Tumor Induction by Plastic Films: Attempt to Correlate Carcinogenic Activity With Certain Physicochemical Properties of the Implant[1,2]

R. L. Carter, F. J. C. Roe, *and* Richard Peto,[3] *Chester Beatty Research Institute, Institute of Cancer Research, Royal Cancer Hospital, London, S.W.3., England; and The Department of the Regius Professor of Medicine, The Radcliffe Infirmary, Oxford, England*

SUMMARY—Forty-eight young-adult CB Wistar rats received a subcutaneous implant of 1 of 3 polyelectrolyte complex films of defined physicochemical properties: These films contained an excess of polyanions ('anionic films'), an excess of polycations ('cationic films'), or equal proportions of polyanions and polycations ('neutral films'). Incisions were made in the flanks of 16 control rats, but film was not inserted. Local tumors were found in each of the 3 test groups, but not in the control group. The incidence rate of local tumors was less for implants of neutral film than for implants of the 2 non-neutral films. In the two latter groups, the incidence rate of local tumors was greater in animals bearing implants of cationic films. Differences were also found in the local tissue responses evoked by the 3 types of plastic: General reactive changes were less marked in connective tissue capsules enclosing neutral films, and ectopic calcification was frequently found near anionic and cationic films, but not near neutral films. Some sarcomas associated with cationic and anionic films were osteogenic. The findings are discussed in relation to the general problem of tumor induction by implanted plastics.—J Nat Cancer Inst 46: 1277–1289, 1971.

INDUCTION of local tumors in experimental animals by various plastics has been repeatedly described. The mechanisms involved are, however, still obscure and the part played by physical as opposed to chemical factors remains controversial (*1*, *2*). A major difficulty is that the detailed chemical composition of many plastics is poorly documented, a situation made worse by the large numbers of additives and contaminants such materials may contain (*3*). Bryson and Bischoff (*2*) wrote recently that "much of the work purporting

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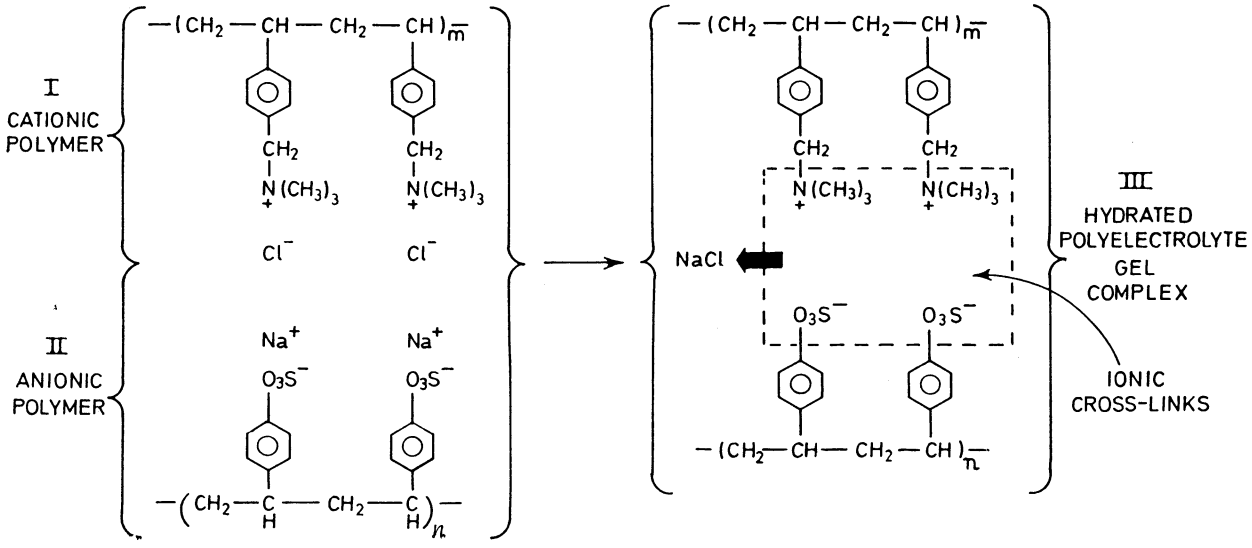
to study the phenomenon of solid-state carcinogenesis is conducted with substances ill-suited for such studies"; but the fact remains that several 'ill-suited' plastics are used in human medicine and their possible deleterious effects must somehow be elucidated. In the last few years, however, certain highly hydrated polymeric materials known as *polyelectrolyte complexes* have been introduced, for which rather detailed information concerning their chemical and physical properties is available (*4–6*). No published account of the carcinogenic effects of these substances is known to us and, in view of the detailed documentation of their physical and chemical properties, they seemed better suited than many other plastics for analysis of the tumor-inducing properties of this type of material. In the present account, the carcinogenic effects of 3 different polyelectrolyte complexes with some defined physicochemical properties are compared.

MATERIALS AND METHODS

Experimental animals.—Sixty-four male CB Wistar rats were used, aged 6–8 weeks and weighing approximately 200 g. They were housed in metal cages in groups of 6 and maintained on a standard cubed diet and water *ad libitum*.

Polyelectrolyte complexes.—The test material ('Ioplex') was manufactured by the Amicon Corporation (Lexington, Mass.) and supplied as thin films. The complex was prepared by coprecipitation of 2 oppositely charged linear polyelectrolytes—a cationic polymer, poly(vinyl-benzyltrimethylammonium) chloride (Formula I), and an anionic polymer, sodium poly(styrene sulfonate) (Formula II), *see* text-figure 1. The product (Formula III) contains both anionic and cationic groups in its structure, and the net charge is determined by the polyanion/polycation ratio. Depending on conditions, the complex can contain an excess of anions, an excess of cations, or equal proportions of both. Complexes of each type were examined. The *anionic* material had an excess polyanion content of 0.5 meq/g of dry resin, the *cationic* complex had an excess polycation content of 0.5 meq/g of dry resin, and the *neutral* material contained equivalent parts of polyanion and polycation. All these complexes contained approximately 50% by weight of water. The films were sterilized in formalin before use, and then washed in 6 changes of sterile distilled water and 2 changes of sterile saline. Despite this repeated washing, it is likely that, because of the water content of the films, some formaldehyde remained trapped in them after sterilization. The structures of the 3 polymers are such that formaldehyde would not react chemically with them.

