D. J. A., 1987, Effects of neutrophil-mediated pulmonary endothelial injury on the localization and metastasis of circulating Walker carcinosarcoma cells. *Invasion Metastasis*, **7,** 183–196.

[157] OSSOWSKI, L., 1988, Plasminogen-activator dependent pathways in the dissemination of human tumor cells in the chick embryo. *Cell*, **52,** 321–328.

[158] OSTROWSKI, L. E., AHSAN, A., SUTHER, B. P., PAGAST, P., BAIN, D. L., WONG, C., PATAL, A., and SCHULTZ, R. M., 1986, Selective inhibition of proteolytic enzymes in an *in vivo* mouse model for experimental metastasis. *Cancer Res.*, **46,** 4121–4128.

[159] OZAKI, T., YOSHIDA, K., USHIJIMA, K., and HAYASHI, H., 1971, Studies on the mechanisms of invasion in cancer. II. *In vivo* effects of a factor chemotactic for cancer cells. *Int. J. Cancer*, **7,** 93–100.

[160] PAGET, S., 1889, The disttibution of secondary growths in cancer of the breast. *Lancet*, **i,** 571–573.

[161] PAINTER, R. G., and GINSBERG, M., 1982, Concanavalin A induces interactions between surface glycoproteins and the platelet cytoskeleton. *J. Cell Biol.*, **92,** 565.

[162] PALMBLAD, J., MALMSTEN, D., UDEN, A. M., RADMARK, O., ENGSTED, L., and SAMUELSSON, B., 1981, Leukotriene B4 is a potent and stereospecific stimulator of neutrophil chemotaxis and adherence. *Blood*, **58,** 658–661.

[163] PEPIN, J. M., and LANGER, R. O. 1985, Effects of dimethyl sulfoxide (DMSO) on bleomycin-induced pulmonary fibrosis. *Biochem. Pharmacol.*, **34,** 2386–2389.

[164] PETERSON, D. M., STATHHOPOULOS, N. A., GEORGIO, D. D., HELUMS, J. D., and MOIKE, J. L., 1987, Shear-induced platelet aggregation requires von Willebrand factor and platelet membrane glycoproteins Ib and IIb-IIIa. *Blood*, **69,** 625.

[165] PHILLIPS, D. R., and AGIN, P. P., 1977, Platelet plasma membrane glycoproteins. Evidence for the presence of nonequivalent disulfide bonds using nonreduced-reduced two-dimensional gel electrophoresis. *J. Biol. Chem.*, **252,** 21.

[166] PHILLIPS, D. R., and SHUMAN, M. A., 1986, *Biochemistry of Platelets* (New York: Academic Press).

[167] PIDARD, D., MONTGOMERY, R. R., BENNETT, J. S., and KUNICKI, T. J., 1983, Interaction of AP-2, a monoclonal antibody specific for the human platelet membrane glycoprotein IIb-IIIa complex, with intact platelets. *J. Biol. Chem.*, **258,** 12582–12586.

[168] POBER, J. S., BEVILACQUA, M. P., MENDRICK, D. L., LAPIERE, L. A., FIERS, W., and GIMBRONE, M. A. JR, 1986, Two distinct monocines, interleukin 1 and tumor necrosis factor, each independently induce biosynthesis and transient expression of the same antigen on the surface of cultured human vascular endothelial cells. *J. Immunol.*, **136,** 1680–1687.

[169] POBER, J. S., GIMBRONE, M. A. JR, LAPIERRE, L. A., MENDRICK, D. L., FIERS, W., ROTHLEIN, R., and SPRINGER, T. A., 1986, Overlapping patterns of activation of human endothelial cells by interleukin 1, tumor necrosis factor, and immune interferon. *J. Immunol.*, **137,** 1893–1896.

[170] POBER, J. S., LAPIERRE, L. A., STOLPEN, A. H., BROCK, T. A., SPRINGER, T. A., FIERS, W., BEVILACQUA, M. P., MENDRICK, D. L., and GIMBRONE, M. A. JR, 1987, Activation of cultured human endothelial cells by recombinant lymphotoxin: comparison with tumor necrosis factor and interleukin species. *J. Immunol.*, **138,** 3319–3324.

