Human Breast Carcinoma: Fibrin Deposits and Desmoplasia. Inflammatory Cell Type and Distribution. Microvasculature and Infarction [1, 2, 3]

Harold F. Dvorak, [4] **G. Richard Dickersin,** [5] **Ann M. Dvorak,** [4] **Eleanor J. Manseau,** [4] *and* **Kathryn Pyne** [4]

ABSTRACT—Study of 14 human infiltrating breast carcinomas revealed new features that shed light on the pathogenesis of tumor stroma formation and on host immunologic defense mechanisms. Fibrin deposits were observed in the stroma of all tumors, particularly at their growing edge. Fibrin may have contributed both to tumor angiogenesis and, with organization, to the formation of the fibrous stroma characteristic of these and other scirrhous carcinomas. We previously proposed similar mechanisms for several animal tumors. All breast carcinomas studied elicited some degree of lymphocytic response at the tumor periphery; lymphocytes penetrated the fibrous tumor stroma poorly, did not exit in significant numbers from central tumor vessels, and, even when greatly outnumbering tumor cells locally, appeared relatively ineffective at tumor cell killing. Microvascular endothelial cell damage was frequently observed and may have been responsible for zones of tumor infarction. Similar observations have been made in skin allografts and animal tumors where rejection was effected principally by microvascular damage and subsequent tissue infarction, not by lymphocyte contact with individual epithelial target cells.—JNCI 1981; 67:335-345.

Recent studies in guinea pigs have shown that two syngeneic hepatocarcinomas, line 1 and line 10, became enveloped by a cocoon-like fibrin gel within a few hours of implantation in the subcutaneous space (*1*). Further, this fibrin gel was apparently induced and modulated by tumor-secreted products acting in concert on extravasated plasma proteins (*2*). The tumor mediators thus far identified include a factor that increases microvascular permeability, a procoagulant, and a plasminogen activator, the last an enzyme activity secreted by many types of tumor cells (*2, 3*). The extensive fibrin gel characteristic of line 1 tumors became organized after about day 3 and was largely replaced by fibrous connective tissue, giving this tumor the appearance of many scirrhous carcinomas of human and experimental animal origin (*1*). Although line 1 tumors were eventually rejected, the mechanisms involved did not seem to depend on direct anatomic contacts between lymphocytes or macrophages and individual tumor cells. Rather, tumor rejection involved a process of infarction resulting from extensive and progressive microvascular injury. Immunologic rejection of first-set vascularized skin allografts has recently been shown to occur by a similar mechanism in several species including humans (*4*). In both tumor and skin allografts it was suggested on morphologic grounds that microvascular endothelial cell injury resulted from the activities of sensitized lymphocytes or their products (*1, 4, 5*).

The more malignant line 10 guinea pig hepatocarcinomas were also surrounded by fibrin deposits; however, these were considerably smaller than those observed about line 1 tumors, did not become organized, and remained relatively constant in size and disposition at the periphery as tumor growth progressed. Line 10 tumors developed little or no fibrous stroma.

Taken as a whole, these results suggested that the stroma of scirrhous tumors might arise from the organization of an abundant preexisting fibrin gel, that is, by a process analogous to wound healing; and that some of the necrosis commonly observed within many malignant tumors might result from microvascular injury that possibly was itself caused by a cellular immune response. To investigate these possibilities and to relate our guinea pig findings to human tumors, we studied a group of surgically excised primary infiltrating ductal breast carcinomas using the improved morphologic methods that had made possible the new findings in line 1 and 10 guinea pig tumors. Portions of this work have been reported in abstract form (*6*).

MATERIALS AND METHODS

Excisional biopsy specimens were obtained from 14 mammary carcinomas removed from 13 patients. (One patient had separate primary tumors in each breast.) Patients ranged in age from 39 to 86 years. Only tissue available for study within 15 minutes of surgical removal was included to ensure optimal fixation. The neoplasms ranged from 2 to 5 cm in diameter, and frozen sections and subsequent permanent hematoxylin and eosin stained sections established the diagnosis in all 14 tumors as infiltrating ductal carcinoma.

Paired, adjacent samples were cut from 10 of the tumors with a sharp razor blade, taking care to include the junctional zone between neoplasm and grossly

ABBREVIATION USED: IF = immunofluorescence.

[1] Received November 13, 1980; accepted March 4, 1981.

[2] Supported by Public Health Service grants CA28471 and CA28834 from the National Cancer Institute.

[3] Research procedures were in accord with the ethical standards of the Human Studies Committee, the Massachusetts General Hospital, and of the Helsinki Declaration of 1975.

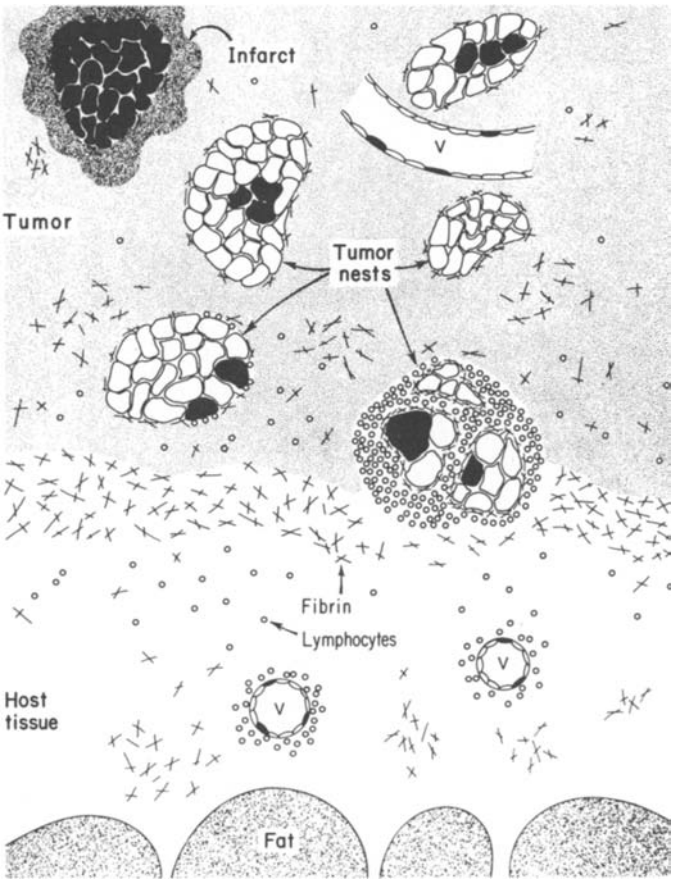
[4] Departments of Pathology, Beth Israel Hospital and Harvard Medical School; and the Charles A. Dana Research Institute, Beth Israel Hospital, Boston, Mass. 02215.