Procedure.—The rats were divided into 4 groups, each consisting of 16 animals. The films were cut into 20 × 20 mm squares and implanted singly into animals in the 3 test groups as follows: anionic



TEXT-FIGURE 1.—Formation of polyelectrolyte complexes.

film (group A), neutral film (group B), cationic film (group C). The implantations were made under ether anesthesia. The skin and superficial subcutaneous tissues of the right flank were laid open, the film was inserted, and the incision was closed with Michel clips. The 16 control rats in group D were not given plastic implants, but incisions were made in the subcutaneous tissues of the right flank under anesthesia as in the test groups A, B, and C.

The animals were examined twice weekly throughout their lives. The experiment ended after 92 weeks when only 3 rats remained alive. Rats were killed earlier if they became sick or developed large local tumors. Full postmortem examinations were made. The implantation sites were removed from every animal, together with other tissues showing macroscopic abnormalities, and fixed in Bouin's solution. All the implantation sites were subsequently cut into thin slices and examined macroscopically, and representative pieces from the middle and ends of each were examined histologically. Paraffin sections were prepared at 5 μ and stained with hematoxylin and eosin and (as required) with Masson's trichrome, periodic acid-Schiff, and Gordon and Sweets' silver impregnation method for reticulin fibers.

RESULTS

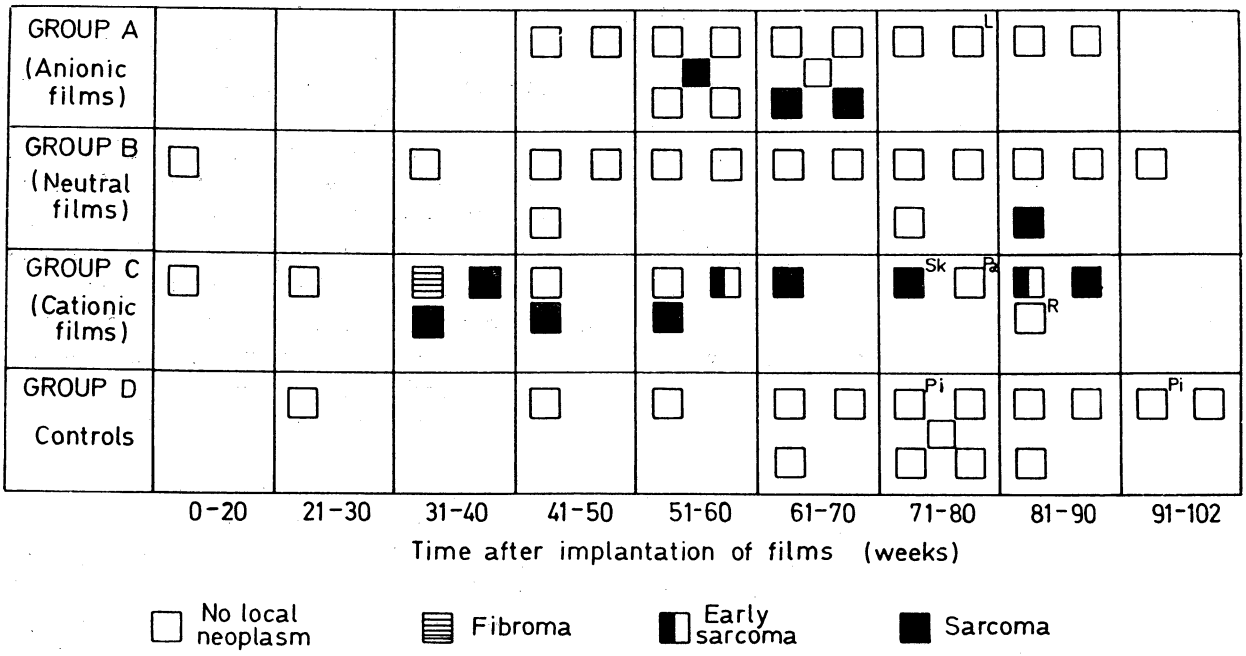
The 3 types of film were well tolerated and all the test rats remained healthy for several months. Apart from a little induration, no significant changes were noted at the implantation sites of living animals until local neoplasms developed. Implants of all 3 films appeared intact at autopsy. However, where local tumors were present, it was sometimes difficult to assess the exact topography of the implants.

In test groups A, B, and C, 13 animals developed swellings at the implantation site before they died or were killed. Of these swellings, 11 were shown later to be neoplasms—1 fibroma and 10 sarcomas. Their histology is discussed in the next section. The 2 remaining swellings—1 in a rat from group A and 1 in a rat from group C—were not tumors, and consisted of masses of granulation tissue and fibrous tissue. During the routine histopathologic examinations of implantation sites, 2 test animals in group C were found to have (presumptive) early sarcomas of microscopic dimensions. No local tumors of any kind were observed in control rats from group D.

To analyze the findings in more detail, we need to define the time of appearance of the various local tumors: This was when the tumors first measured 10 mm in at least 2 diameters at right angles. Text-figure 2 summarizes observations in the 4 groups to show the times of development of neoplasms at the implantation sites and the times of death of rats without local tumors. (The times of death of 2 rats with microscopic local lesions are also shown, but obviously the times of appearance of these lesions cannot be given in the terms defined above; they were therefore excluded from the statistical analysis which follows.) The histologic types of the local tumors and the times of their appearance are also shown in table 1. The rates of incidence of neoplasms at the implantation site in rats of groups A, B, and C were compared actuarially (*7*, *8*). Table 2 shows the observed numbers of local tumors in these animals, together with the numbers that would have been expected if the incidence rates had been the same in all 3 groups. The differences between the observed and the expected numbers of tumors in the 3 groups are too extreme to be due to chance alone ($\chi_2^2 = 8.5$; $P<0.05$). We conclude that the cationic film is more carcinogenic than the neutral film, with the anionic film apparently intermediate in carcinogenic activity. The discovery of 2 impalpable early sarcomas at the sites of implantation in rats of group C strengthens the evidence that the cationic film is the most strongly carcinogenic. On the other hand in terms of palpable tumors, the numbers of observations are insufficient to show that the carcinogenic activity of the anionic film is either significantly less than that of the cationic film or significantly more than that of the neutral film. The only fact established is that, at the 5% level of significance, the carcinogenic activity of all 3 films is not the same.

