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Arrest and Extravasation of Neoplastic Cells

An Electron Microscopy Study of Serial Sections at Sequential Stages *

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Summary. The morphological aspects of the arrest and extravasation of malignant cells of human origin (K-562 cell line) in the lungs of athymic (nude) and asplenic-athymic (lasat) mice were studied by electron microscopy examination of serial sections. The specimens were obtained at sequential stages after the sc inoculation into newborn mice of 10^7 malignant cells. K-562 cells (10^5) were also injected iv into control groups of nude and lasat mice to assess the influence of the route of inoculation on the in vivo behavior of K-562 cells. Our results demonstrated that K-562 cells were arrested and proliferated within the pulmonary capillaries without the participation of platelets or fibrin. The neoplastic cells extravasated by attrition and penetration of the endothelium (rather than by diapedesis) and continued to proliferate in the interstitial tissue of the lung, developing into neoplastic nodules. Following iv injection, K-562 cells induced the formation of platelet-tumor cell aggregates within the pulmonary capillaries. However, under these conditions, the neoplastic cells did not adhere to the endothelium nor did they proliferate or extravasate. These aggregates were flushed out by the circulation, restoring the permeability of the capillaries.

Key words: Human malignant cells – K-562 cell line – Vascular arrest – Endothelial attrition – Extravasation – Nude and lasat mice – Electron microscopy

Characterization of the mechanisms of arrest and extravasation of neoplastic cells will add to the understanding of the events occurring during the complex process of dissemination of malignant tumors. This knowledge, in turn, may have practical implications, such as the formulation of new therapeutic approaches to metastases, the most important complication of neoplastic growth.

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The emphasis of clinical and experimental studies has been on the importance of platelets and fibrin in the arrest of blood-borne neoplastic cells within the capillaries of target organs (Dvorak et al. 1979, 1980; Gasic et al. 1973; Gastpar 1980; Hilgard 1980; Hiramoto et al. 1960; Warren 1973; Wood 1958). In line with these reports, several investigators have proposed that the entrapment of neoplastic cells by an intravascular (iv) clot may facilitate their extravasation by diapedesis (Ludatscher et al. 1967; Sindelar et al. 1975; Warren and Vales 1972; Wood 1958). These conclusions do not correlate well with the histopathological features of human neoplasia and are at variance with the experimental results reported by other authors (Baserga 1955; Chew et al. 1976; Dingemans et al. 1978; Fisher 1967; Hagmar 1970; Locker et al. 1970; Winterbauer et al. 1968).

Recently, we developed a compromise model of disseminated neoplastic growth by subcutaneous (sc) injection of K-562 cells derived from a human leukaemia (Lozzio and Lozzio 1975) into hereditarily athymic (nude) and asplenic-athymic (lasat) *newborn* mice (Lozzio et al. 1976; Machado et al. 1976). The neonatal "priming" led to the development – in about 60% of the mice – of multiple neoplastic nodules in the lungs and kidneys, as well as infiltration of the lymph nodes and meningeal space (Lozzio et al. 1976; Machado et al. 1981). The visceral distribution of disseminated growths was consistently reproduced from one inoculum of cells to the next over a 2-year period. In contrast, K-562 cells did not proliferate after iv injection into *1-month old* mice. Also, K-562 cells, inplanted in *adult* nude mice, grew as sc tumors which did not disseminate (Machado et al. 1977). With regard to the production of neoplastic growths in distant organs, the K-562 cells gradually spread by entering the systemic circulation of the host at the site of sc inoculation. This type of inoculation avoided the limited anatomical distribution forced by iv injection of neoplastic cells which occurs in other systems (Chew et al. 1976). Thus, under well-controlled conditions, our model is suitable for conducting a variety of studies, including repeated ultrastructural examination of tissues at selected intervals.

We have investigated the involvement of platelets and fibrin in the intravascular arrest of K-562 cells and the pattern of extravasation of these malignant cells by electron microscopy. Since dissemination and arrest of neoplastic cells is a dynamic process, significant information could not be obtained from isolated morphological observations; we therefore, examined large numbers of serial sections from specimens obtained at sequential stages. This procedure introduced the time factor into the analysis of the images and facilitated the interpretation of spatial relationships between neoplastic and normal cells. The lungs of nude mice were particularly suitable for these studies because pulmonary capillaries have a non-fenestrated endothelium, vascular distribution in the alveolar walls is relatively simple, and perivascular tissue is scarce.

Material and Methods

Mice. BALB/c nude and lasat mice of both sexes in the 17th and 10th generations, respectively, (Lozzio et al. 1976; Machado et al. 1976) were used for the inoculation of tumorigenic cells. Breeders, litters, and experimental mice were kept in specific pathogen-free (SPF) enclosures. Transplantation, autopsies, and other procedures were carried out in the SPF enclosures.

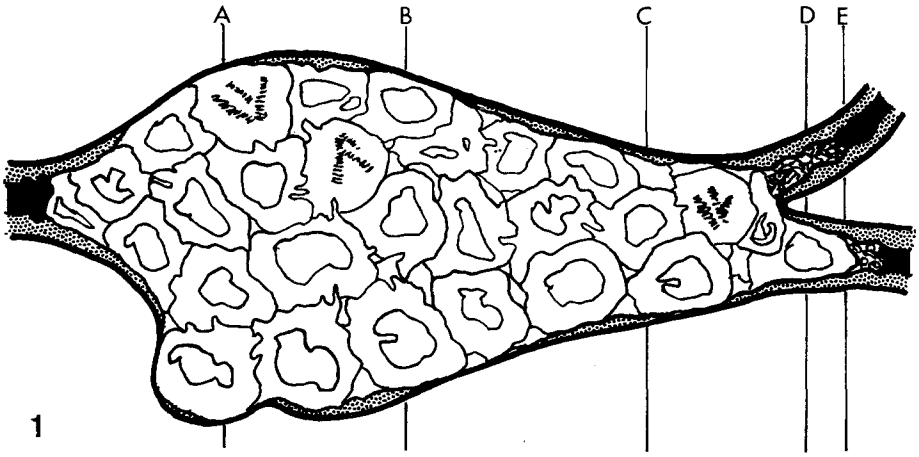


Fig. 1. A schematic drawing of a K-562 cell intravascular nodule. The letters represent the planes of section for the following figures: A – Fig. 4; B – Fig. 3; C – Fig. 13; D – Fig. 14; and E – Fig. 15 inset. The following are the abbreviations used in the figures: *Ac,* alveolar cell; *As,* alveolar space; *c,* capillary; *col,* collagen; *End,* endothelium, endothelial cell; *Er,* erythrocyte; *f,* fibrin; *Fib,* fibroblast; *G,* granulocyte; *Kc,* K-562 cell; *l,* lipid; *M,* macrophage; *mit,* mitosis; *Pl,* platelet

Heterotransplantation of Tumorigenic Cells. The K-562 cell line (Lozzio et al. 1975) used in these studies was established in our laboratories more than 10 years ago and originated from a patient with chronic myelogenous leukaemia. This cell line is consistently tumorigenic in immunodeficient mice (Machado et al. 1977). The cells have a readily identifiable ultrastructural morphology (Maxwell et al. 1979) and contain the Philadelphia (Ph^{1+}) chromosome and chromsomal translocations t15;17 (Lozzio et al. 1975).