[5] Departments of Pathology, Massachusetts General Hospital and Harvard Medical School, Boston, Mass. 02114.

normal breast tissue. The first member of the pair consisted of a wedge of tissue measuring approximately 1.0-1.5 cm^2 and 0.2-0.3 cm thick. This was immediately frozen for IF studies. The second piece of the paired samples, and the only piece taken in 4 cases, was a strip of tissue of similar dimensions that was placed immediately in dilute Karnovsky's fixative (*7*) at room temperature. After about 5 minutes, the stiffened tissue was bisected longitudinally, and both halves were reimmersed in fixative for an additional 5 hours at room temperature and subsequently transferred to 0.1 *M* cacodylate buffer (pH 7.4) at 4°C. After trimming, tissue included approximately 4-7 mm of neoplasm, the tumor-benign tissue junction, and several millimeters of benign breast tissue, all in continuity. One piece was then processed for giant 1-μm Epon sections, Giemsa staining, and light microscopy; the other half was minced into smaller pieces and processed for electron microscopy (*1*).

Fibroadenomas were obtained from 5 patients, and normal or fibrocystic breast tissue was obtained from 5 others. These biopsies were processed for IF and for 1-μm sections in the same manner as the carcinomas.

The samples frozen for IF were sectioned at 4 μm and stained with fluoresceinated rabbit antiserum to human fibrinogen, γ-globulin, albumin, and the third component of complement (C3) (*1*). In all instances, one section was stained with toluidine blue for study by conventional light microscopy in parallel with IF. The activity and specificity of the antisera were substantiated as previously described (*8, 9*). Antifibrinogen antibodies have specificity for fibrinogen, fibrin, and certain large degradation products of both fibrin and fibrinogen. Therefore, the antigens detected in tissue sections with this antiserum are referred to collectively as "Fib," even though, as discussed elsewhere (*8, 9*), fibrillar deposits in tissue that bind this antiserum almost certainly represent fibrin. Absorption of anti-Fib sera with purified fibronectin or human serum linked to agarose beads did not affect staining; however, absorption with a washed fibrin clot removed all anti-Fib staining.



TEXT-FIGURE 1.—Schematic diagram summarizing newly recognized features of human breast tumors. Fibrin deposits are maximal at the tumor-host junction and surround individual peripherally disposed tumor cell nests. Less fibrin is observed in more central portions of the tumor. Vessels (V) of benign breast tissue are cuffed by lymphocytes, whereas tumor-penetrating vessels are not. Lymphocytes penetrate only for short distances into the scirrhous tumor stroma (*stippled area*) but may envelop peripheral tumor cell clumps in high lymphocyte:tumor cell ratios. Some tumor cells (*blackened*) are necrotic. Of these, some are associated with lymphocytes, whereas others lie in the centers of tumor nests well away from contacts with infiltrating cells. Entire tumor nests and surrounding collagen may undergo infarction. Tumor infarction may be secondary to microvascular endothelial cell damage (*blackened endothelial cells*).

RESULTS

Infiltration of Ductal Breast Carcinomas

In agreement with many previous histologic descriptions of this common tumor (*10-12*), all carcinomas in this study consisted of variably sized groupings of anaplastic epithelial cells separated by fibrous stroma (fig. 1A). Because of the manner in which our sections were oriented, we were able to distinguish peripheral from more central portions of each tumor and to concentrate our attention on the tumor-host junction (figs. 2 and 3). A number of new features were recognized and are illustrated schematically in text-figure 1.

Fibrin deposits.—These were present in all 14 tumors studied. Fibrin could be identified with difficulty in Giemsa-stained, Epon sections in one-half of the tumors. However, as for experimental line 1 tumors that had progressed to the scirrhous stage (*1*), even abundant fibrin deposits were difficult to visualize in 1-μm Epon sections when embedded in fibrous connective tissue, and they were easily missed. Fibrin was reliably identified by electron microscopy (fig. 1B) by virtue of its fibrillar structure and characteristic periodicity (*13*), but electron microscopy was not useful as a screening procedure because of the extensive sampling problems involved. IF (fig. 2) with highly specific antisera provided the simplest and most sensitive approach for reliable identification and rough quantitation of Fib in breast tumors and was positive in all of the 10 carcinomas in which it was applied. However, as noted above, we cannot be certain that all Fib staining represents cross-linked fibrin.

Fib was most abundant in the loose connective tissue at the tumor-host interface and in the immediately surrounding benign breast tissue (fig. 2A). It appeared as a fibrillar meshwork inserted between bundles of collagen and fat, independent of the extent of lymphocyte infiltration (*see* below). Although sometimes traversed by Fib strands, areas of lymphocyte infiltration were largely free of Fib deposits (figs. 2B and 2C). Peripherally situated tumor cell nests were often enveloped by a prominent rim of Fib that separated them from the surrounding connective tissue (fig. 2D). Sometimes, Fib strands also penetrated into tumor nests and surrounded smaller groups of tumor cells (fig. 2E). Small peripheral tumor clumps that were surrounded by numerous lymphoid cells were often enveloped by particularly prominent bands of Fib-staining material that separated them from the lymphocytes (figs. 2E, 2F).

Fib staining was less extensive in more central portions of the tumor. The Fib deposits surrounding tumor clumps were less abundant, more discontinuous, and were often lacking altogether. However, small patchy Fib deposits remained scattered in the abundant fibrous stroma.

IF staining with antibodies directed at albumin and C3 were negative; variable numbers of inflammatory cells were stained with antibodies to immunoglobulin, as have been described by others in many different tumors (*14*).

Inflammatory cells:Relation to tumor and blood vessels.—It has been known for many years that inflammatory cells commonly appear in association with breast carcinomas and other malignant tumors, and there is disagreement in the literature as to whether such infiltrates lead to a better clinical prognosis (*12, 15-17*). The techniques used here permitted a better characterization of the types and precise localization of infiltrating cells (figs. 3 and 4).

The vast majority of inflammatory cells were lymphocytes, but macrophages, plasma cells, and sometimes neutrophils were also present in small numbers. Basophils and eosinophils were rarely encountered, but mast cell hyperplasia was present in 11 of 14 tumors (*18, 19*) (figs. 3C and 4B). The inflammatory infiltrate was largely confined to the tumor-benign breast tissue interface. Inflammatory cells tended to be concentrated about small veins and venules and rarely penetrated the tumor by more than 1 mm in any significant numbers. Only occasional lymphocytes and macrophages were found in more central portions of any of the 14 carcinomas studied. Although contacts between such inflammatory cells and tumor cells did occur, they were infrequent and involved less than 1% of tumor cells. In two cases inflammatory cells at the tumor-benign breast junction were particularly abundant and formed the principal milieu enveloping nests of peripheral tumor cells (figs. 2E and 3D). Cell counts revealed local lymphocyte-to-tumor cell ratios of 25:1 or higher. In both cases, lymphocytes formed numerous anatomic contact with tumor cells nests, and a few of these tumor cells (generally, <10%) appeared damaged or frankly necrotic (fig. 3D).

Lymphocytes not uncommonly enveloped individual nonmalignant mammary ducts in the benign breast tissue peripheral to the carcinoma and sometimes did so with an intensity that matched the infiltration about malignant tumor cells. Such lymphocytic accumulations are, of course, not uncommon in fibrocystic disease and other benign breast lesions. Thus elicitation of a mononuclear cell infiltrate cannot be considered specific to carcinomas.

