Vascular Patterns in Primary and Secondary Pulmonary Tumors in the Dog

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THE ADVENT OF NEW TECHNIQUES for the treatment of human lung cancer by administration of chemotherapeutic agents through bronchial arteries has given new importance to the study of the vascular supply of lung tumors.

This study of spontaneous canine neoplasms was undertaken to see if the vascular patterns in such instances were sufficiently similar to those in man to justify their use as a model in future investigations.

Materials and Methods

Dogs with a diagnosis of pulmonary disease compatible with primary or secondary neoplasia were obtained for this study in cooperation with practicing veterinarians.

Angiographic studies were performed on 1 dog in vivo. Hyapaque, 90%, was injected into the main pulmonary artery and six exposures per second were taken for 6 sec. An aortic catheter was placed at the arch and a similar injection and exposure series taken.

Two postmortem injection techniques were employed to facilitate anatomic studies of the vasculature of these canine lungs. Of 13 attempted injections, 9 were successful, including that in the animal which had had angiography in vivo. Four specimens were injected inadequately for interpretation. The vinylite corrosion-cast method of Liebow *et al* [1] was used successfully in five cases. In these, the vinylite mass was injected into the systemic arteries of the thorax through a cannula tied into the aorta just beyond the aortic valve. The aorta was ligated at the diaphragm prior to injection. After removal of the thoracic organs en bloc, vinylite masses of different colors were injected into the pulmonary arteries and pulmonary veins through cannulas tied into the main pulmonary artery and the left atrium. The remainder of the tissue was then digested with concentrated solutions of NaOH. In order to better visualize smaller vessels and to see their relationships with the other tissues, 4 dogs received injections of a modified Schlesinger gelatin mass.[2]

The gelatin masses, injected at monitored physiologic pressures in the arteries and slightly above physiologic pressure in the pulmonary veins, were selected to penetrate arterioles and venules but only a small part of the capillary bed. After the gelatin had solidified (45–60 min) the lungs were fixed with 10% formaldehyde by bronchial perfusion at a pressure of 20–25 cm of water. Fixed lungs were cut into

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¾-in. thick slices; after gross examination selected slices were embedded in gelatin and cut into serial, 300-μ thick, whole-lobe sections by the method of Gough and Wentworth.[3] These sections were then cleared in three changes of glycerin and mounted in Saran Wrap plastic envelopes for examination under the dissecting microscope. By using these serial sections, small vessels within individual tumor nodules were traced from the capillary network to parent arteries and veins of sufficiently large size for unequivocal indentification. Several tumor nodules were examined from each dog. Representative blocks of these lungs were also taken for routine histologic sections stained with hematoxylin and for elastica by the method of Verhoeff [4] with Mallory's counterstain (EVG).[5]

Results

In accord with tradition, all systemic arteries (those originating from the aorta or one of its branches and having an elastic structure typical of systemic arteries) within the lung are called bronchial arteries. In abnormal lungs, such as the ones in our study, some of these arteries arise from unnamed mediastinal, pleural, and intercostal arteries, as well as from normal pre-existing bronchial arteries.

In the one animal studied by angiography, there were multiple metastatic lesions in both lungs. On the pulmonary artery angiogram, the vessels were well filled in both lungs. Some branches of the pulmonary artery were distorted and partially compressed by tumor nodules (Fig 1), but no "tumor blush" or new branches of the pulmonary artery could be identified. The systemic angiogram demonstrated very rapid filling of bronchial arteries, including several which appeared to have proliferated into the tumor nodules (Fig 2). The early filling and the large size of these vessels were interpreted as evidence of significant systemic blood flow to the tumor-filled regions of lung.

Cast Corrosion Preparation

The five cast preparations that were successful represented four primary pulmonary carcinomas and one metastatic urinary bladder carcinoma. Of the four specimens with primary lung tumors, three also had intrapulmonary metastases. The tumor types and probable sites of the primary tumors are listed in Table 1.

All casts demonstrated proliferated bronchial arteries and increased caliber of the normal bronchial arteries, suggesting increased flow and distribution during life (Fig 3–5).

The bulk of large tumors was frequently present only as an empty space without any injected structures (Fig 3). This in part was due to necrosis and vascular obliteration in large masses, but also in part to the fact that many intratumor vessels were smaller than usually can be filled with the vinylite injection mass. If these fine branches took up the