[171] POLVERINI, P. J., and LEIBOVICH, S. J., 1984, Induction of neovasculaturization *in vivo* and endothelial proliferation *in vitro* by tumor-associated macrophages. *Lab. Invest.*, **51,** 635–642.

[172] POUPON, M. F., PAUWELS, C., JASMIN, C., ANTOINE, E., LASCAUX, V., and ROSA, B., 1984, Amplified pulmonary metastases of a rat rhabdomyosarcoma in response to nitrosourea treatment. *Cancer Treat. Rep.*, **68,** 749–758.

[173] PYTELA, R., PIERSCHBACHER, M. D., and RUOSLAHTI, E., 1985, Identification and isolation of a 140-Kd cell surface glycoprotein with properties expected of a fibronectin receptor. *Cell*, **40,** 191–198.

[174] PYTELA, R., PIERSCHBACHER, M. D., and RUOSLAHTI, E., 1985, A 125/115-kDa cell surface receptor specific for vitronectin interacts with the arginine-glycine-aspartic acid adhesion sequence derived from fibronectin. *Proc. Natl. Acad. Sci., USA*, **82,** 5766–5770.

[175] RAO, C. N., MARGULIES, I. M. K., TRALKA, T. S., TERRANOVA, V. P., MADRI, J. A., and LIOTTA, L., 1982, Isolation of a subunit og laminin and its role in molecular structure and tumor cell attachment. *J. Biol. Chem.*, **257,** 9740–9744.

[176] RAO, M. C., BARSKY, S. H., TERRANOVA, V. P., and LIOTTA, L., 1983, Isolation of a tumor cell laminin receptor. *Biochem. Biophys. Res. Commun.*, **111,** 804–808.

[177] RAZ, A., and BEN-ZE'EV, A., 1987, Cell-contact and architecture of malignant cells and their relationship to metastasis. *Cancer Metastasis Rev.*, **6,** 3–21.

[178] ROTHLEIN, R., DUSTIN, M. L., MARLIN, S. D., and SPRINGER, T. A., 1986, A human intercellular adhesion molecule (ICAM-1) distinct from LFA-1. *J. Immunol.*, **137,** 1270–1274.

[178a] ROZHIN, J., ROBINSON, D., STEVENS, M. A., LAH, T. T., HONN, K. V., RYAN, R. E., and SLOANE, B. F., 1987, Properties of a plasma membrane-associated cathepsin B-like cysteine proteinase in metastatic B16 melanoma variants. *Cancer Res.*, **47,** 6620–6628.

[179] RUOSLAHTI, E., and PIERSCHBACHER, M. D., 1987, New perspectives in cell adhesion: RGD and intergrins. *Science*, **238,** 491–497.

[180] SANTOSO, S., ZIMMERMANN, U., NEPPERT, J., and MUELLER-ECKHARDT, C., 1986, Receptor patching and capping of platelet membranes induced by monoclonal antibodies. *Blood*, **67,** 343–349.

[181] SATO, H., and SUZUKI, M., 1976, Deformability and viability of tumor cells by transcapillary passage with reference to organ affinity in metastasis in cancer. *Fundamental Aspects of Metastasis*, edited by L. Weiss (Amsterdam: North-Holland), pp. 311–317.

[182] SCHMID-SCHONBEIN, G. W., 1986, Rheology of leukocytes. *Handbook of Bioengineering*, edited by R. Skalak and S. Chien (New York: Pergamon), chap. 13.

[183] SCHMID-SCHONBEIN, G. W., 1987, Leukocyte kinetics in the microcirculation. *Bioheology*, **24,** 139–151.

[184] SHAH, S. V., BARICOS, W. H., and BASCI, A., 1987, Degradation of human glomerular basement by stimulated neutrophils. Activation of a metalloproteinase(s) by reactive oxygen metabolites. *J. Clin. Invest.*, **79,** 25–31.

[185] SHOENFELD, Y., TAL, A., BERLINER, S., and PINKHAS, J., 1986, Leukocytosis in non-hematological malignancies—a possible tumor-associated marker. *J. Cancer Res. Clin. Oncol.*, **111,** 54–58.

[186] SINDELAR, W. F., TRALKA, T. S., and KETCHAM, A. S., 1975, Electron microscopic observations on formation of pulmonary metastases. *J. Surg. Res.*, **18,** 137.