K-562 cells from 6- to 7-day-old suspension cultures were concentrated by centrifugation, then resuspended in Eagle's minimal essential medium (MEM) at the rate of 20×10^7 cells/ml. *Newborn* (24–48 h old) nude and lasat mice each received a single sc injection containing 10^7 cells in 50 µl of MEM. The injection was always made in the dorsal area and no further treatment was given. To ascertain the influence of the route of inoculation on disseminated neoplastic growth, 10^5 K-562 cells were administered into the tail veins of 1-month-old nude mice; needles and syringes were not treated with anticoagulants. The size of *newborn* mice did not permit using them for iv injection.

Histopathological Examination. Mice injected sc were killed at intervals from 1 to 9 h and from 1 to 94 days after injection. Mice receiving cells iv were killed at 1, 30, and 60 min intervals after injection. Lung specimens were fixed in 3% glutaraldehyde in 0.05 M sodium cacodylate buffer at pH 7.2 for 2 h, rinsed 3 times in buffer, and post-fixed for 1 h in 2% osmium tetroxide in 0.1 M sodium cacodylate at pH 7.2. After dehydration, the specimens were embedded in Epon 812. Thick sections were stained with toluidine blue O solution and examined by light microscopy (LM) to locate intravascular K-562 cells and extravascular neoplastic nodules. Serial, ultra-thin sections of the block were then cut. The morphology of the intravascular accumulations of K-562 cells was reconstructed by continuing serial sectioning until the K-562 cells were no longer visible in the pulmonary tissue (Fig. 1). The sections were mounted on Formvar- and carbon-coated slot grids, stained with uranyl acetate and lead citrate, and examined and photographed in a Zeiss EM 9-S electron microscope.

Cytogenetic Studies. Chromosome analyses of K-562 cells, using standard techniques (Lozzio et al. 1975), were made prior to transplantation and in cells cultured from neoplastic growths excised from the lungs. At least 20 cells were studied each time.

Nomenclature. Intravascular neoplastic nodules and intravascular neoplastic cell accumulations are terms used in this report to designate the proliferation of K-562 cells at the site of arrest preceding extravasation. We have not used the terms thrombi or emboli because the former indicates coagulative phenomena and the latter implies plugging of capillaries by blood-borne groups of cells or tissue fragments, neither of which apply to this model.

Results

General Findings. We have previously found that, as usually happens in heterotransplantation of neoplastic cells, a large number of K-562 cells underwent necrosis at the site of injection and in the circulation of the nude mice. Between 20 and 30 days after inoculation, the remaining viable K-562 cells gave rise to multiple pulmonary neoplastic growths. During this process, the haematopoietic tissue and peripheral blood of the mice were normal.

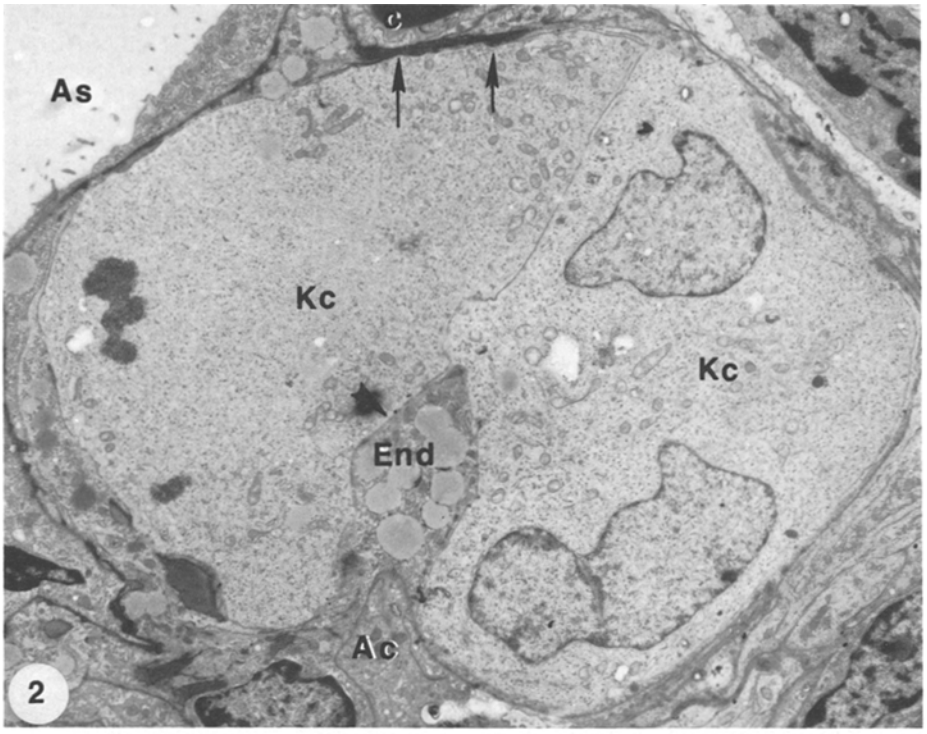
Morphology and Chromosomal Analyses of K-562 Cells. The morphology of K-562 cells composing neoplastic growths in the lungs of nude and lasat mice was identical to that of the cells proliferating in suspension culture. The karyotype examination of the cells from 12 pulmonary neoplastic nodules demonstrated the presence of the Philadelphia (Ph^{1+}) chromosome and the characteristic chromosomal translocation t15;17.

Electron Microscopy Examinations. Although strict limits cannot be drawn between the various phases of proliferation of K-562 cells in the pulmonary tissue, the process can be arbitrarily divided into three main stages: 1) intravascular arrest; 2) endothelial penetration; and 3) extravasation.

Intravascular Arrest of K-562 Cells. K-562 cells, many of them displaying mitotic figures, were found within pulmonary capillaries as early as 3 h after sc inoculation. During the next 5 days, mitotic division of the K-562 cells proceeded steadily (Fig. 2). The number of cells gradually increased and, finally, nodules which completely filled the vascular lumen were formed. The surface of these nodules closely adhered to the endothelial cells. Minute and homogeneous electron-dense deposits, possibly representing non-striated, monomeric fibrin, were present in only a few specimens. In serial sections from the earliest and subsequent stages of intravascular arrest, neither platelets nor fibrin with identifiable periodicity were interspersed between K-562 cells or between these cells and the endothelium.