Histopathology of Neoplasms Induced by Polyelectrolyte Complex Films

Except for 1 fibroma, the 11 palpable implantation site tumors were all malignant. Six were



TEXT-FIGURE 2.—Development of local tumors and other neoplasms in rats bearing implants of anionic, cationic, and neutral polyelectrolyte films. For local tumors, the times indicated relate to the dates on which the tumors first reached 10 × 10 mm in their two greatest diameters. L = malignant lymphoma, Sk = squamous carcinoma of skin of forelimb, Pa = exocrine adenoma of pancreas, R = adenocarcinoma of rectum, Pi = chromophobe adenoma of pituitary gland.

TABLE 1.—Incidence of tumors at implantation site

Group	Number of rats	Local tumors			Time of appearance of tumors palpable before death (weeks)
		Fibromas	Sarcomas	'Early sarcomas'*	
A—anionic film	16	0	3	0	59–69
B—neutral film	16	0	1	0	83
C—cationic film	16	1	6	2	33–81
D—controls	16	0	0	0	—

*Discovered at necropsy.

TABLE 2.—Test for inhomogeneity in response to local carcinogenicity of implanted films

Group	Type of film	Observed No. of rats with palpable tumors	Expected No. of rats with palpable tumors after adjustment for survival differences
A	Anionic	3	3. 9
B	Neutral	1	4. 2
C	Cationic	7	2. 9

Significance of heterogeneity: $\chi^2_2 = 8.5$; $P < 0.05$

typical spindle cell sarcomas with no outstanding features, but the remaining four (2 in group A and 2 in group C) were pleomorphic tumors and showed extensive osteogenic activity (figs. 1, 2). Some consisted of sheets of polygonal osteoblast-like cells, separated by small deposits of osteoid; elsewhere there were larger patches of organized bone. Multinucleate giant cells resembling osteoclasts were frequent. There was no evidence of cartilage formation. Most palpable lesions contained small fragments of intact plastic, but there

was rarely any trace of the connective-tissue pocket which had previously enclosed the implanted film. The tumors showed a variable amount of local invasion in the body wall, but distant metastases were not seen.

Early sarcomas: These microscopic lesions were found in 2 rats. They had not yet obliterated the capsule surrounding the implant and appeared as small nodules arising within the capsule and extending inward into the central space and outward into the adjacent dermis (fig. 3). Some sections showed well-preserved tumor cells lying free in the center of the pocket close to the implant (figs. 4–6).

Histopathologic Changes in Implantation Sites From Animals Without Local Tumors

The connective-tissue pockets surrounding the films were intact and showed various changes. In some, the walls contained granulation tissue, abundant reticulin fibers, siderophages, and chronic inflammatory cells (figs. 7, 8); others appeared virtually inactive and consisted of avascular hyaline collagen (fig. 9). Inert-looking capsules were more common around implants of neutral films (group B); reactive changes were more prominent in capsules enclosing anionic or cationic films (groups A and C). One apparent difference between the tissue responses evoked by neutral and non-neutral films was the formation of ectopic bone (fig. 8). No such foci were seen round neutral films, but they were observed near 2 anionic and 4 cationic films. Bone found in these sites was usually well formed, sometimes containing normal-looking marrow spaces. Foreign-body giant cells were rare and there was no histologic evidence to suggest that the films could be absorbed.

Other Histologic Changes

Test rats (groups A–C): The few other neoplasms observed in these animals are recorded in text-figure 2. Most animals had advanced bronchiectasis and cystic nephritis, which are almost universal findings in old, conventionally maintained Wistar rats.

Control rats (group D): Neoplasms in this group are also shown in text-figure 2. Degenerative pulmonary and renal diseases were common. No abnormalities were found in the flanks where the control incisions had been made.

DISCUSSION

Previous unpublished accounts cited by Markley *et al.* (*4*) suggest that polyelectrolyte complexes implanted into various sites—the femoral arteries of cats, the eyes and circulatory systems of dogs, the brains of rabbits—evoke little or no tissue response. But it is clear from the present results that such materials induce considerable local changes when they are implanted into the subcutaneous tissues of the rat. The reactions evoked resemble those associated with many other implanted plastics (*1*, *2*) and, taken as a whole, they do not merit detailed discussion. Obvious similarities include the persistence of the material at the implantation site, its encapsulation by connective tissue, and the development of local sarcomas. The long latent period of these neoplasms is characteristic and so is their pleomorphism: Osteogenic sarcomas, for example, have been described in relation to other implanted plastics (*9*, *10*). The occurrence of 'early' microscopic sarcomas in 2 rats is of interest, but comparable lesions previously observed near implanted plastics (*11*) have been described in relation to subcutaneously injected carcinogens (*12–14*). The appraisal of these changes has been discussed elsewhere (*12*, *14*) but their significance is still uncertain. In particular, it is not clear what proportions progress to overt sarcomas, remain unchanged, or regress; second, their true incidence in implantation sites cannot be determined without the examination of serial sections of all specimens. For these reasons they were excluded from the actuarial analysis reported here.

Of greater interest in the present context are certain differences in the tissue response evoked by the 3 types of film. Most obvious is the statistically significant difference between the lower rate of tumor development near implants of neutral films (group B) and the higher rate of tumor development near cationic films (group C); the rate with anionic films (group A) appears to be intermediate. But there are other qualitative and quantitative differences between the tissue responses in the 3 groups, which have been noted from the routine examination of several pieces of tissue from each

implantation site, irrespective of their macroscopic appearance at autopsy. Thus ectopic bone formation in (non-neoplastic) capsules was seen in several rats in groups A and C with implants of anionic or cationic films, but never in rats from group B with implants of neutral films. Other reactive changes were also more prominent in the capsules enclosing the non-neutral materials than in those enclosing neutral films.