Table 1. Canine Lung Tumors That Had Received Injections

Dog. No.	Primary site	Tumor type	Degree	Cast	Gelatin	Comments
1	Lung	Undiff Ca	1°	x		BA → tumor; PV Drainage
2	Lung	Undiff Ca	1°M	x		BA → tumor; multifocal or multimeta.
3	Lung Kidney	Adeno Ca Trans Ca	1°	x		BA → tumor
4	Bladder	Trans Ca	M	x		BA → tumor; Ba ↔ PA; angiogram
5	Lung	Adeno Ca	1°M	x		BA → tumor
6	Lung Skin	Adeno Ca Poorly diff Ca	1°M M		x	Prim & meta: BA & PA → tumor; PV & BV drainage; BA ↔PA; PV ↔ BV
7	Lung	Undiff Ca	1°M		x	BA ↔ PA; BA → tumor
8	Lung	Poorly diff Ca	1°M		x	BA & PA → tumor
9	Lung	Undiff Ca	1°M		x	BA & PA → tumor

injected material, they frequently were insufficiently supported to survive intact the digestion. Evidence for this was that small branches were found free in the digestion vat. Injections into smaller nodules were more successful throughout, and in these the space occupied by the tumor nodule was filled with a twisted complex of bronchial arteries (Fig 4). Anastomoses of bronchial to pulmonary arteries were not demonstrated in the corrosion casts.

Some pulmonary arteries were displaced or otherwise distorted by the tumor masses (Fig 4). No proliferated or enlarged branches of the pulmonary artery were identified in any of the casts; but some branches were absent, presumably because they had been destroyed by the tumor or occluded by thrombi (Fig 5).

Pulmonary veins also were sometimes flattened and displaced by tumor nodules. Venous drainage of the neoplastic tissue was not demonstrated adequately with the vinylite injections; we did not attempt injections to azygous veins.

Four dogs were given injections of the gelatin mass. All had intrapulmonary metastases. Although different colors were used when giving injections to each of the different vessels, it was immediately apparent that identification of small vessels by color alone was inadequate. The presence of anastomoses and reverse or retrograde flow of gelatin made it imperative that definite identification of vessels be supported by other means. To achieve this end, serial sections of whole lung were made and small vessels traced to obvious parent vessels which could be properly identified. Supplemental information was obtained by examining EVG-stained histologic preparations of vessels to determine the distribution of the elastic layers indicating whether the vessel was a pulmonary or bronchial artery.

All preparations demonstrated proliferated bronchial arteries into tumor nodules and masses. Figure 6 demonstrates a solitary tumor nodule supplied by a proliferated bronchial artery arising alongside a bronchus. The supplying bronchial vessel divided into many smaller vessels providing the main blood supply to the tumor nodule (Fig 8). There was no evidence of pulmonary artery supply in this instance.

The relationships of the pulmonary arteries to tumors was studied in detail. Grossly, the tumor nodule in Fig 7 was blue, suggesting that a pulmonary arterial supply was present. An EVG preparation from a similar nodule in another dog (Fig 9) demonstrated tumor growing within alveoli, the septums remaining intact. The tumor had grown as a cancerous pneumonia, obtaining its nourishment by diffusion across the capillary walls. No evidence of bronchial artery supply was noted in this area. Of specific note was the absence of any evidence of pulmonary artery proliferation (Fig 10 and 11). The pulmonary artery capillary bed remained intact, providing a blood supply to tumor growing within the previously air-occupied alveolar lumens. This form of pulmonary artery supply was demonstrated in all four specimens that had received injections. Large nodules frequently had a bronchial artery supply in their centers, while the advancing edge of the nodule grew as a cancerous pneumonia, obtaining its nourishment from diffusion across the still present pulmonary artery capillary bed. Whenever bronchial artery proliferation was noted, concomitant evidence of destruction of alveolar septums was found. The triggering mechanism for bronchial artery proliferation appeared to be this destruction of pulmonary parenchyma or capillary bed.

Bronchial artery to pulmonary artery anastomosis occurred in two of the four dogs. As seen in Fig 12, a bronchial artery has penetrated a pulmonary artery and a common lumen resulted.

Gough sections demonstrated many pulmonary artery to bronchial artery anastomoses (Fig 13). The direction of the flow of these shunts was not determined.

Venous drainage of tumor nodules was found to involve both the pulmonary and bronchial veins (Fig 14). The extent of proliferation of veins and the extent of involvement of the azygous system was not studied.

Discussion

Several studies of the vascular supply of primary and metastatic tumors in the lungs of man have been reported over the past 30 years. A bronchial arterial proliferation into and supply of primary tumors has

been established repeatedly. The source of nutrient vessels to metastatic nodules in human lungs, however, has remained a matter of dispute.

In 1938 Wood and Miller [6] studied one patient with metastatic uterine sarcoma and concluded that the blood supply was probably from the pulmonary arteries. Cudcowicz and Armstrong [7] drew similar conclusions from their postmortem studies of one patient with metastatic gastric carcinoma and one with metastases from a lung carcinoma originating in the contralateral lung. In both of these investigations the conclusions were based on indirect evidence, as neither study had achieved injection into the vessels within the tumor nodules. In 1956 Liebow demonstrated a bronchial artery supply to metastases of a renal cell carcinoma [8] in one patient studied by the vinylite corrosion cast technique.