[187] SLOANE, B. F., and HONN, K. V., 1984, Cysteine proteinases and metastasis. *Cancer Metastasis Rev.*, **3,** 249–263.

[187a] SLOANE, B. F., ROZHIN, J., HATFIELD, J. S., CRISSMAN, J. D., and HONN, K. V., 1987, Plasma membrane-associated cysteine proteinases in human and animal tumors. *Exp. Cell Biol.*, **55,** 209–224.

[188] SOBEL, M. E., YAMAMOTO, T., DECROMBRUGHE, B., and PASTAN, I., 1981, Regulation of procollagen messenger ribonculeic acid levels in Rous sarcoma virus transformed chick embryo fibroblasts. *Biochemistry*, **20,** 2678–2684.

[189] SOBERMAN, R. J., HARPER, T. W., BETTERIDGE, D., LEWIS, R. A., and AUSTEN, K. F., 1985, Characterization and separation of the arachidonic acid 5-lipoxygenase and linoleic acid W-6 lipoxgenase (arachidonic acid 15-lipoxygenase) of human polymorphonuclear leukocytes. *J. Biol. Chem.*, **260,** 4508–4515.

[190] SOLUM, N. A., and OLSEN, T. M., 1984, Glycoprotein Ib in the Triton-insoluble (cytoskeletal) fraction of blood platelets. *Biochem. Biophys. Acta*, **799,** 209.

[191] SOMMER, A., BREWER, M. T., THOMPSON, R. C., MOSCATELLI, D., PRESTA, M., and RIFKIN, D. B., 1987/8, Primary structure of a human placental angiogenesis factor and cDNA sequence of a clone coding for a homologous protein from a human hepatoma cell line. *Biochem. Biophys. Res. Commun.*,

[192] STARKEY, J. R., LIGGITT, H. D., JONES, W., and HOSICK, H. L., 1984, Influence of migratory blood cells on the attachment of tumor cells to vascular endothelium. *Int. J. Cancer*, **34,** 535–543.

[193] STEEL, G. G., and ADAMS, K., 1977, Enhancement by cytotoxic agents of artificial pulmonary metastasis. *Br. J. Cancer*, **36,** 653–658.

[194] STEWART, R. A. N., and HACKER, M. P., 1984, Enhancement of B16 melanoma pulmonary colonization in mice following bleomycin in hyperoxia. *Proc. Am. Assoc. Cancer Res.*, **25,** 60.

[195] STRAULI, P., and WEISS, L., 1977, Cell locomotion and tumor penetration. *Eur. J. Cancer*, **13,** 1–12.

[196] SUZUKI, S., ARGRAVES, W. S., PYTELA, R., ARAI, H., KRUSIUS, T., PIERSCHBACHER, M. D., and ROUSLAHTI, E., 1986, cDNA and amino acid sequences of the cell adhesion protein receptor recognizing vitronectin reveal a transmembrane domain and homologies with other adhesion protein receptors. *Proc. Natl. Acad. Sci., USA*, **83,** 8614–8618.

[197] TABILIO, A., ROSA, J. P., TESTA, U., KIEFFER, N., NURDEN, A. T., DELCANIZO, M. C. J., BRETON-GORIUS, J., and VAINCHENKEN, W., 1984, Expression of platelet membrane glycoproteins and alpha-granule proteins by a human erythroleukemia cell line (HEL). *Embo J.*, **3,** 453–459.

[198] TAKAHASHI, M., FUJIWARA, M., KISHI, K., SAKAI, C., SANADA, C., SANADA, M., MORIYAMA, Y., and SHIBATA, A., 1985, CSF producing all bladder cancer: case report and characteristics of the CSF produced by tumor cells. *Int. J. Cell Cloning*, **3,** 294–303.

[199] TANAKA, N. G., TOHGO, A., and OGAWA, H., 1986, Platelet-aggregation activities of metastasizing tumor cells. V. *In situ* roles of platelets in hematogeneous metastasis. *Invasion Metastasis*, **6,** 209–224.

[200] TAO, T.-W., and JOHNSON, L. K., 1982, Altered adhesiveness of tumor cells surface variants with reduced metastasizing capacity—reduced adhesiveness to vascular wall components in culture. *Int. J. Cancer*, **30,** 763–766.