Fig. 2. Two K-562 cells, one in mitosis, occupy the lumen of a pulmonary capillary 5 days after sc inoculation. Most of the endothelium appears compressed. An endothelial cell containing numerous lipid droplets is visible (*arrowhead*), and electron-dense material, which is possibly non-striated fibrin, is adjacent to one cell (*arrows*). Platelets are not observed (×4,300)

Fig. 3. Ten days after sc inoculation, actively proliferating K-562 cells form a medium-sized, intravascular, neoplastic cell nodule within a pulmonary capillary. This micrograph shows a capillary bend, producing an image of "inward-thrusting" endothelial cells (*arrowheads*). The endothelium is diffusely compressed (*arrows*) (×2,400)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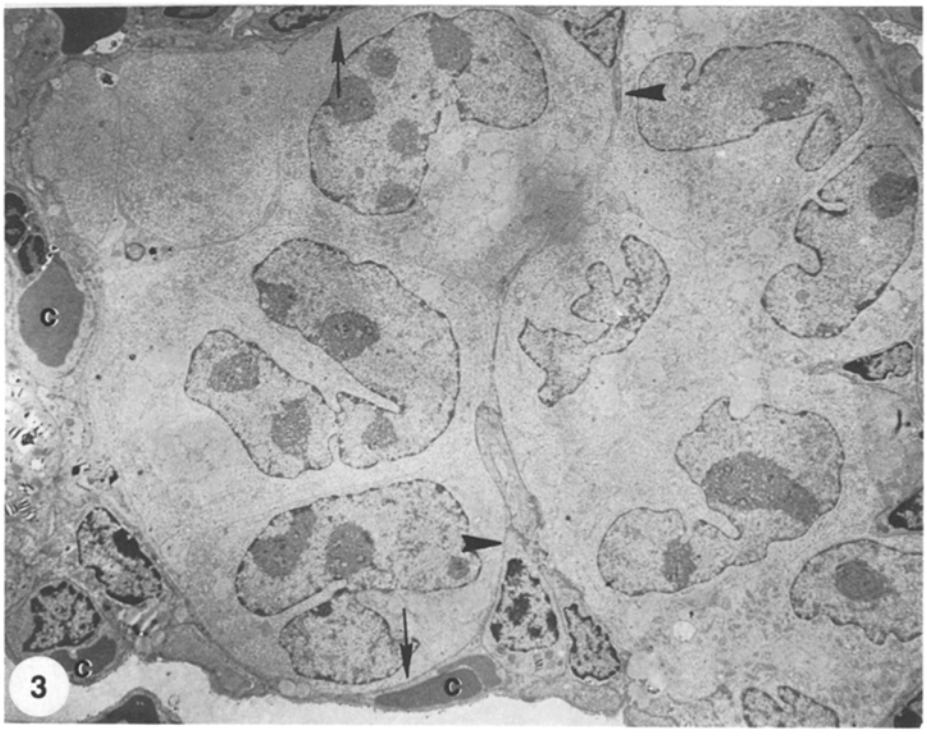
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Endothelial Penetration. After 10 days, the intravascular nodules composed of K-562 cells dilated the vessels, compressing and stretching the endothelium (Fig. 3). Large areas of the vascular wall exhibited only a thin layer of endothelial cytoplasm separating the neoplastic cells from the basement membrane.

Examination of the serial sections demonstrated that the intravascular nodules of K-562 cells were roughly fusiform with a wider medial area tapering toward each end. In transverse sections, the medial area contained 10 to 30 K-562 cells, depending on the size of the nodule (Fig. 4). The K-562 cells, linked by numerous interdigitating cytoplasmic processes, were tightly packed within the vascular lumen (Fig. 5). A few cells contained lipid droplets but no signs of necrosis were seen, demonstrating that the intercellular diffusion of plasma met the metabolic needs of the actively divided neoplastic cells. The absence of cellular injury, coupled with variations in cellular shape and numerous mitotic divisions, indicated that the K-562 cells were able to withstand the mechanical trauma of compression within the expanding intravascular neoplastic nodules.

In many areas, cytoplasmic processes of K-562 cells appeared as isolated vesicles within adjoining endothelial cells. However, the examination of serial sections demonstrated these vesicles to be continuous with the neoplastic cells. The cytoplasmic extensions appeared either as short, round stubs or as finger-like projections of varied thicknesses and lengths (Figs. 6–9). The cytoplasmic processes were not associated with particular areas of the intravascular nodule; instead, they were randomly distributed over the surface of the K-562 cells. In the numerous serial sections examined, the processes did not penetrate into the junctions between endothelial cells.

As a result of the diffuse stretching and compression and of the penetration by extensions of the neoplastic cells, signs of cell injury (such as dense residual autophagosomes, blurring or organelle membranes, and focal accumulations of lipids) were observed in the endothelium (Figs. 6–9). Nevertheless, simultaneous morphological changes associated with lytic enzymatic activity and phagoby the neoplastic cells were not found. This lack of phagocytic ability is characteristic of K-562 cells in vitro and was maintained during the proliferation in the tissues of the mice.

Numerous gaps developed in the endothelium in association with the compression and penetration by neoplastic cells extensions. Through these endothelial gaps, K-562 cells adhered directly to the vascular basement membrane (Fig. 10). The examination of adjoining serial sections demonstrated that the width and shape of these gaps varied widely. They were always limited by markedly compressed endothelial cells and, in some areas, remnants of the endothelial cytoplasm were still present between the neoplastic cells and the basement membrane.

Deep invaginations of whole neoplastic cells into the adjacent pulmonary interstitial tissue were seen (Fig. 11). These protruding cells were surrounded by an extremely thin endothelial layer and the basement membrane and maintained continuity with the rest of the intravascular nodule by means of wide areas of intercellular contact and cytoplasmic interdigitations.