Tumor blood vessels.—An important negative finding was the observation that tumor blood vessels (i.e., vessels traversing the tumor stroma and supplying central portions of the tumor mass) were seldom surrounded by lymphocytes, and then only in small numbers. This is in striking contrast to similar sized venules at the tumor periphery (figs. 3B-D, 4A and 4C) that regularly supported extensive inflammatory cell diapedesis and that were characterized by cuffs of enveloping lymphocytes.

The small blood vessels within and immediately adjacent to breast tumors underwent a number of intrinsic changes (figs. 3D, 4A-4D). These were of four types: *a*) hypertrophy (activation) of both endothelial cells and pericytes; *b*) endothelial cell division (hyperplasia); *c*) injury to or frank necrosis of endothelial cells; and *d*) basal lamina alterations, including regular and irregular duplication, deposition of debris, and nodular thickening. The hypertrophy and hyperplasia observed are consistent with a growing microvasculature, and similar types of endothelial cell and pericyte changes (enlarged cell size, large open nucleus with dispersed chromatin, increased cytoplasmic mass with numerous organelles, and cell division) may be observed, for example, in healing wounds (*20, 21*). Unlike healing wounds, however, individual endothelial cells of tumor vessels commonly showed evidence of injury, and it was not uncommon to observe entire vessel profiles lined by damaged or frankly necrotic endothelial cells. Even when extensive, endothelial injury was seldom associated with luminal thrombosis. Both vessels at the tumor periphery and also more central vessels that penetrated the tumor stroma were affected. Direct anatomic contacts were not observed between any type of inflammatory cell and damaged endothelium. However, lymphocytes were present in vessel lumina. They were also observed within the walls of small vessels at the tumor edge and presumably represented cells in the process of diapedesis. The lymphocyte cuffs enveloping peripheral vessels were separated from endothelial cells by basal lamina and often by pericytes as well. Injury to pericytes was not observed.

Tumor necrosis.—While tumor cell injury associated with lymphocyte or other inflammatory cell contact was uncommonly seen, some degree of tumor necrosis, apparently independent of lymphocyte associations, was observed in all 14 tumors studied (fig. 5) (*22, 23*). In its mildest form, individual cells in the centers of

tumor nests, the cells farthest removed from the blood supply, exhibited signs of injury, including vacuolization, cytoplasmic swelling and lucency, and frank necrosis. Neutrophils were sometimes observed at sites of individual tumor cell necrosis. In other instances, entire nests of tumor cells, or several adjacent nests and the intervening stroma, appeared necrotic, often accompanied by focal microhemorrhages. This tumor cell necrosis was of the "coagulative" type in that anatomic landmarks (i.e., distinction between stroma and tumor nests) and profiles of individual tumor cells were preserved for a considerable period after cell death. Indeed, this pattern persisted until macrophages invaded the necrotic areas to phagocytose the debris (figs. 5B–5D).

Benign Breast Lesions

For comparison with intraductal carcinomas, 10 biopsies of benign breast tissue were also examined (2 normal, 3 fibrocystic disease, 5 fibroadenomas). The microscopic features of these biopsies, as studied in 1-μm Epon sections, generally corresponded to those of classic descriptions. IF staining for Fib was negative in normal breast tissue and also in fibrocystic disease. However, moderate staining for Fib was observed in 4 of the 5 fibroadenomas studied. Patchy Fib deposits analogous to those described in ductal carcinomas were observed at the tumor-breast junction and to a lesser extent in the mature fibrous connective tissue of the tumor stroma. Staining with the other antisera was negative. In the single fibroadenoma that was Fib-negative, the stroma was almost completely hyalinized, and epithelial elements were infrequent, suggesting that the tumor had ceased to grow.

DISCUSSION

The fibrin found in the stroma of human breast carcinomas, as that associated with guinea pig line 1 and 10 tumors, most probably arises from the extravasation and coagulation of plasma fibrinogen (*1*, *2*). The pathogenesis of these events is uncertain, but at least three possibilities may be considered: *a*) Guinea pig tumor cells, both in vivo and in short-term cultures, release biologically active mediators that enhance vascular permeability (allowing transudation of fibrinogen and other plasma proteins) and that trigger coagulation (*2*). Whether human breast cancer cells secrete similar mediators has not yet been determined. *b*) Increased microvascular permeability and fibrin deposition are regular components of delayed type hypersensitivity reactions in both humans and experimental animals (*5*, *19*). The lymphocyte-rich infiltrate concentrated at the periphery of breast cancers may represent the expression of delayed type hypersensitivity and could be involved in the fibrin deposition associated with these tumors. That this could provide the entire explanation is unlikely in view of the paucity of lymphocytes about fibroadenomas which nonetheless had fibrin deposits. *c*) Local synthesis of fibrinogen by tumor or by stromal cells is a theoretical possibility, though unlikely in our view.

Whatever the mechanism, our data indicate that fibrin deposition is a factor that must be reckoned with in attempting to understand the biology of human breast tumors. As a component of most types of tissue injury, fibrin deposition is thought to be involved in the as yet poorly defined pathways leading to wound healing (*24*). Wound healing involves both angiogenesis and fibroplasia, i.e., the production of granulation tissue followed eventally by scar formation. In guinea pigs, implants of fibrin alone led to angiogenesis (*25*) equivalent to that induced by tumor cells in both quantity and kinetics (*1*). Given these circumstances, fibrin itself (*26*), or some product associated with fibrin production or catabolism, may account for tumor angiogenesis. Indeed, the possibility that the tumor angiogenesis factor described by Folkman (*27*) acts by enhancing vessel permeability and activating the clotting system deserves serious investigation (*28*).

Given these considerations, "organization" of the abundant fibrin deposits found at the periphery of breast tumors into fibrous connective tissue may explain the pathogenesis of tumor desmoplasia. Data from guinea pig tumors are consistent with this possibility. The line 1 guinea pig carcinoma is enveloped at early intervals by an abundant fibrin gel that is replaced after a few days by fibrous connective tissue as the tumor assumes a scirrhous character (*1*). In contrast, the line 10 carcinoma, associated with little fibrin gel, acquires little fibrous stroma (*1*). Also consistent with this hypothesis is the finding of fibrin deposits in fibroadenomas, benign tumors comprised of epithelial tumors cells and abundant fibrous stroma. Taken together, these data suggest that the scirrhous stroma characteristic of many human and animal tumors, both benign and malignant, may arise from the organization of a preexisting fibrin gel. They further predict that, other things being equal, the extent of fibrin gel deposition around tumors, and the persistence of that gel in the face of antagonistic tumor and host enzymes (e.g., plasminogen activator), may be proportional to the amount of fibrous connective tissue that eventually replaces the fibrin. Thus our data predict that scirrhous tumors are those that lay down and maintain an abundant fibrin gel that can subsequently be organized by fibrous stroma as in normal wound healing.

An alternate possibility has been suggested, namely, that breast tumors provide their own scirrhous stroma by synthesizing collagen and elastin (*29–32*). However, subsequent work has shown that breast tumor cells synthesize basement membrane collagen (type IV), whereas breast tumor stroma, as scar tissue in wound healing, is comprised largely of type I collagen [(*33*) and Liotta LA: Personal communication].