[201] TERRANOVA, V. P., WILLIAMS, J. E., and LIOTTA, L. A., 1984, Modulation of the metastatic activity of melanoma cells by laminin and fibronectin. *Science*, **226,** 982–985.

[202] THIAGARAJAN, P., SHAPIRO, S., SWERERLITSCH, L., and MCCORD, S., 1987, A human erythroleukemic cell line synthesizes a functionally active glycoprotein IIb/IIIa complex capable of binding fibrinogen. *Biochem. Biophys. Acta*, **924,** 127–134.

[203] THOMPSON, W. D., SHIACH, K. J., FRASER, R. A., MCINTOSH, L. C., and SIMPSON, J. G., 1987, Tumours acquire their vasculature by vessel incorporation, not vessel in growth. *J. Pathol.*, **151,** 323–332.

[204] THORGEIRSSON, U. P., LIOTTA, L. A., KALEBIC, T., MARGULIES, I. M., THOMAS, K., RIOS-CANDELORE, M., and RUSSO, R. G., 1982, Effect of natural protease inhibitors and a chemoattractant on tumor cell invasion *in vitro*. *J. Natl. Cancer Inst.*, **69,** 1049–1054.

[205] TILL, G. O., JOHNSON, K. J., KUNKEL, R., and WARD, P. A., 1982, Intravascular activation of complement and acute lung injury. *J. Clin. Invest.*, **69,** 1126–1135.

[206] TOHGO, A., TANAKA, N. G., and OGAWA, H., 1986, Platelet aggregating activities of metastasizing tumor cells. IV. Effects of cell surface modification on thrombin generation, platelet aggregation and subsequent lung colonization. *Invasion Metastasis*, **6,** 58–68.

[207] TURPEENNIEMI-HUJANEN, T., THORGEIRSSON, U. P., HART, I. R., GRANT, S. S., and LIOTTA, L. A., 1985, Expression of collagenase IV (basement membrane collagenase) activity in murine tumor cell hybrids that differ in metastatic potential. *J. Natl. Cancer Inst.*, **75,** 99–103.

[208] TUSZYNSKI, G. P., TATIANA, B. G., ROTHMAN, V. L., KNUDSEN, K. A., and GASIC, G. J., 1987, Thrombospondin. A potentiator of tumor cell metastasis. *Cancer Res.*, **47,** 4130–4133.

[209] TYAGI, J. S., HIRANO, H., MERLINO, G. T., and PASTAN, I., 1983, Transcriptional control of the fibronectin gene in chick embryo fibroblasts transformed by Rous sarcoma virus. *J. Biol. Chem.*, **258,** 5787–5793.

[210] VAN DEN BRENK, H. A. S., STONE, M., KELLY, H., ORTON, C., and SHARPINGTON, C., 1984, Promotion of growth of tumour cells in acutely inflammed tissues. *Br. J. Cancer*, **30,** 246–260.

[211] VAUPEL, P., and MULLER-KLIESER, W., 1983, Interstitieller Raum and Mikromilieu in malignen tumoren. *Mikrozirk Forsch Klin*, Vol. 2 (Basel: Karger).

[212] VANPUTTEN, L. M., KRAM, K. L. J., VAN DIERENDONCK, H. H. C., SMINK, T., and FUZY, M., 1975, Enhancement by drugs of metastatic lung nodule formation after intravenous tumour cell injection. *Int. J. Cancer*, **15,** 588–595.

[213] VARANI, J., and FANTONE, J. C., 1982, Phorbol myristae acetate-induced adherence of Walker 256 carcinosarcoma cells. *Cancer Res.*, **42,** 190–197.

[214] VARANI, J., DIXIT, V. M., FLIGIEL, S. E. G., MCKEEVER, P. E., and CAREY, T. E., 1986, Thrombospondin-induced attachment and spreading of human squamous carcinoma cells. *Expl. Cell Res.*, **167,** 376–390.

[215] VLODAVSKY, I., ATZMON, A. R., and FUKS, Z., 1982, Tumor cell attachment to the vascular endothelium and subsequent degradation of the subendothelial extracellular matrix. *Expl. Cell Res.*, **140,** 149–159.