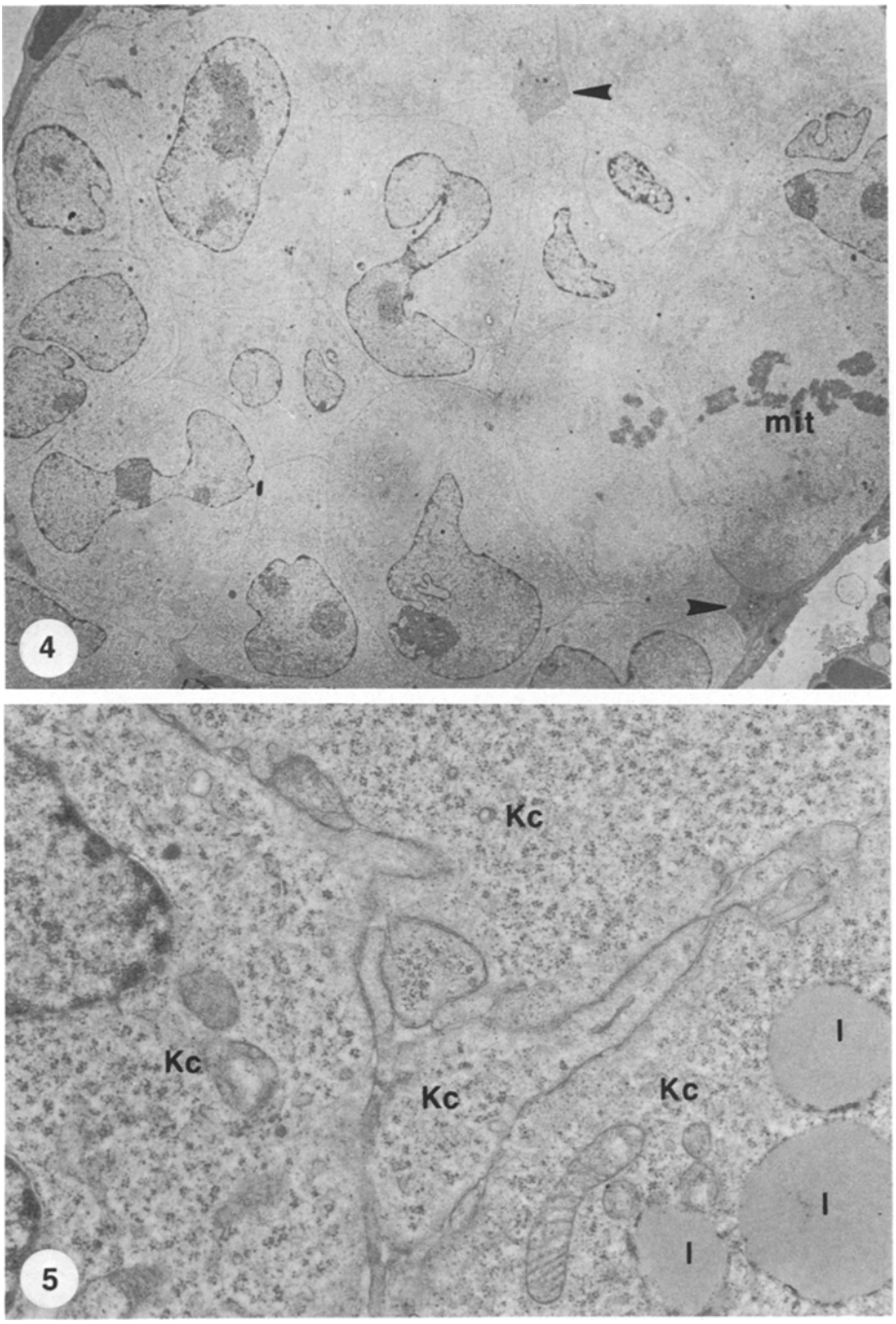
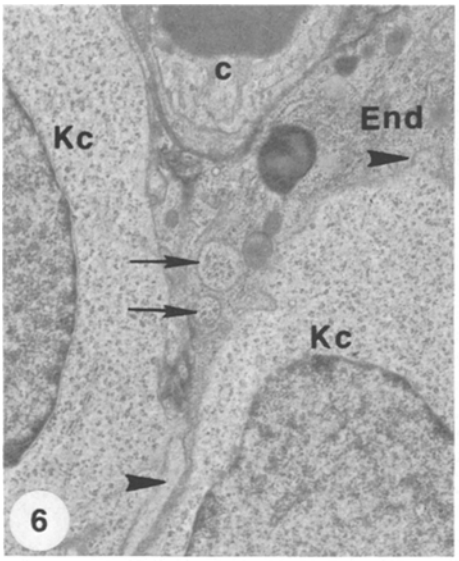


Fig. 4. Medial area of a large intravascular nodule formed by numerous K-562 cells, some in mitosis. The endothelial wall is stretched and compressed but does not show interruptions. Two platelets are present in this nodule (*arrowheads*), an unusual finding in this model. Fibrin deposits are not observed ($\times 1{,}800$)

Fig. 5. Numerous cytoplasmic interdigitations link the intravascular K-562 cells. One cell contains a few lipid droplets. ($\times 20{,}000$)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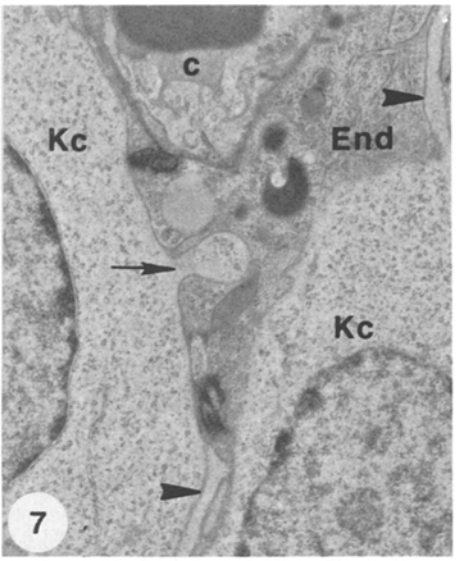
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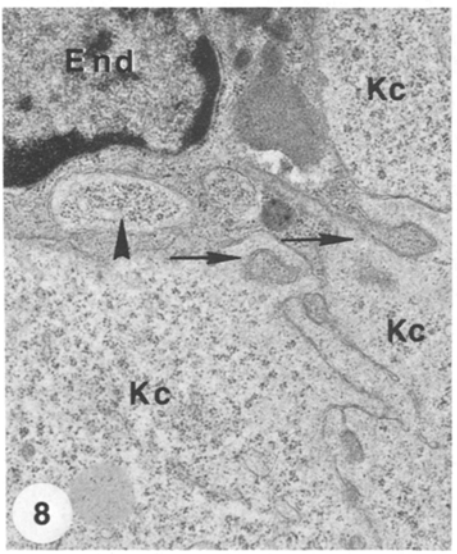
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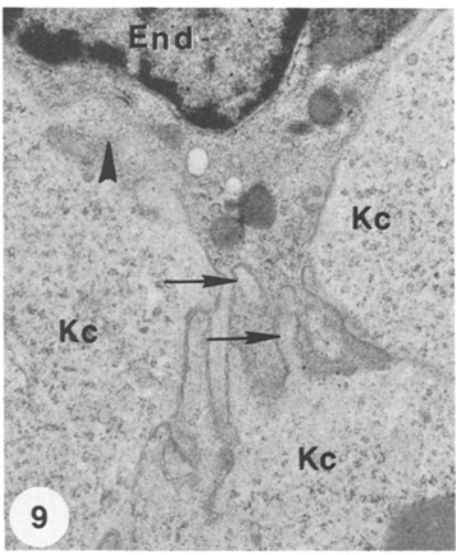
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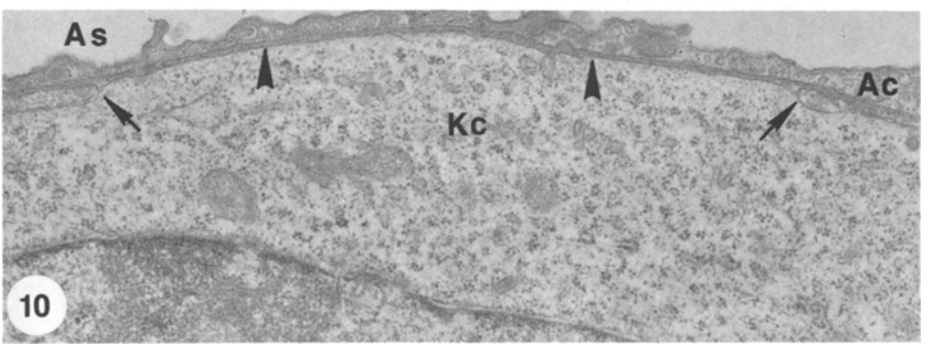
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In random spots, vascular alteration was even more advanced, as seen by thinning and, finally, the disappearance of areas of the basement membrane. As a result, the cell membranes of neoplastic and alveolar pulmonary cells were in direct contact (Fig. 12). Processes of the K-562 cells had also advanced between the endothelial remnants and were in contact with other pulmonary interstitial cells and close to adjoining capillaries. All these changes, although limited to restricted areas of the capillaries, preceded a more extensive disruption of the vascular barrier.