Although inflammatory cells, mostly lymphocytes, were abundant, at least focally, at the tumor-benign tissue interface in 10 of our 14 cases, relatively few penetrated the scirrhous tumor stroma to any significant extent. Moreover, even in cases where significant

numbers of inflammatory cells enveloped peripheral tumor cell nests (figs. 2E, 3D, and 4A), the extent of tumor cell killing observed was modest and the vast majority of tumor cells appeared to be undamaged. Various possibilities have been invoked to explain the failure of the immune response to rid the host of tumor (*34-36*), but none of these are proved as yet. Alternatively, the data may indicate that, as in first-set skin allograft rejection (*4*), lymphocyte contact-mediated killing of graft epithelium is a relatively slow and inefficient process and that even this meagre defense is inoperative in more central and scirrhous portions of tumor where lymphocytes penetrate with difficulty.

Related to this discussion is a negative finding: the relative failure of vessels that penetrate tumor to support diapadesis of lymphocytes. By contrast, lymphocytes and other inflammatory cells regularly formed aggregates about small vessels at the tumor-benign breast junction.

Thus the host may be thrice disadvantaged in its immunologic defenses against breast tumors. Lymphocytes that have emigrated from vessels at the tumor-host junction appear to have only a limited capacity to kill tumor cells despite vast local numerical superiority; lymphocytes find great difficulty in penetrating the fibrous tumor stroma; and finally lymphocytes seem unable to emigrate from blood vessels that do traverse tumor. Acting in concert, these factors could effectively protect tumors from host defenses, creating for the tumor de facto an immunologically privileged site within a host that may be otherwise immunologically competent.

The endothelial cell injury and necrosis affecting tumor and interface blood vessels are identical to changes previously described in delayed type hypersensitivity skin reactions (*5, 19, 37*), in first-set human skin allograft rejection (*4*), and in the immunologic rejection of the line 1 guinea pig hepatocarcinoma (*1*). The mechanisms of vessel damage are unclear, but, by analogy with the other examples cited, may involve the activities of lymphocytes or certain of their products. This is particularly likely for junctional vessels cuffed by lymphocytes.

As noted above, necrosis of individual peripherally disposed tumor cells was associated with anatomic contacts with lymphocytes. However, a much more extensive form of necrosis affected entire tumor cell clumps and intervening stroma. Common to many malignant tumors, this necrosis was of the "coagulative" type that is generally associated with ischemic injury (*29*). Coagulation tumor necrosis developed without apparent anatomic contacts between tumor and inflammatory cells and closely resembled the infarct-like patterns occuring in rejecting first-set skin allografts (*4*) and vascularized line 1 tumors (*1*). Perhaps this tumor necrosis developed on an ischemic basis, secondary to the microvascular injury described above. Indeed, others have also hypothesized a vascular mechanism of necrosis in breast caarcinomas but with routine histology were not able to find a vascular lesion (*22, 23, 38*). Others have also described tumor injury and rejection in the absence of anatomic contacts between inflammatory and tumor cells (*39, 40*).

Recently, an abundant literature has emphasized the role of macrophages in tumor rejection (*41-44*). On the basis of studies of cells isolated from tumor homogenates, several authors have suggested that human and animal tumors are heavily infiltrated by macrophages. In contrast, we found relatively few macrophages in our series of breast tumors, except about sites of preexisting coagulative tumor necrosis. Here macrophages accumulated in large numbers and phagocytozed cell debris. We therefore urge caution in drawing conclusions about the importance and frequency of macrophages in host tumor defenses based on approaches that do not preserve normal intercellular relationships (*45*). We do agree, however, that macrophages, in contrast to lymphocytes, can penetrate dense tumor stroma, a property that may be related to their abundant proteases.

REFERENCES

(*1*) DVORAK HF, DVORAK AM, MANSEAU EJ, WIBERG L, CHURCHILL WH. Fibrin gel investment associated with line 1 and line 10 solid tumor growth, angiogenesis, and fibroplasia in guinea pigs. Role of cellular immunity, myofibroblasts, microvascular damage, and infarction in line 1 tumor regression. JNCI 1979; 62:1459-1472.

(*2*) DVORAK HF, ORENSTEIN NS, CARVALHO AC, et al. Induction of a fibrin-gel investment: An early event in line 10 hepatocarcinoma growth mediated by tumor-secreted products. J Immunol 1979; 122:166-174.

(*3*) REICH E. Plasminogen activator: Secretion by neoplastic cells and macrophages. In: Reich E, Rifkin DB, Shaw E, eds. Proteases and biological control. New York: Cold Spring Harbor Press, 1975:333-341.

(*4*) DVORAK HF, MIHM MC JR, DVORAK AM, BARNES BA, MANSEAU EJ, GALLI SJ. Rejection of first-set skin allografts in man. The microvasculature is the critical target of the immune response. J Exp Med 1979; 150:322-337.

(*5*) DVORAK HF, GALLI SJ, DVORAK AM. Expression of cell-mediated hypersensitivity in vivo—recent advances. Int Rev Exp Pathol 1980; 21:119-194.

(*6*) DICKERSIN GD, MANSEAU EJ, DVORAK AM, DVORAK HF. Fibrinous/fibrous stroma in human breast carcinoma: Possible role in tumor protection from cell mediated immunity. Lab Invest 1980; 42:112.

(*7*) KARNOVSKY MJ. The ultrastructural basis of capillary permeability studied with peroxidase as a tracer. J Cell Biol 1967; 35:213.

(*8*) COLVIN RB, JOHNSON RA, MIHM MC JR, DVORAK HF. Role of the clotting system in cell-mediated hypersensitivity. J Exp Med 1973; 138:686-698.

(*9*) COLVIN RB, DVORAK HF. Fibrinogen/fibrin on the surface of macrophages: Detection, distribution, binding requirement, and possible role in macrophage adherence phenomena. J Exp Med 1975; 142:1377-1390.

(*10*) MCDIVITT RW, STEWART FW, BERG JW. Tumors of the breast. Atlas of tumor pathology. Washington, D.C.: Armed Forces Institute of Pathology, 1968.

(*11*) FISHER ER, GREGORIO RM, FISHER B. The pathology of invasive breast cancer. A syllabus derived from the findings of the National Surgical Adjuvant Breast Project (protocol 4). Cancer 1975; 36:1-85.

(*12*) TREMBLAY G. Stromal aspects of breast carcinoma. Exp Mol Pathol 1979; 31:248-260.

(*13*) MCKAY DG, MARGARETTEN W. The role of the platelet in DIC. In: Brinkhous KM, Shermer RW, Mostofi FK, eds. The platelet. Baltimore: Williams & Wilkins, 1971:273-284.