Various explanations may be suggested for the greater activity of the anionic and cationic polyelectrolyte complexes. The differences in response exclude the possibility that tumor induction was due to formalin trapped in the films during sterilization, since all 3 films were similarly exposed. In any case, trapped formalin is likely to have been rapidly leached from films by tissue fluids after implantation. All 3 materials remained intact and provided a mechanical barrier "disconnecting" the surrounding connective tissues. This mechanical factor is common to all 3 types of material, but important differences are found in their respective surface properties. For example, the surfaces of anionic films are thrombo-resistant *in vivo*, whereas cationic films are not (*6*). Anionic surfaces contain bound groups such as sulfate and sulfonate ions and are thought to simulate a heparinized surface. Differences in permeability are also likely to be involved—and these provide an obvious point of distinction between solid-state carcinogenesis brought about by semipermeable plastic films and by solid, wholly impermeable, implants such as metals (*1*, *2*). Polyelectrolyte complexes are highly permeable to water and small molecule solutes, but transport of larger substances and certain ions is selective and determined by factors such as the specific ion-binding capacity of the complex, its water content, and—with non-neutral complexes—the operation of the Donnan equilibrium (*5*). (These properties make polyelectrolyte complexes well-suited for use as dialysis membranes.) Since the complexes may also release some of their own component ions, the normal distribution of ions and other substances in the adjacent tissues is likely to be profoundly altered, the nature and degree of such alterations varying according to the composition and properties of the films concerned. The potential consequences are complex, since the local accumulation of ions and other compounds may affect tissues either directly or indirectly by altering extracellular *p*H or osmotic pressure. Damage may be inflicted at a cellular level on modalities such as membrane permeability and enzyme function. Outside cells, the normal polymerization and cross-linking of soluble collagen monomers to form collagen fibrils may be impaired; pathologic processes such as ectopic bone formation may be favored, possibly as a result of changes in ground substance. The main long-term consequence of sustained local damage of this sort is frustration of the normal healing processes, and there is increasing evidence that this is sometimes a crucial factor in predisposing to the eventual formation of local sarcomas (*14–19*). The morphologic changes found in the present study, particularly in relation to anionic and cationic films, are compatible with these suggestions, though there is an obvious need to examine the early changes evoked by polyelectrolyte complexes during the first few days and weeks after implantation.

Two final comments may be made about the capsules enclosing the implants: 1) It is obvious that, once the films are encapsulated, many local physical and chemical effects of the implant will be confined to one small zone of connective tissue. 2) The present results indicate that the presence of a capsule is not the sole determinant of subsequent tumor development. The neutral films were encirled by thick capsules which were at least as dense as those seen around non-neutral films, yet the incidence of local tumors in the first group was comparatively low.

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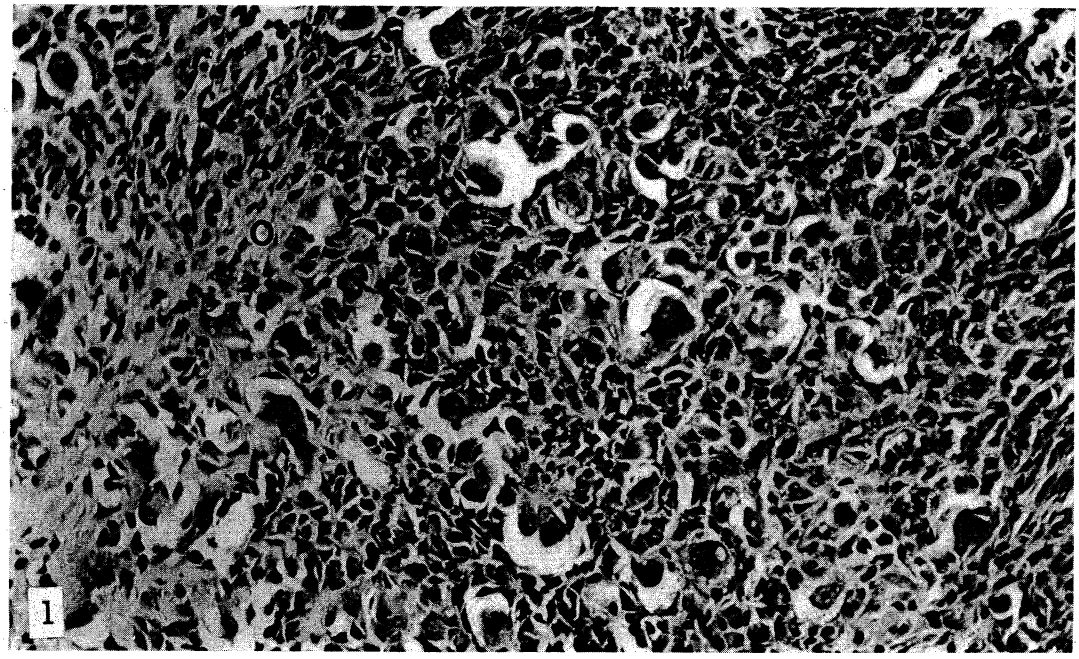