The cytoplasm of K-562 cells did not show morphological modifications throughout the development of vascular attrition. Similarly, as in the early stages of cell arrest, fibrin deposits and platelets were not seen between the neoplastic cells or between them and the endothelial and basement membranes during this phase of endothelial penetration.

Serial sections obtained from areas toward both ends of the intravascular nodule contained a decreasing number of neoplastic cells until, finally, only a few or even a single K-562 cell occupied the vascular lumen (Figs. 13 and 14). In these sections, many endothelial cells were nearly normal when compared with control mice. However, as in the sections containing multicellular nodules, focal areas of compression by the neoplastic cells and the development of endothelial gaps were observed. The intravascular nodules composed of K-562 cells caused a marked circulatory stasis. As a consequence, the adjoining vessels were plugged by erythrocytes, granulocytes, platelets, and deposits of electron dense material which was probably a mixture of plasma proteins and non-striate fibrin. Neoplastic cells were not present within the thrombosed capillaries (Fig. 15 inset).

Extravasation of K-562 Cells. The continuity of the vascular wall was totally disrupted by degenerative changes of the endothelium and basement membrane induced by the K-562 cells. Wide cytoplasmic projections of the neoplastic cells surrounded the necrotic endothelial and alveolar cells and bundles of collagen fibrils (Figs. 15,16,16 inset).

Fig. 6. Cytoplasmic processes of K-562 cells appear as round vesicles apparently contained in the adjoining endothelial cell (*arrows*). The endothelial cell shows autophagosomes and smaller, dense bodies, probably lipids. There are also long, thin processes piercing the endothelium (*arrowhead*) (× 13,500)

Fig. 7. Serial sectioning demonstrates the continuity between the intravascular K-562 and one "intraendothelial" vesicle observed in Fig. 6. This cytoplasmic process is short and wide (*arrow*). The adjoining K-562 cells, on the contrary, exhibit long and thin cytoplasmic penetrations into the endothelial cell (*arrowheads*) (× 13,500)

Figs. 8,9. Serial sections showing the continuity of multiple finger-like penetrations (*arrows*) of endothelium by K-562 cells. A large, intraendothelial "vesicle" observed in Fig. 8 corresponds to the tip of a wide projection of a K-562 cell in Fig. 9 (*arrowhead*). Portions of dark endothelial cytoplasm can be seen surrounded by the K-562 cytoplasmic projections (× 18,000)

Fig. 10. Extensive area of direct contact between a K-562 cell and the capillary basement membrane (*arrowheads*). Endothelial cytoplasm forms the limits of this gap (*arrows*) (× 20,000)