(*14*) WITZ IP. Tumor-bound immunoglobulins: In situ expressions

of humoral immunity. Adv Cancer Res 1977; 25:95-148.
(15) BLACK MM, OPLER SR, SPEER FD. Microscopic structure of gastric carcinomas and their regional lymph nodes in relation to survival. Surg Gynecol Obstet 1954; 98:725-734.
(16) IOACHIM HL. Guest editorial: The stroma reaction of tumors: An expression of immune surveillance. J Natl Cancer Inst 1976; 57:465-475.
(17) HAMLIN IM. Possible host resistance in carcinoma of the breast: A histological study. Br J Cancer 1968; 22:383-401.
(18) FISHER ER, FISHER B. Role of mast cells in tumor growth. Arch Pathol 1965; 79:185-191.
(19) DVORAK HF, MIHM MC JR, DVORAK AM, et al. Morphology of delayed type hypersensitivity reactions in man. I. Quantitative description of the inflammatory response. Lab Invest 1974; 31: 111-130.
(20) VRACKO R. Basal lamina scaffold—anatomy and significance for maintainance of orderly tissue structure. A review. Am J Pathol 1974; 77:314-346.
(21) PAPADIMITRIOU JM, WOODS AE. Structural and functional characteristics of the microcirculation in neoplasms. J Pathol 1975; 116:65-72.
(22) COOPER EH, BEDFORD AJ, KENNY TE. Cell death in normal and malignant tissues. Adv Cancer Res 1975; 21:59-120.
(23) FISHER ER, PALEKAR AS, GREGORIO RM, REDMOND C, FISHER B. Pathological findings from the national surgical adjuvant breast project (protocol No. 4). IV. Significance of tumor necrosis. Human Pathol 1978; 9:523-530.
(24) FLOREY HW, ed. General pathology. Philadelphia: Saunders, 1970.
(25) DUMONDE DC, GLYNN LE. The reaction of guinea pigs to autologous and heterologous fibrin implants. J Pathol Bacteriol 1965; 90:649-657.
(26) SHUBIK P, FELDMAN R, GARCIA H, WARREN BA. Vascularization induced in the cheek pouch of the Syrian hamster by tumor and nontumor substances. J Natl Cancer Inst 1976; 57:769-774.
(27) FOLKMAN J. Tumor angiogenesis. Adv Cancer Res 1974; 19:331-358.
(28) DVORAK HF, ORENSTEIN NS, DVORAK AM. Tumor secreted mediators and the tumor microenvironment: Relationship to immunologic surveillance. Lymphokine Rep 1981; 2:203-233.
(29) SHIVAS AA, MACKENZIE A. The origins of stromal reaction in breast carcinoma. J R Coll Surg Edinb 1974; 19:345-350.
(30) DOUGLAS JG, SHIVAS AA. The origins of elastica in breast carcinoma. J R Coll Surg Edinb 1974; 19:89-93.
(31) AL-ADNANI MS, KIRRANE JA, MCGEE JO'D. Inappropriate production of collagen and prolyl hydroxylase by human breast cancer cells in vivo. Br J Cancer 1975; 31:653-660.
(32) ROESEL RA, CUTRONEO KR, SCOTT DF, HOWARD EF. Collagen synthesis by cloned mouse mammary tumor cells. Cancer Res 1978; 38:3269-3275.
(33) LIOTTA LA, FOIDART JM, GEHRON ROBEY P, MARTIN GR, GUILLINE PM. Identification of micrometastases of breast carcinoma by presence of basement membrane collagens. Lancet 1979; 2:146-147.
(34) HENNEY CS. Mechanisms of tumor cell destruction. In: Green I, Cohen S, McCluskey RT, eds. Mechanisms of tumor immunity. New York: Wiley, 1977:55-86.
(35) PREHN RT. Guest editorial: Immunostimulation of the lymphodependent phase of neoplastic growth. J Natl Cancer Inst 1977; 59:1043-1049.
(36) JERRELLS TR, DEAN JH, RICHARDSON GL, MCCOY JL, HERBERMAN RB. Role of suppressor cells in depression of in vitro lymphoproliferative responses of lung cancer and breast cancer patients. JNCI 1978; 61:1001-1009.
(37) DVORAK AM, MIHM MC JR, DVORAK HF. Morphology of delayed-type hypersensitivity reactions in man. II. Ultrastructural alterations affecting the microvasculature and the tissue mast cells. Lab Invest 1976; 34:179-191.
(38) TANNOCK IF, STEEL GG. Quantitative techniques for study of the anatomy and function of small blood vessels in tumors. J Natl Cancer Inst 1969; 42:771-782.
(39) PERRY LL, BENACERRAF B, MCCLUSKEY RT, GREENE MI. Enhanced syngeneic tumor destruction by in vivo inhibition of suppressor T cells using anti-I-J alloantiserum. Am J Pathol 1978; 92:491-506.
(40) VAAGE J, GANDBHIR L. Local cellular responses associated with dormancy and regresion of a syngeneic C3H mammary carcinoma. Cancer Immunobiol Immunother 1978; 4:263-268.
(41) HASKILL JS, PROCTOR JW, YAMAMURA Y. Host responses within solid tumors. I. Monocytic effector cells within rat sarcomas. J Natl Cancer Inst 1975; 54:387-393.
(42) ALEXANDER P, ECCLES SA, GAUCI CL. The significance of macrophages in human and experimental tumors. Ann NY Acad Sci 1976; 276:124-133.
(43) WOOD GW, GOLLAHON KA. Detection and quantitation of macrophage infiltration into primary human tumors with the use of cell-surface markers. J Natl Cancer Inst 1977; 59:1081-1087.
(44) ADAMS DO, SNYDERMAN R. Guest editorial: Do macrophages destroy nascent tumors? JNCI 1979; 62:1341-1345.
(45) DVORAK HF, DVORAK AM. Inflammatory cells that infiltrate tumors: Identification and characterization by improved morphological and immunohistological methods. In: Haskill SJ, ed. Antitumor defense mechanisms: In vitro and in vivo. New York: Marcel Dekker. In press.