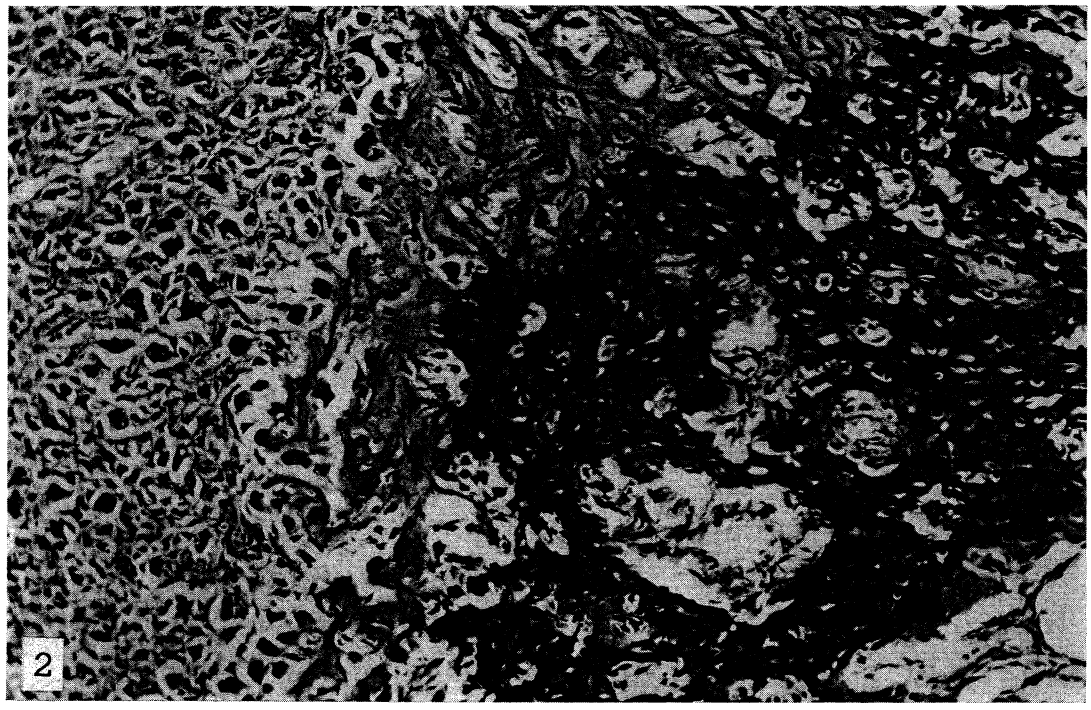


FIGURE 1.—Implantation site tumor; anionic film; 74 weeks. Osteogenic portion of pleomorphic sarcoma. Osteoblasts, osteoclast-like giant cells, and a small amount of osteoid deposition (O). Hematoxylin and eosin. × 200

FIGURE 2.—Implantation site tumor; cationic film; 49 weeks. Osteogenic regions of pleomorphic sarcoma showing large focus of poorly organized bone. Periodic acid-Schiff. × 130

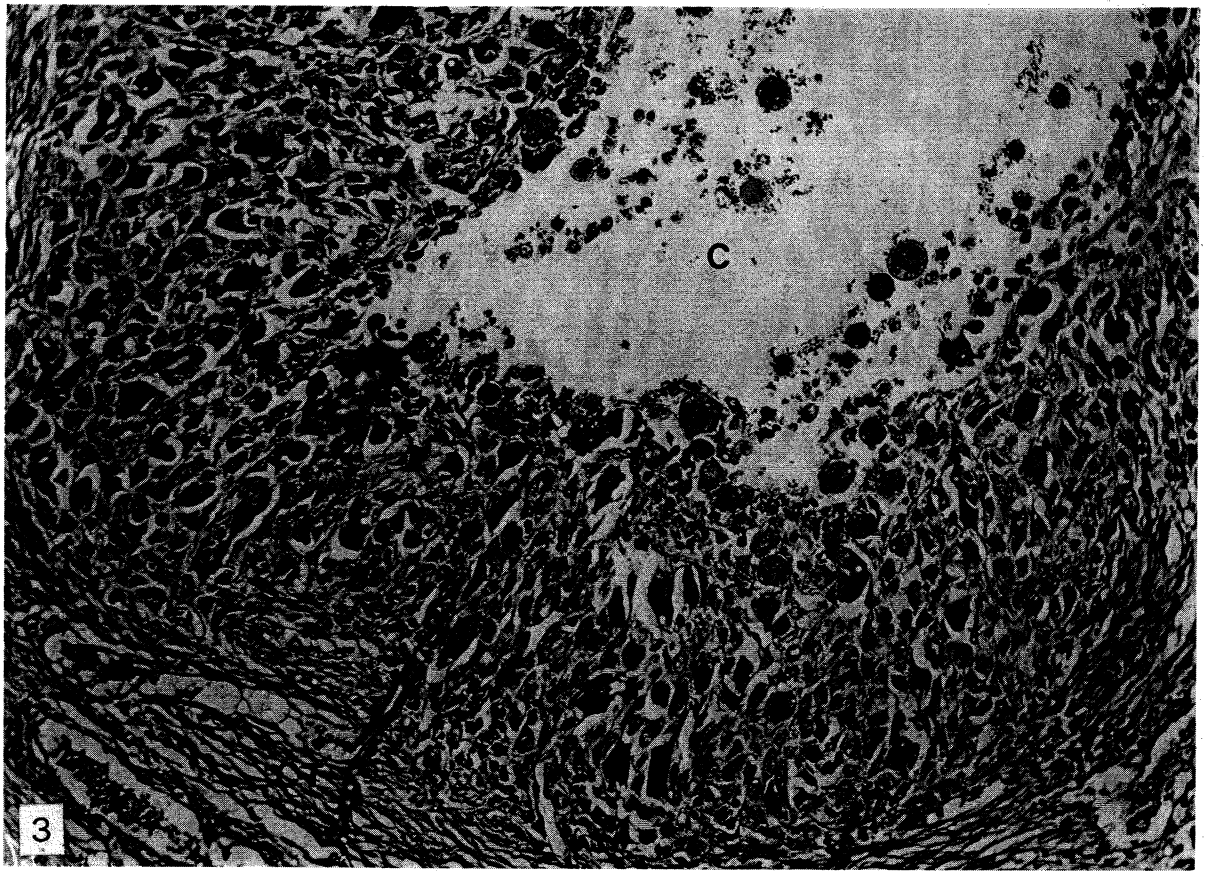


FIGURE 3.—Implantation site tumor; anicnic film; 54 weeks. Focus of pleomorphic tumor cells. Center (C) of pocket which contained implanted plastic is still intact, as is surrounding connective tissue (CT). Masson's trichrome. × 160