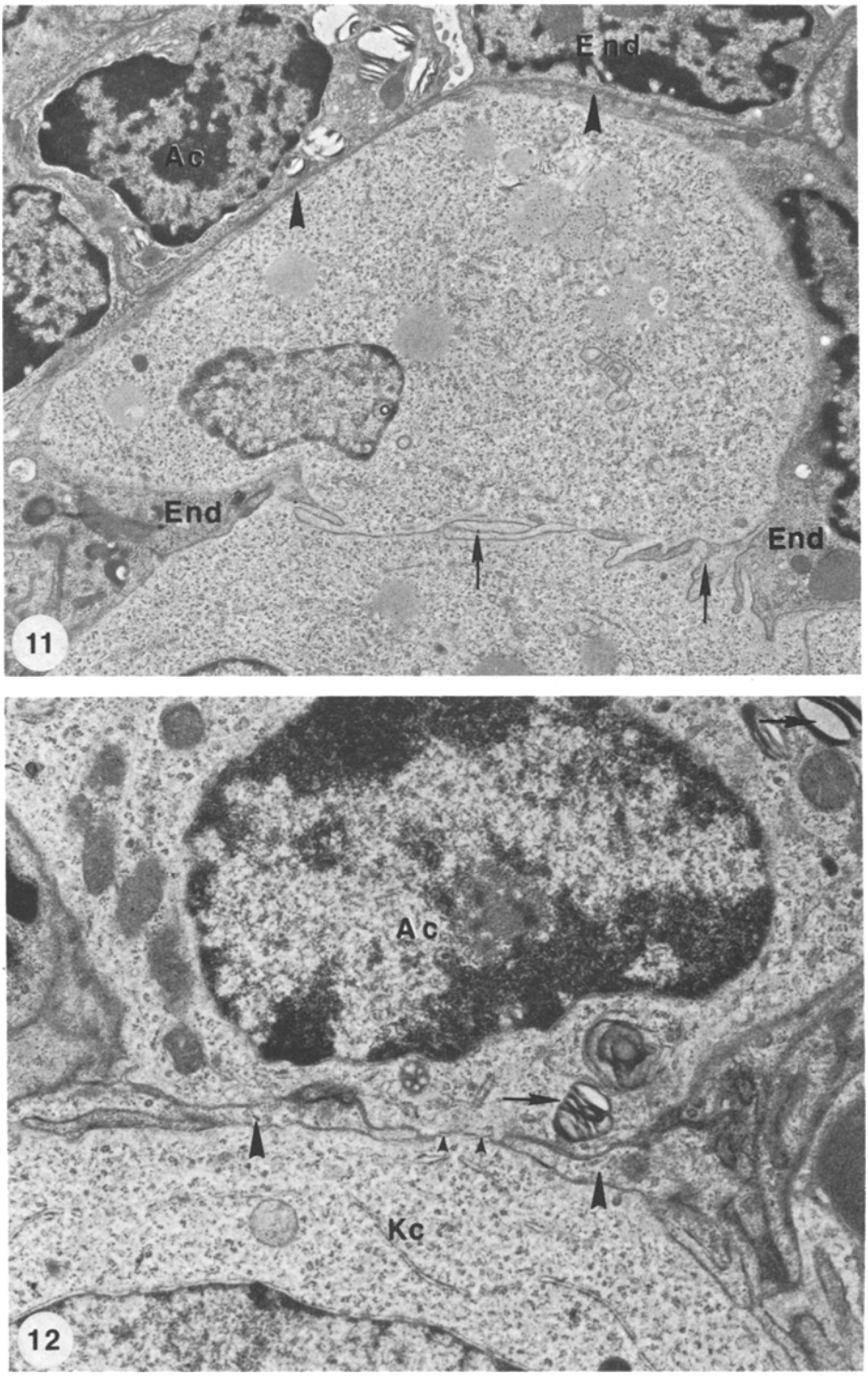
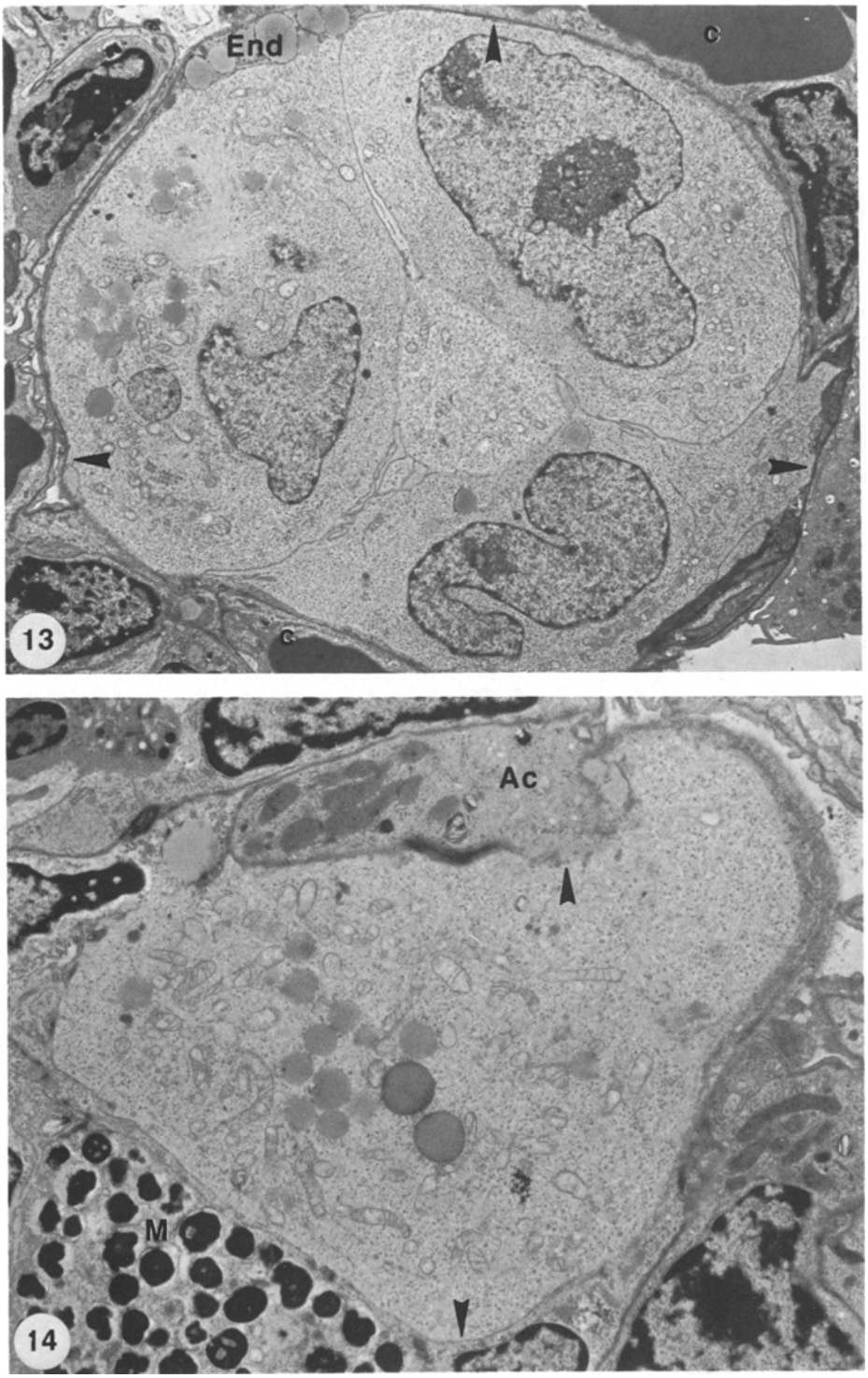
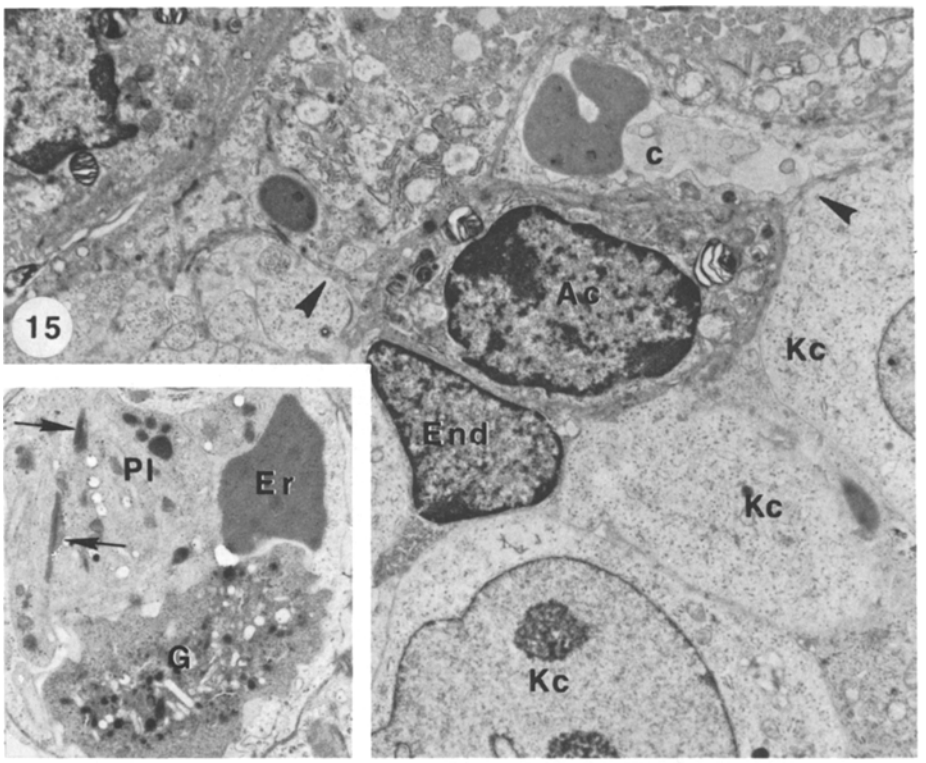


Fig. 11. K-562 cell forming an invagination into the surrounding pulmonary tissue. This protruding cell maintains extensive interdigitations with the adjoining intravascular K-562 cell (*arrows*). Despite the pronounced protrusion of the neoplastic cell into normal tissues, the basement membrane (*arrowheads*) and endothelial lining remain intact ($\times 6{,}000$)

Fig. 12. This micrograph shows a K-562 cell in direct contact with an alveolar cell (*small arrowheads*) which displays the characteristic lamellar lipid bodies (*arrows*). Portions of endothelium (*arrowheads*)