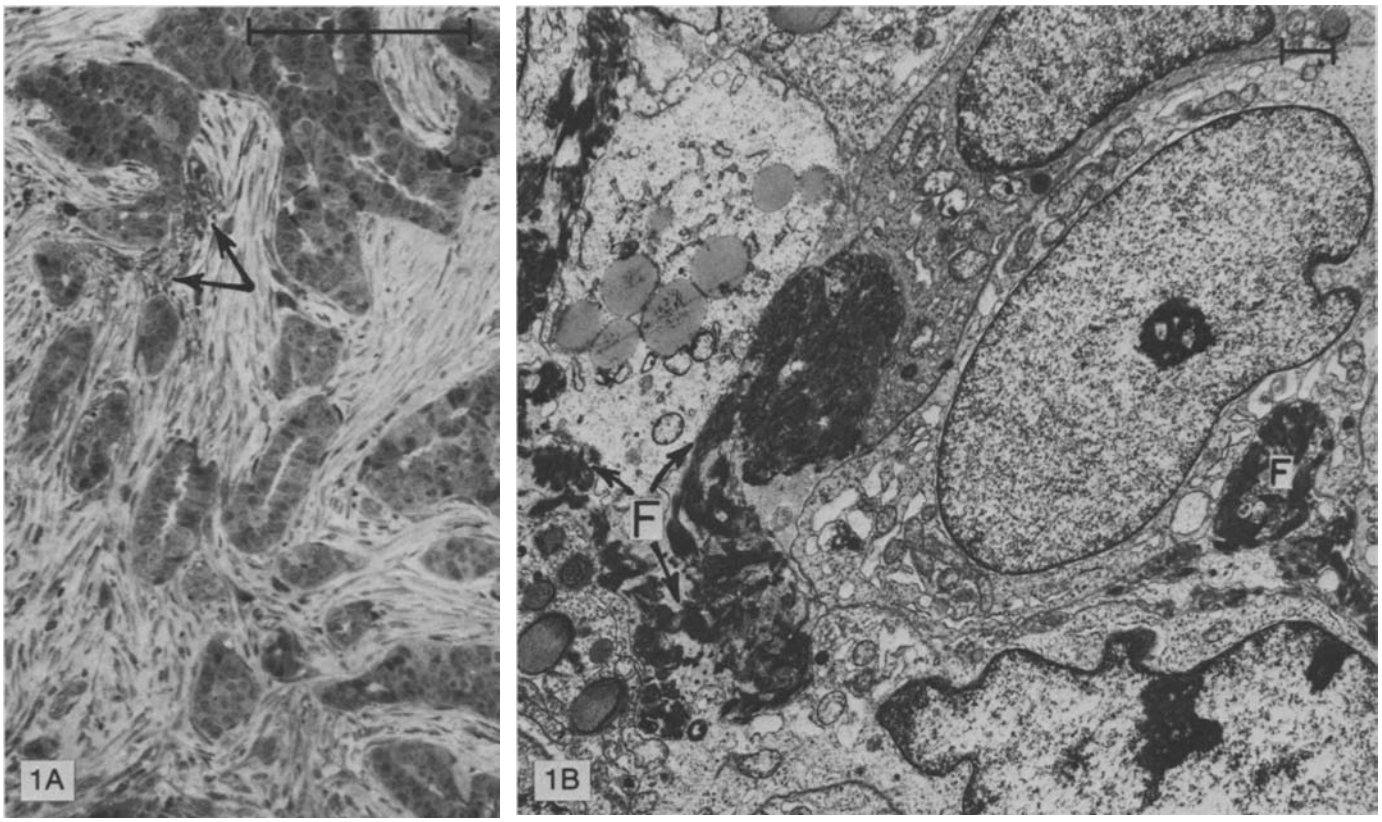


FIGURE 1.—1A) Typical intraductal breast carcinoma illustrating tumor cell nests and cords separated by scirrhous stroma in a 1-μm-thick Epon section. *Arrows* indicate focus of increased elastic tissue around a tumor nest. 1B) Electron micrograph illustrating fibrin deposits (F) between individual tumor cells. 1A) *Bar*=100 μm. × 250. 1B) *Bar*=1.0 μm. × 6,720

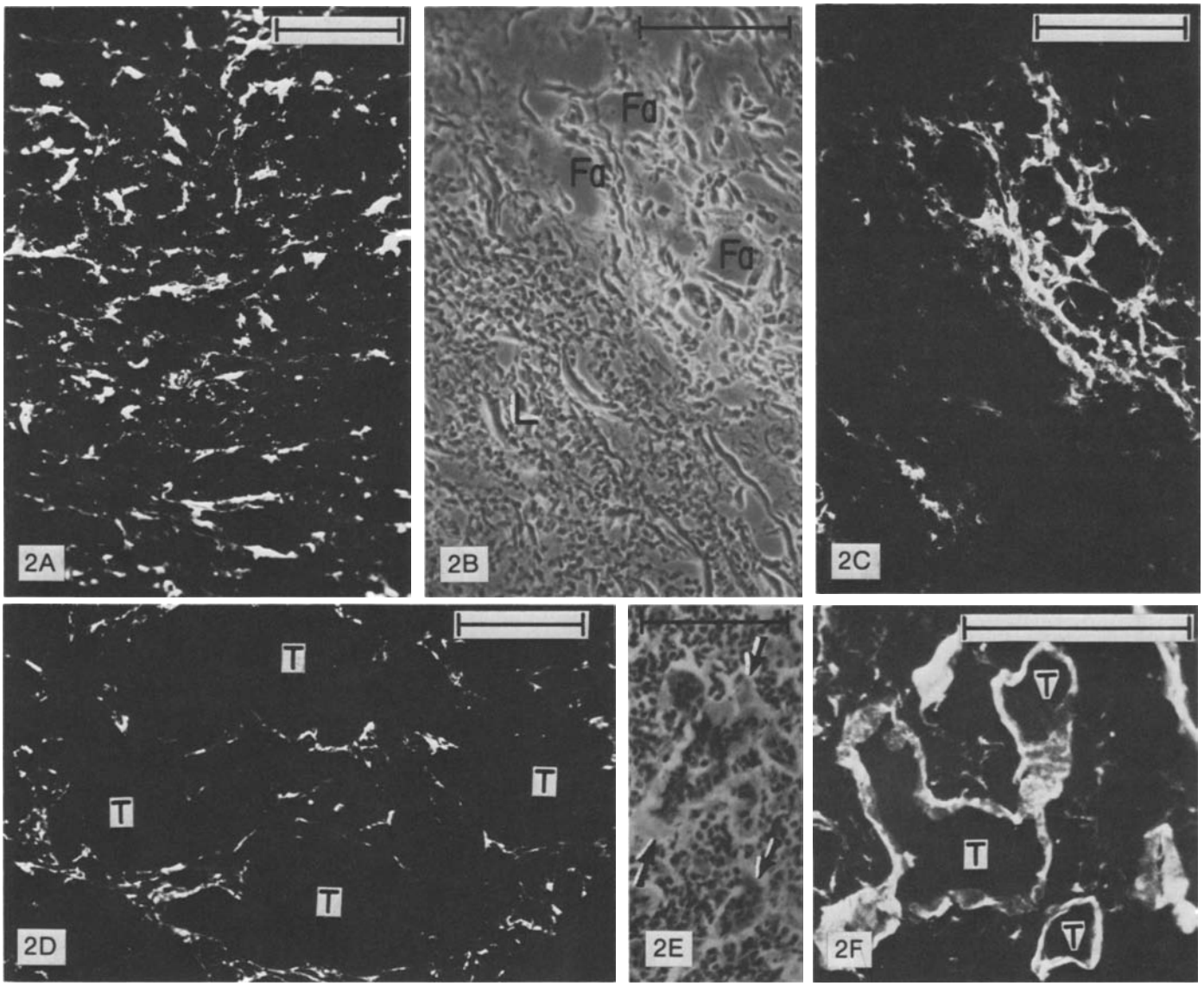
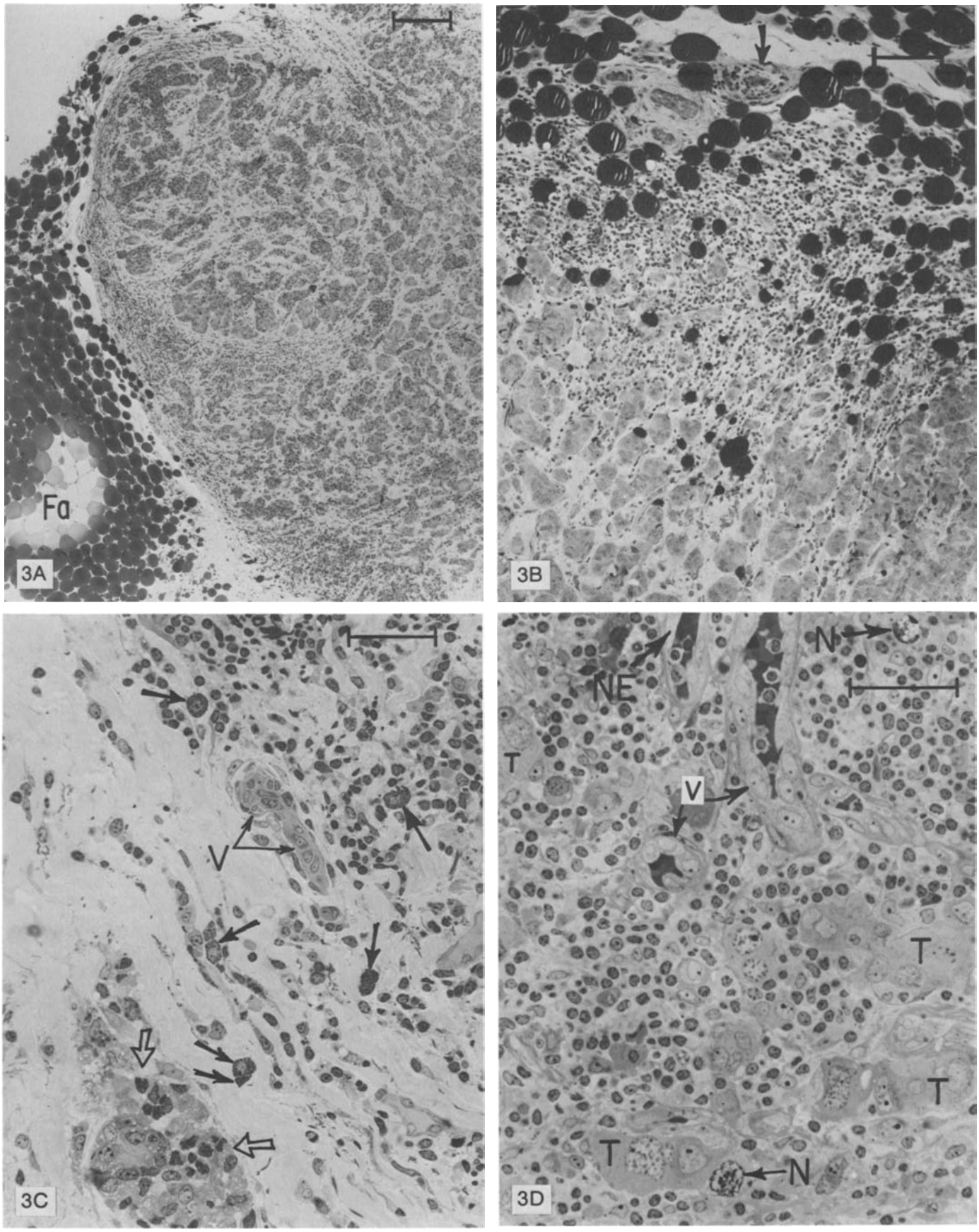