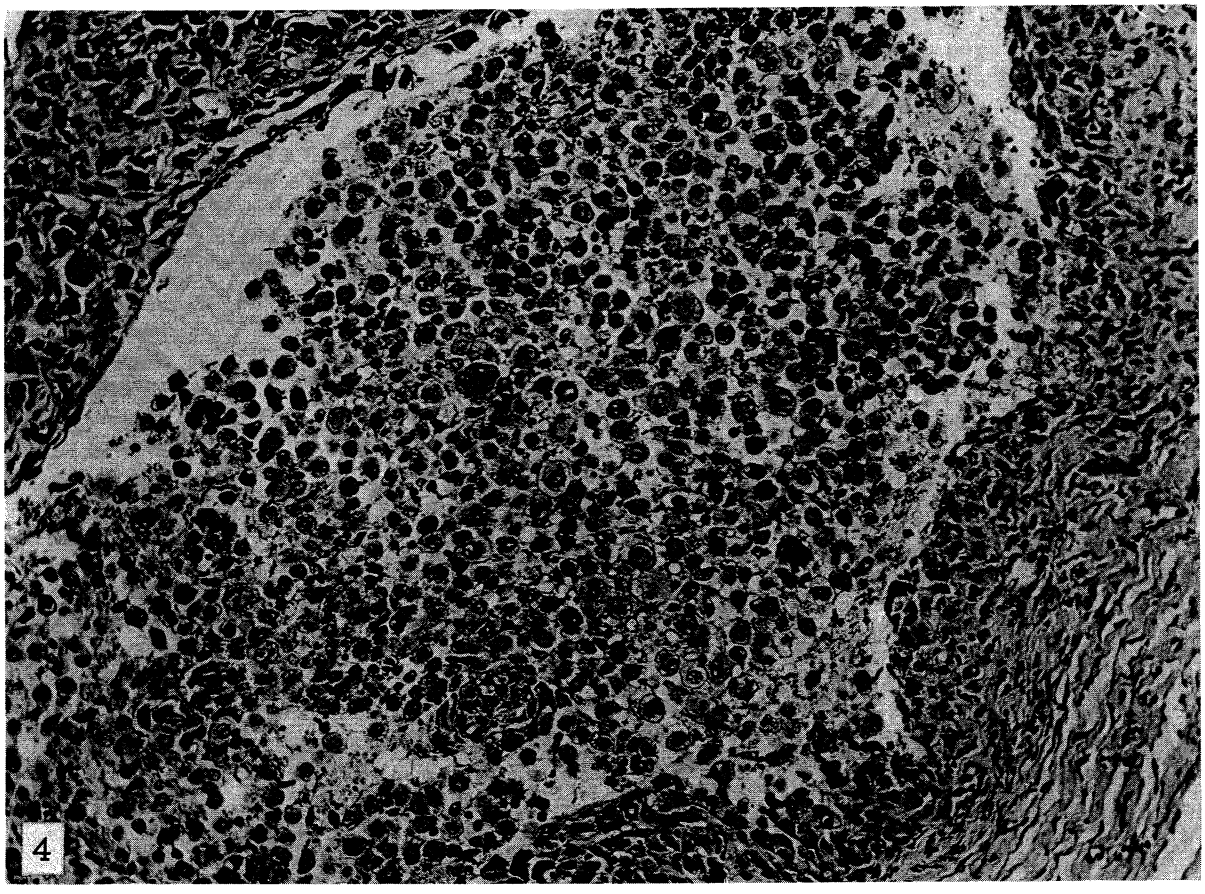
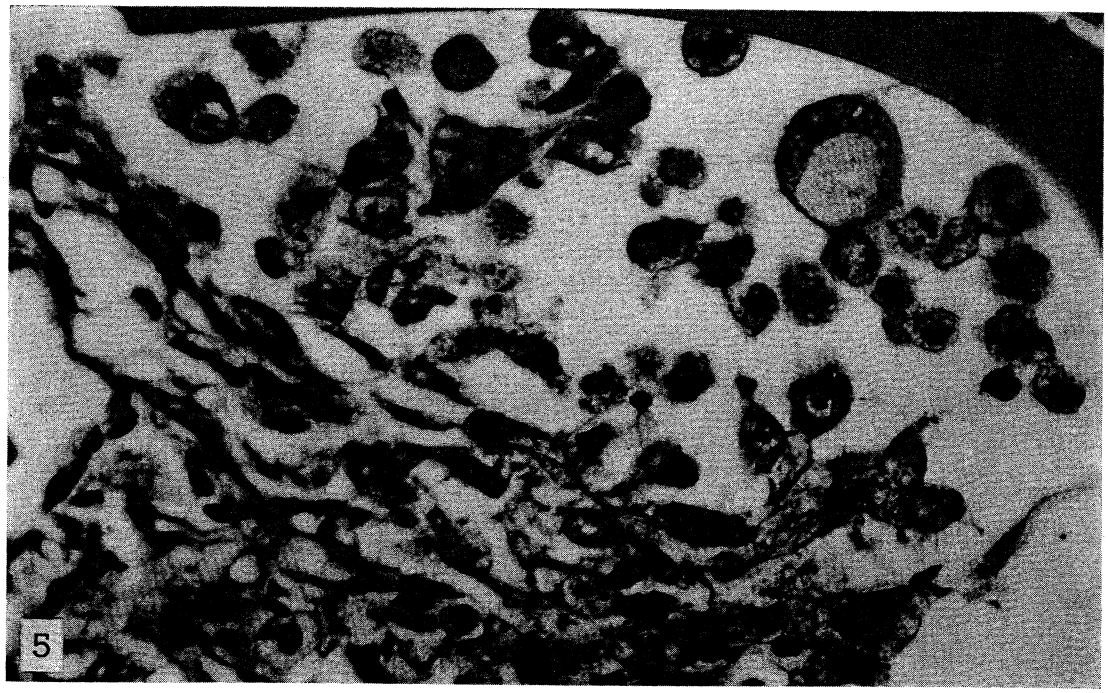