[216] VLODAVSKY, I., FUKS, Z., BAR-NER, M., ARIAV, Y., and SCHIRRMACHER, V., 1983, Lymphoma cell-mediated degradation of sulfated proteoglycans in the subendothelial extracellular matrix: relationship to tumor cell metastasis. *Cancer Res.*, **43,** 2704–2711.

[217] WALTHER, H. E., 1948, *Krebsmetastasen* (Basel: Schwabe), pp. 296–297.

[218] WARBURTON, M. J., FERNS, S. A., KIMBELL, R., RUDLAND, P. S., MONOGHAND, P., and GUSTERSON, B. A., 1987, Loss of basement membrane deposits and development of invasive potential by virally-transformed rat mammary cells are independent of collagenase production. *Int. J. Cancer*, **40,** 270–277.

[219] WARD, P. M., and WEISS, L., 1985, Effects of single versus triple intravenous injections of B16 melanoma cells on the development of pulmonary tumors in mice. *Int. J. Cancer*, **36,** 519–521.

[220] WARREN, B. A., 1979, Tumor angiogenesis. *Tumor Blood Circulation*, edited by H.-I. Peterson (Boca Raton, FL: CRC Press), pp. 49–75.

[221] WEISS, L., 1965, Studies on cell deformability. I. Effect of surface charge. *J. Cell Biol.*, **26,** 735–739.

[222] WEISS, L., 1979, Dynamic aspects of cancer cell populations in metastasis. *Am. J. Pathol.*, **97,** 601–608.

[223] WEISS, L., 1980, Cancer cell traffic from the lungs to the liver: an example of metastatic inefficiency. *Int. J. Cancer*, **25,** 385–392.

[224] WEISS, L., 1985, *Principles of Metastasis* (Orlando, FL: Academic Press), pp. 3–9.

[225] WEISS, L., 1985, *Principles of Metastasis*, (Orlando: Academic Press), pp. 134–159.

[226] WEISS, L., 1985, *Principles of Metastasis*, (Orlando, FL: Academic press), pp. 162.

[227] WEISS, L., 1986, A critical overview of the metastatic porcess. *Mechanisms of Cancer Metastasis: Potential Therapeutic Implications*, edited by K. V. Honn, W. E. Powers and B. F. Sloane (Boston, MA: Martinus Nijhoff), pp. 23–40.

[228] WEISS, L., 1986, Metastatic inefficiency: causes and consequences. *Cancer Rev.*, **3,** 1–24.

[229] WEISS, L., 1987, The hemodynamic destruction of circulating cancer cells. *Biorheology*, **24,** 105–115.

[230] WEISS, L., 1988, Biomechanical destruction of cancer cells in the heart: a rate-regulator for hematogenous metastasis. *Invasion Metastasis*, **8,** 228–237.

[231] WEISS, L., 1988, Biomechanical destruction of cancer cells in the skeletal muscle: a rate-regulator for hematogenous metastasis. *Clin. Expl. Metastasis* (In press).

[232] WEISS, L., and CLEMENT, K., 1969, Studies on cell deformability: some rheological considerations. *Expl. Cell. Res.*, **58,** 379–387.

[233] WEISS, L., and DIMITROV, D. S., 1984, A fluid mechanical analysis of the velocity, adhesion and destruction of cancer cells in capillaries during metastasis. *Cell Biophys*, **6,** 9–22.

[234] WEISS, L., and DIMITROV, D. S., 1986, Mechanical aspects of the lungs as cancer cell-killing organs during hematogenous metastasis. *J. Theor. Biol.*, **121,** 307–321.

[235] WEISS. L., DIMITROV, D. S., and ANGELOVA, M., 1985, The hemodynamic destruction of intravascular cancer cells in relation to myocardial metastasis. *Proc. Natl. Acad. Sci., USA*, **82,** 5737–5741.

[236] WEISS, L., and GLAVES, D., 1983, Cancer cell damage at the vascular endothelium. *Ann. NY Acad. Sci.*, **416,** 681–692.

[237] WEISS, L., GRUNDMANN, E., TORHORST, J., HARTVEIT, F., MOBERG, I., EDER, M., FENOGLIO-PREISER, C. M., NAPIER, J., HORNE, C. H. W., LOPEZ, M. J., SHAW-DUNN, R. I., SUGAR, J., DAVIES, J. D., DAY, D. W., and HARLOS, J. P., 1986, Hematogenous metastatic patterns in colonic carcinoma: an analysis of 1541 necropsies. *J. Pathol.*, **150,** 195–203.