FIGURE 2.—Fib deposits in breast carcinomas recognized by IF. 2A) Abundant Fib between collagen bundles (*black areas*) in the loose connective tissue at the tumor-benign breast interface. *Bar*=100 μm. × 190. 2B and 2C) Phase-contrast (2B) and IF (2C) photomicrographs of the same field at edge of tumor, illustrating dense lymphocyte infiltrate (L) and loose fibrofatty connective tissue (*upper right*). Fib staining is largely confined to the connective tissue. Fa=fat cells (2B). *Bar*=100 μm. × 230. 2D) Fib deposits outlining nests of tumor cells (T). *Bar*=100 μm. × 190. 2E) Small peripheral tumor cell clumps (*arrows*) are separated from an extensive surrounding lymphocytic infiltrate by lightly staining material that by IF (2F) exhibits strongly positive Fib staining. Fig 2E represents a toluidine blue-stained frozen section from the same block as fig. 2F. 2E) *Bar*=100 μm. × 230. 2F) *Bar*=100 μm. × 350.

FIGURE 3.—Inflammatory cell infiltrate at tumor-benign breast interface. 3A) Lymphocytes at junction of tumor (*right*) with fat (Fa) of benign breast (black globules, *left*) infiltrate tumor patchily and irregularly for a short distance. *Bar*=200 μm. × 50. 3B) Higher magnification of lymphocyte infiltrates at tumor-benign tissue junction. Lymphocytes surround a small venule (*arrow*) and penetrate only a short distance into tumor below. Black globules represent fat cells of breast. *Bar*=100 μm. × 130. 3C) Increased mast cells (*solid arrows*) in the inflammatory infiltrate at tumor-benign breast junction. Focal interactions between mononuclear cells, mostly lymphocytes, and a tumor cell clump leading to focal tumor cell killing (*open arrows*). V=venule. *Bar*=50 μm. × 320. 3D) Small peripheral clumps of tumor cells (T) in a sea of inflammatory cells, mostly lymphocytes but including occasional macrophages and plasma cells. Occasional tumor cells appear necrotic (N), but most appear undamaged. Endothelium of small venules (V) exhibit hypertrophy and rarely in this field, necrosis (NE). All are 1-μm-thick Giemsa-stained Epon sections. *Bar*=50 μm. × 400