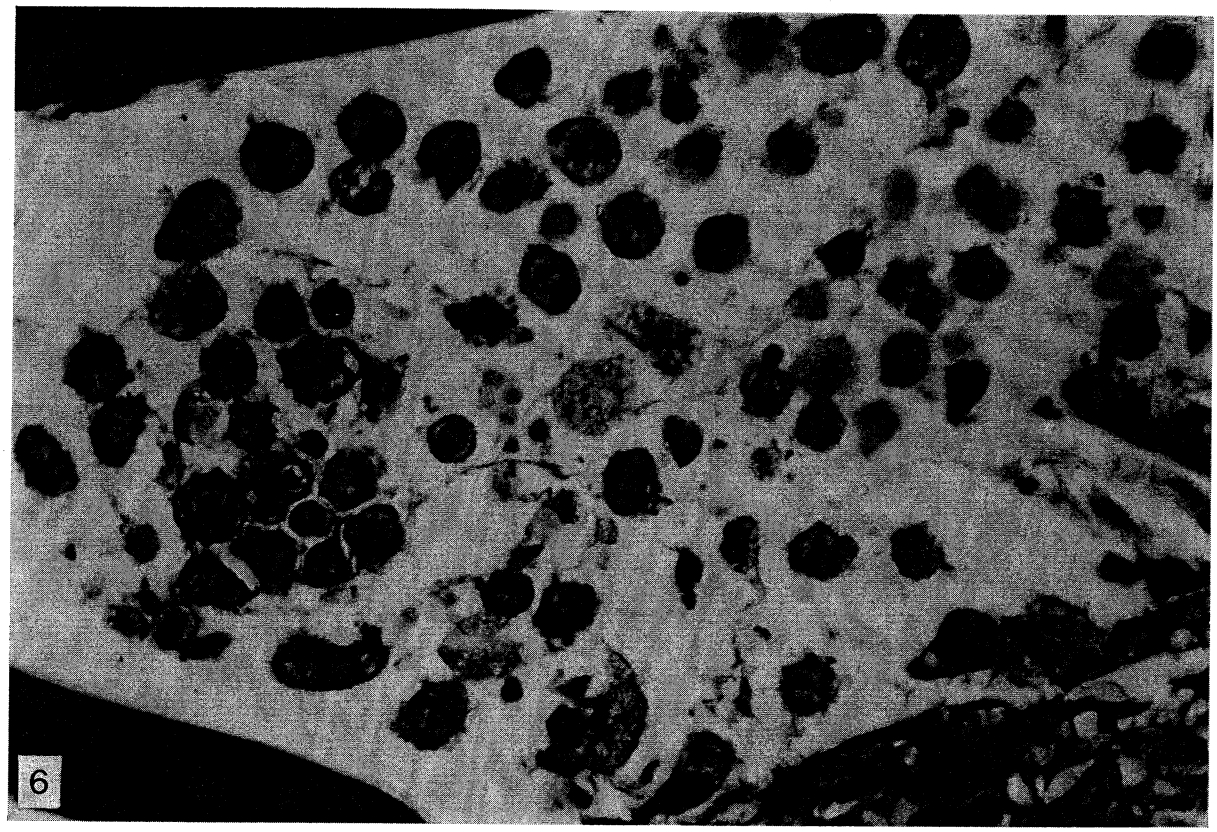
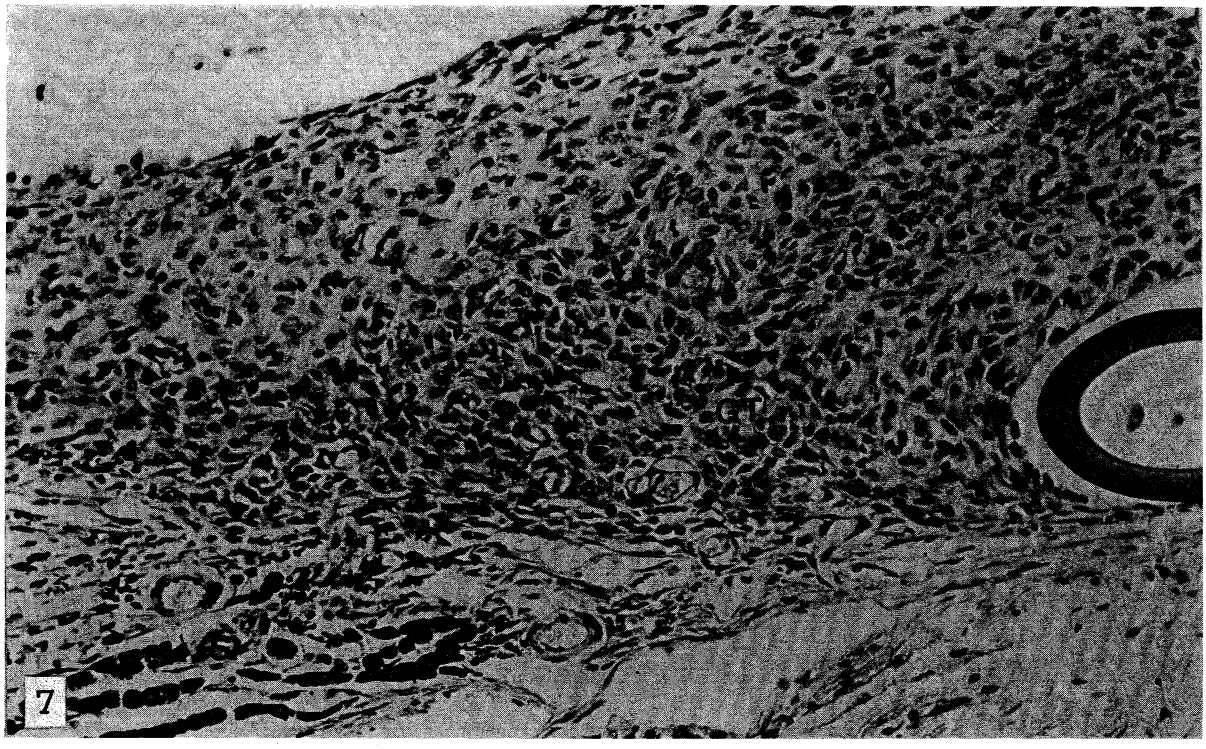