Figs. 13,14. Serial sections towards the end of the intravascular neoplastic nodule shown in Fig. 4. The number of cells decreases to 4 (Fig. 13) until, finally, part of the cytoplasm of only one neoplastic cell is present within the capillary (Fig. 14). Compression of endothelium and endothelial gaps are also observed in these sections (*arrowheads*) ($\times 4{,}200$, $\times 7{,}500$)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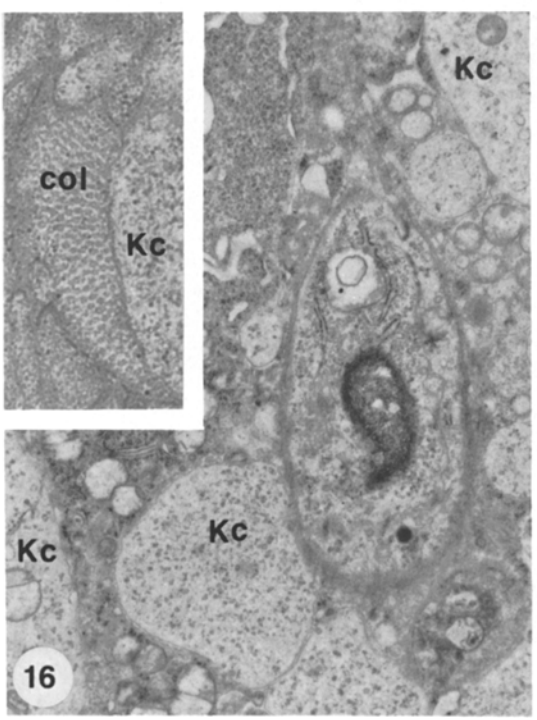
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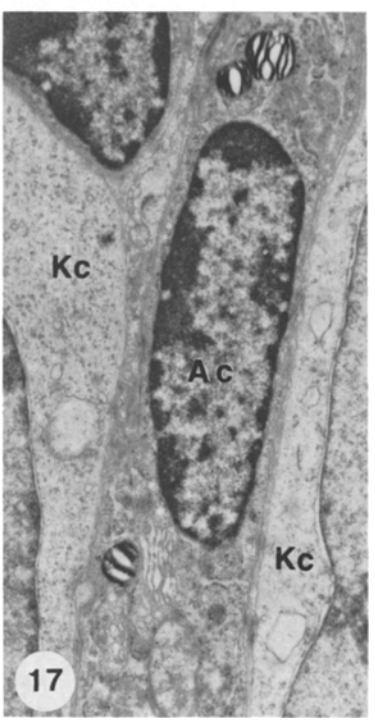
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Many alveolar cells flanked by K-562 cells acquired a fibroblast-like shape and could only be recognized by the presence of the characteristic cytoplasmic lamellar lipid bodies (Fig. 17). Reconstitution of the capillary wall was not seen. On the contrary, continuity was maintained between the neoplastic cells in the interstitial tissue of the lung and the intravascular nodules. The extravasated neoplastic cells proliferated, forming large neoplastic nodules which had a sarcomatous appearance with a newly formed fibrovascular stroma derived from the host (Fig. 18). Capillary basement membranes in the stroma were not developed until the extravascular growth reached a considerable size.

Intravascular Injection of K-562 Cells. In sections of lungs obtained 1 to 5 min after iv injection, K-562 cells appeared within the pulmonary vessels. The cells showed signs of necrosis and were surrounded by large aggregates of partially degranulated platelets and electron-dense homogeneous material (Fig. 19). These aggregates did not adhere firmly to the endothelium and, 60 min later, platelet accumulations and K-562 cells had disappeared; the vascular lumen appeared permeable and contained only normal blood cells.

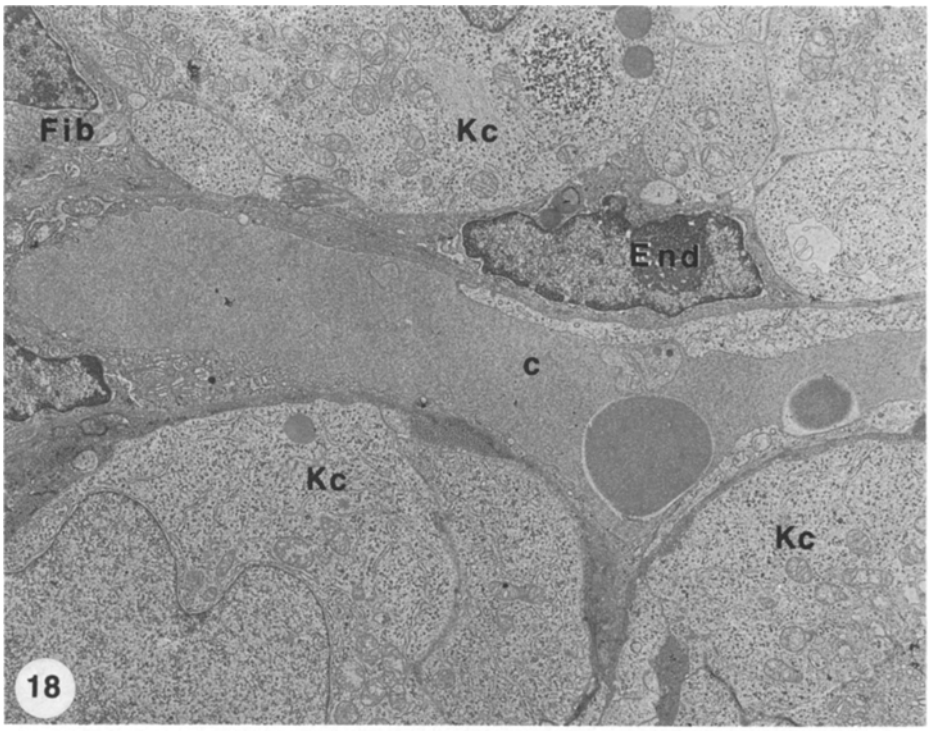
Discussion

The examination of numerous serial sections of the lungs from a few hours to several days after sc injection demonstrated that, following their arrival at the pulmonary capillaries, the K-562 cells adhered among themselves and to the endothelium without interposition of platelets and fibrin. The appearance of small, homogeneous, dense deposits, presumably containing non-striated, monomeric fibrin, was rare. The large number of sections examined made it obvious that immunohistochemical procedures for fibrin would not have resulted in significant differences. A lack of involvement of the coagulation system in intravascular cell arrest is not unique to the K-562 cells and has been observed in human neoplasia and experimental models (Baserga and Saffiotti 1955; Chew et al. 1976; Dingemans et al. 1978; Fisher and Fisher 1967; Hagmar 1970; Locker et al. 1970; Winterbauer et al. 1968). On the contrary, several authors (Dvorak et al. 1979, 1980; Gasic et al. 1973; Gastpar 1980; Hilgard 1973; Hiramoto et al. 1960; Warren 1973; Wood 1958) have proposed that platelets and

Fig. 15. Wide projections of the intravascular K-562 cells surround endothelial and alveolar cells and advance deeply into the pulmonary interstitial tissue (*arrowheads*). The continuity of the vascular wall has been destroyed. No signs of phagocytic activity by neoplastic cells is seen (×3,500). *Inset:* Section of capillary in continuity with the area occupied by the intravascular neoplastic nodule. The lumen is obstructed by platelets, granulocytes, and erythrocytes and possibly fibrin (*arrows*) as a result of the circulatory stasis (×7,500)

Fig. 16. Projections of K-562 cells appear amidst necrotic cells of the pulmonary interstitial tissue (×10,000). *Inset:* K-562 cell in contact with collagen of the interstitial tissue of the lung (×28,000)

Fig. 17. An alveolar cell appears compressed by two K-562 cells advancing into the interstitial pulmonary tissue (×7,500)