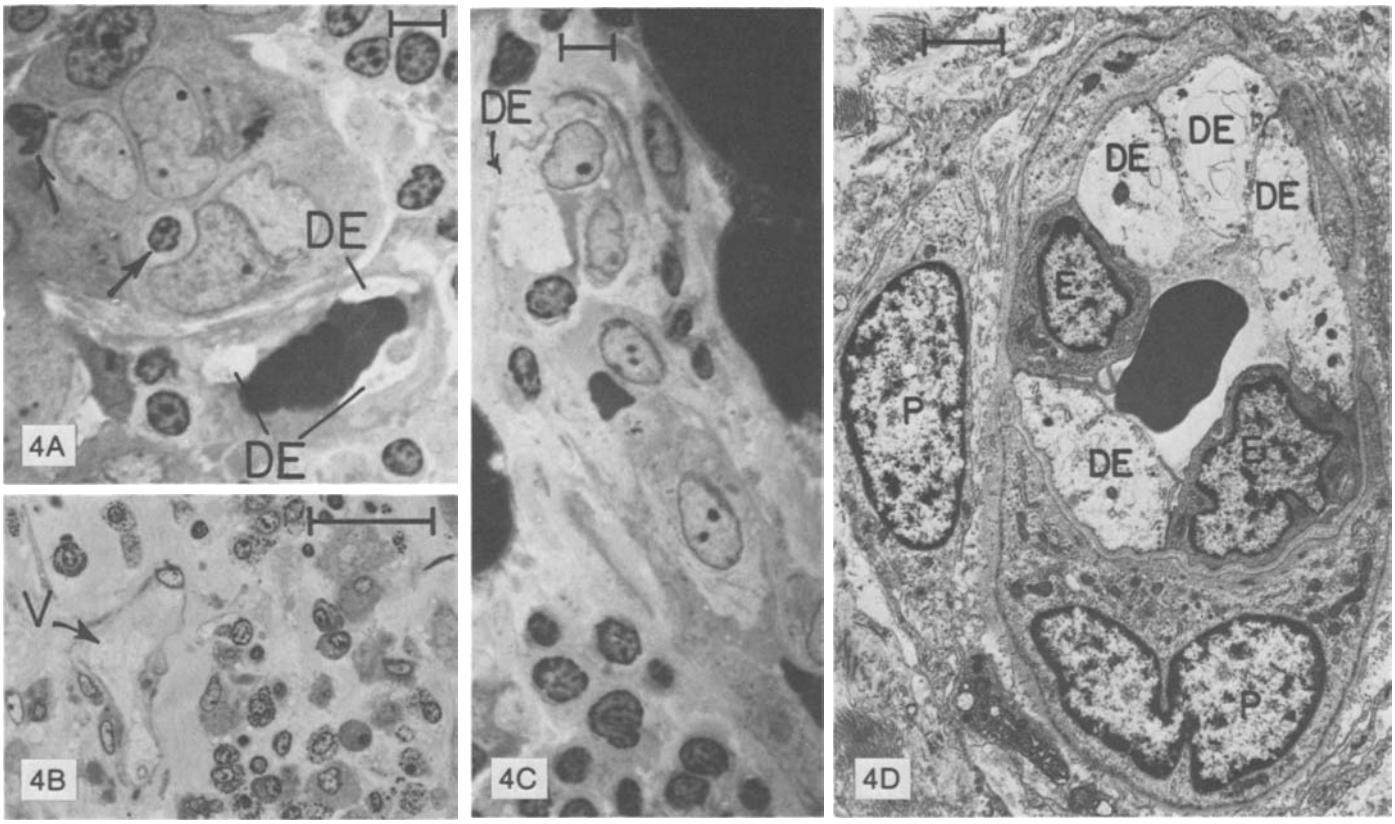


FIGURE 4.—Tumor microvasculature. Venules exhibit extensive damaged endothelium (DE), as evidenced by cytoplasmic lucency and in some instances swelling. *Note* normal appearances of tumor cells in 4A, despite close associations with lymphocytes (*arrows*). In 4B, *note* venule (V) with damaged endothelium and surrounding mast cell hyperplasia. In the electron micrograph (4D), four endothelial cells are damaged (DE), whereas immediately adjacent pericytes (P) and endothelial cells (E) are undamaged. 4A) *Bar*=10 μm. × 650. 4B) *Bar*=50 μm. × 320. 4C) *Bar*=10 μm. × 650. 4D) *Bar*=2 μm. × 5,000

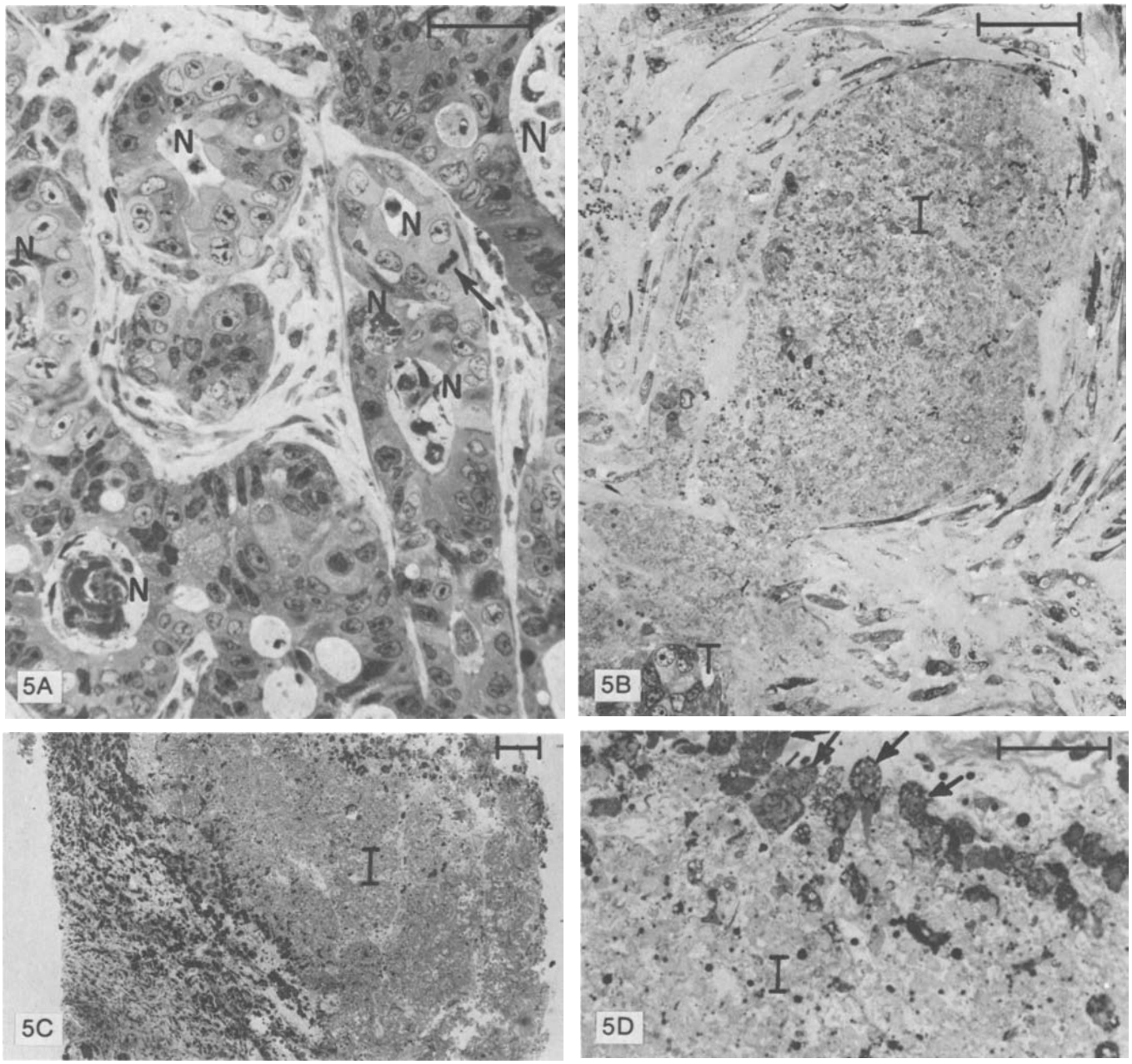


FIGURE 5.—Focal central tumor cell necrosis (N) in 5A progressing to infarction (I) in 5B), without associated inflammatory cell contact. *Note* that zone of infarction extends irregularly beyond tumor cells into surrounding fibrous connective tissue. 5A and 5B) *Bar*=50 μm. × 320. 5C) At later times, infarcted tumor (I) is surrounded by phagocytic macrophages, here staining black because their cytoplasm contains abundant phagocytized osmium-reactive lipid. *Bar*=100 μm. × 64. 5D) Higher magnification of 5C, illustrating lipid-filled macrophages (*arrows*) at edge of infarct (I). Outlines of individual necrotic tumor cells are still recognizable, a characteristic feature of coagulation necrosis. *Bar*= 2 μm. × 350. All are 1-μm Epon sections.