FIGURE 4.—'Early sarcoma'; cationic film; 54 weeks. Mass of tumor cells in central space of pocket. Surrounding wall is infiltrated by tumor (*top left corner*) but dermal connective tissues (*lower right corner*) are still intact. Masson's trichrome. × 160





FIGURES 5, 6.—Early sarcoma; cationic film; 84 weeks. Tumor cells free in central space of connective tissue pocket formerly surrounding film (F). Periodic acid-Schiff. × 590



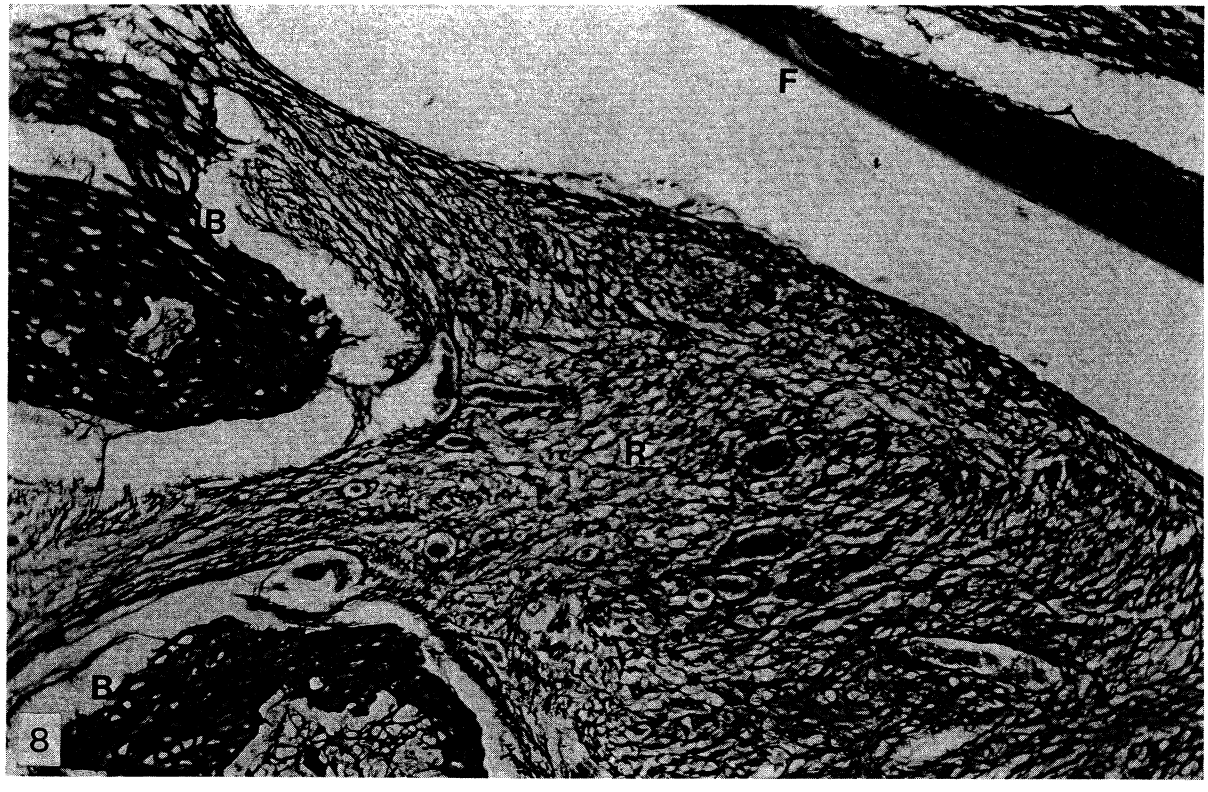


FIGURE 7.—Capsule surrounding anionic plastic; 80 weeks. Wall enclosing film (F) contains active granulation tissue (GT) and focus of siderophages (S). Hematoxylin and eosin. × 200

FIGURE 8.—Capsule surrounding cationic plastic; 77 weeks. Wall of capsule encloses loop of film (F) and contains patches of well-formed bone (B) with recognizable marrow spaces. Reticulin fibers (R) abundant. Silver impregnation. × 130

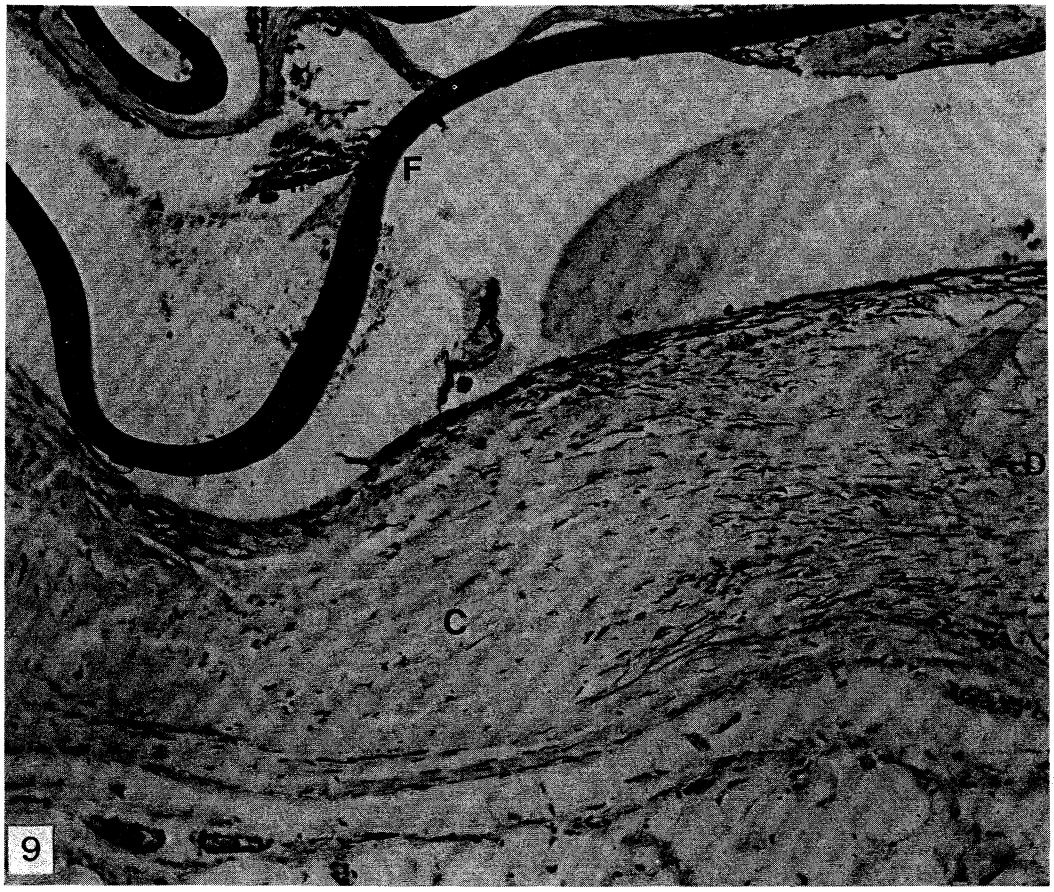


FIGURE 9.—Capsule surrounding neutral plastic; 78 weeks. Film (F) is enclosed by dense pocket of avascular collagen fibers (C). Hematoxylin and eosin. × 130