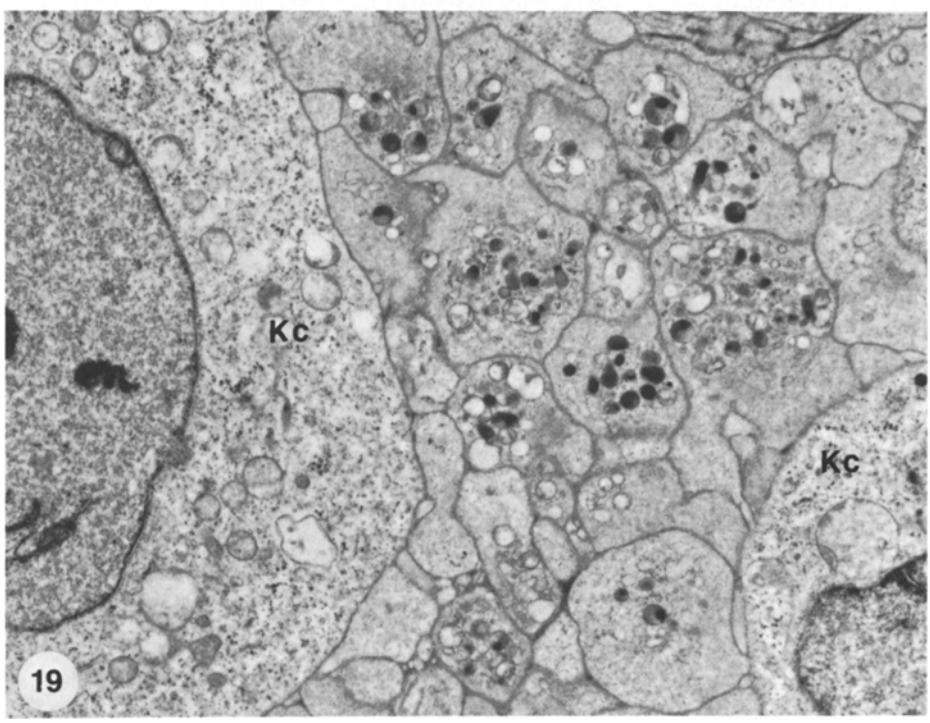


Fig. 18. K-562 cells proliferating in the pulmonary extravascular compartment display a sarcomatous pattern. Newly formed capillaries and fibroblasts form the stroma of the neoplastic growth (× 6,000)

Fig. 19. Aggregate of partially degranulated platelets surrounding K-562 cells 5 min after iv injection. The neoplastic cells show swelling and disruption of mitochondria (× 9,000)

fibrin are important factors affecting the arrest and fixation of neoplastic cells. However, platelet and fibrin accumulations in human neoplasias – the bottom line for any experimental model – are more probably thrombotic complications arising from endothelial injury rather than from the specific characteristics of neoplastic growth. Winterbauer et al. (1968) found that only 20% of 366 patients with metastases of various types of malignant tumors also had associated pulmonary thrombosis and tumor emboli. Furthermore, fibrin deposits, when present, appeared in the central part of the neoplastic emboli and thus were probably not a causal factor in tumor cell arrest.

The failure to produce disseminated neoplastic growth by iv injection of K-562 cells despite the induction of large aggregates of platelets indicates that factors other than the coagulation system were important for the arrest and proliferation of neoplastic cells. It must be pointed out that most experiments on the formation of metastases have been done iv injection of neoplastic cells, a procedure that reproduces only the implantation phase of metastatic spread (Foulds 1969; Weiss 1980). Besides, since that system permits the sudden entrance into the host's bloodstream of a large number of neoplastic cells, it is hardly comparable with the slow and continuous cell release that occurs in "spontaneous" malignant tumors. The coating of the cells by the host's plasma proteins resulting from the iv injection is certainly important because this event alone may trigger platelet adhesion (Vroman et al. 1977). Transient intravascular aggregates of platelets and fibrin may be induced in the lungs of nude mice by iv injection with either malignant neoplastic cell lines, normal human lymphocytes, or inert latex particles (Machado et al. unpublished results).

The mechanism of extravasation of neoplastic cells is also a matter of controversy. Experimental studies made by light and electron microscopy and microcinematography led some investigators (Ludatscher et al. 1967; Sindelar et al. 1975; Warren et al. 1972; Wood 1958) to propose that malignant cells leave the vascular lumen by diapedesis in a fashion similar to that of nomal blood cells; other have demonstrated a transendothelial passage (Dingemans et al. 1978) or a direct destruction of the vascular wall by neoplastic cells (Baserga et al. 1955; Chew et al. 1976; Locker et al. 1970). Diapedesis of isolated malignant cells and their successful proliferation in the extravascular compartment could conceivably be the exception rather than the rule in human oncology (Willis 1952). In contrast, vascular destruction from within by proliferating neoplastic cells is frequently observed in early developmental stages of metastasis of human neoplasias.

Based on our results, we can exclude diapedesis as a mechanism of extravasation of K-562 cells. In fact, the intravascular proliferation of the neoplastic cells produced a marked dilation of the pulmonary capillaries and compression and piercing of the endothelial cells. Under these conditions, hypoxia of the vascular wall and interference by the neoplastic cells with the replacement of cells shed during the turnover of the endothelium (Sherwin 1976) were likely to occur. As a consequence, the neoplastic cells adhered to the vascular basement membrane. The displacement of the endothelium by neoplastic cells may be responsible for the basement membrane destruction because endothelial cells are involved in the biosynthesis and deposition of that vascular lamina (Murphy

and Carlson 1978; Rhodin 1968). We have already pointed out that signs of lytic activity by the K-562 cells were not observed.

Cytoplasmic extensions interdigitated, holding the intravascular K-562 cells in compact, nodule arrangements. This close linking between the proliferating malignant cells is more amenable to an extravasation in toto by endothelial attrition and alteration of the basement membrane than it is to the diapedesis of isolated tumor cells. The disruption of the capillary wall may allow the plasma (which is the milieu in which the blood-borne malignant cells had survived) to flow into the extravascular compartment. This could provide the neoplastic cells a receptive environment until the neovascularization is established.

Although mechanical and haemodynamic phenomena may be influential in the arrest of tumor cells, other factors must play a role in this complex process (DeWys 1972). Among those factors, a specific fixation of K-562 cells to the endothelium and basement membrane cannot be ruled out with the information available.

Obviously, comparison of data from experimental models should be made with caution as conditions differ. However, our results and those of other authors demonstrate that alternative (and not mutually exclusive) pathways of arrest and extravasation of neoplastic cells exist. In fact, although neoplastic growths share certain characteristics, individual tumors display distinctive behavior. Therefore, the results of a particular experimental system do not warrant the formulation of dogmatic conclusions on the mechanism(s) of neoplastic dissemination and, do not permit extrapolation without critical appraisal. We feel that new, compromise models, such as the one reported here, must be developed for other malignant cells of human origin. Neither in vitro experiments nor iv injections of murine neoplastic cells will provide all the information needed to elucidate the multiple events occurring during malignant cell spread. Furthermore, the validity of experimental results must always be evaluated by comparison with the information afforded by human pathology of cancer.

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