[238] WEISS, L., and HARLOS, J. P., 1972, Some speculations on the rate of adhesion of cells to coverslips. *J. Theor. Biol.*, **37,** 169–179.

[239] WEISS, L., and HARLOS, J. P., 1972, Short-term interactions between cell surfaces. Progr. *Surface Sci.*, **1**, 355–405.

[240] WEISS, L., and HARLOS, J. P., 1987, Unpublished data.

[241] WEISS, L., ORR, F. W., and HONN, K. V., 1988, Interactions of cancer cells with the microvasculature during metastasis. *FASEB J.*, **2**, 12–21.

[242] WEISS, L., and SUH, O. W., 1985, The quantitation of some aspects of invasion *in vivo* and *in vitro*. *Basic Mechanisms and Clinical Tremtment of Tumor Metastasis*, edited by M. Toriss and Y. Yoshida (New York: Academic Press), pp. 23–40.

[243] WEISS, L., WARD, P. M., HARLOS, J. P., and HOLMES, J. C., 1984, Target organ patterns of tumors in mice following the arterial dissemination of B16 melanoma cells. *Int. J. Cancer*, **33**, 825–830.

[244] WEISS, L., WARD, P. M., and HOLMES, J. C., 1983, Liver to lung traffic of cancer cells. *Int. J. Cancer*, **32**, 79–83.

[245] WEISS, L., PEPPIN, G., ORTIZ, X., RAGSDALE, C., and TEST, S. T., 1985, Oxidative autoactivation of latent collagenase by human neutrophils. *Science*, **227**, 747–749.

[246] WENCEL-DRAKE, J. D., PLOW, E. F., KUNICKI, T. J., WOODS, V. L., KELLER, D. M., and GINSBERG, M. H., 1986, Localization of internal pools of membrane glycoproteins involved in platelet adhesive responses. *Am. J. Pathol.*, **124**, 324–334.

[247] WILLIS, R. A., 1952, *The Spread of Tumours in the Human Body* (London: Butterworth), pp. 284–285.

[248] WOOD, S., 1958, Pathogenesis of metastasis formation observed *in vivo* in the rabbit ear chamber. *Arch. Pathol.*, **66**, 550–568.

[249] WOODS, D. L. JR, WOLFF, L. E., and KELLER, D. M., 1986, Restins platelets contain a substantial centrally located pool of glycoprotein IIb–IIIa complex which may be accessible to some but not other extracellular proteins. *J. Biol Chem.*, **261**, 15242.

[250] WOOLLEY, D. E., 1984, Collagenolytic mechanisms in tumor cell invasion. *Cancer Metastasis Rev.*, **3**, 361–372.

[251] WOOLLEY, D. E., 1984, Proteolytic enzymes of invasive cells. *Invasion. Experimental and Clinical Implications*, edited by M. M. Mareel and K. C. Calman (Oxford: Oxford University Press), pp. 228–251.

[252] YOGEESWARAN, G., 1983, Cell surface glycolipids and glycoproteins in malignant transformation. *Adv. Cancer Res.*, **38**, 289–350.

[253] ZEIDMAN, I., 1961, The fate of circulating cancer cells. I. Passage of cells through capillaries. *Cancer Res.*, **121**, 38–39.

[254] ZEIDMAN, I., and BUSS, J. M., 1952, Transpulmonary passage of tumor cell emboli. *Cancer Res.*, **12**, 731–733.

[255] ZUCKER, S., LYSIK, R. M., RAMAMURTHY, N. S., GOLUB, L. M., WIEMAN, J. M., and WILKIE, D. P., 1985, Diversity of melanoma plasma membrane proteinases: inhibition of collagenolytic and cytolytic activities by minocycline. *J. Natl. Cancer Inst.*, **75**, 517–525.

[256] ZUCKER, S., LYSIK, R. M., WIEMAN, J., WILKIE, D. P., and LANE, B., 1985, Diversity of human pancreatic cancer cell proteinases: role of cell membrane metalloproteinases in collagenolysis and cytolysis. *Cancer Res.*, **45**, 6168–6178.