Newton and Preger [9] used selective bronchial arteriography in vivo and described bronchial supply to a metastatic melanoma and a metastatic synovial sarcoma. Noonan, Margulis, and Wright [10] used a postmortem micropaque injection into bronchial arteries and demonstrated branching of the bronchial arteries into metastatic nodules of breast carcinoma in two patients. The vessels within metastatic nodules in their other patients did not fill with the injection mass. In 1967 Milne [11] reported an extensive roentgenographic study of lungs obtained by surgical resection and at necropsy. The bronchial and the pulmonary arteries were given injections separately, and roentgenograms were made after each injection. Milne concluded that although "central" metastases generally had bronchial circulation, the peripheral metastases usually obtained their circulation from the pulmonary arteries.

Wright [12] reported a very detailed study from which he concluded that bronchial arteries supplied most metastatic nodules. He described one patient with metastatic choriocarcinoma, however, in which the margins of the tumor nodules were supplied by small branches of the pulmonary artery. The centers of these nodules were necrotic, and no bronchial artery supply was identified. Harley *et al* [13] conducted a study of primary and metastatic lung tumors in man at the same time and with the same techniques as our study of canine tumors. Their findings appeared to be identical to those described in this paper. From our observations and from those of Harley *et al*, it is clear that "vascular supply" of tumors must be considered in two aspects—source of new vessel growth and source of nutrition.

In terms of the biology of vascular proliferation in the lung, past experience with a variety of spontaneous and induced lesions has led

to the concept that after the pulmonary arteries complete their developmental growth they are incapable of further proliferation. The bronchial arteries, however, are able to respond in various situations by proliferation of new channels and by enlargement of old ones.

Thus, in chronic inflammatory lesions such as tuberculosis, bronchiectasis, and necrotizing granulomatosis of childhood,[14] the bronchial arteries enlarge and proliferate extensively in the granulation tissue. Although parts of the capillary bed and many small pulmonary arteries are destroyed by these lesions, new branches of the pulmonary artery do not form. Similarly, in experimental pulmonary artery ligation in the dog[15] and rat,[16] and in induced pulmonary hypertension in the dog,[17] it is only the bronchial arteries which proliferate. The situation in regard to pulmonary and bronchial veins has not yet been studied adequately to draw certain conclusions. In pulmonary vein ligation, however, all proliferation appears to be from bronchial veins and from minute channels in granulation tissue.[18]

In the past three decades, the reports of pulmonary artery supply to metastatic pulmonary neoplasms have stood as an exception to the general "rule" of vascular proliferation in the lung. Our study and that of Harley *et al*[13] confirm that, as in the other lesions cited above, only bronchial arteries proliferate in primary and in metastatic pulmonary neoplasms.

In terms of supply of nutrition and, similarly, vascular access to tumor nodules for chemotherapy via selective catheterization, the problem is not so simple. In instances where neoplastic cells grow within alveoli or on the surfaces of the alveolar septums without destroying the septums themselves or their parent pulmonary arteries, the normal alveolar capillaries persist and, hence, supply nutrition to the tumor by diffusion without formation of new vessels. This situation occurs in bronchiolar alveolar tumors and when the tumor growth pattern can be described as a cancerous pneumonia. It is also very common, although not universal, at the very peripheral margin of tumor nodules which more centrally are supplied by newly proliferated systemic arteries. Furthermore, as in other situations where tissue destruction and bronchial artery proliferation occur, significantly large anastomoses develop between the systemic and pulmonary arteries.

As vascular invasion, compression, thrombosis, and recanalization develop and progress in both the systemic and pulmonary arterial systems, the hemodynamic relationships at these anastomotic sites may change repeatedly. Thus, blood flow within a particular branch of the pulmonary artery may be derived from the right ventricle in the normal

fashion or from the left ventricle via proliferated and enlarged systemic arteries. When such anastomoses are sufficiently large, reversal of direction of flow in the pulmonary artery may occur, and the systemic blood may gain access to other branches of the pulmonary artery in regions of lung parenchyma that are not invaded by tumor. Also, for example, by recanalization of pulmonary artery thrombi and tumor invasion of the parent vessels of collateral systemic channels, conditions may be achieved by which right ventricular blood will flow through proliferated systemic arteries into tumor nodules. It is apparent that direction of blood flow at any one focus is not predicted easily from examination of local vessels alone. It is also evident that angiograms, while giving accurate information about blood flow at any one time, can be seriously misleading if interpreted to reflect vascular proliferation without consideration of these other facets of the problem.

A distinction between primary and metastatic pulmonary nodules was not emphasized in the main body of this report. The findings strongly suggested that, in fact, there were no differences in type of supply or nourishment. A fortuitous control was obtained in the one case of both primary and metastatic tumor in the same lung. Three of four lungs receiving injections of gelatin had intrapulmonary metastases from a primary lung tumor. No distinction could be made between these nodules. The evidence suggests that both primary and metastatic pulmonary tumors in the dog induce a similar vascular reaction, and the basic patterns of growth are similar.

A comparison of the two injection techniques used in this study reveals a few major differences. The vinylite corrosion method presented an excellent three-dimensional representation of bronchial vessels to tumor. However, because the tissues, both normal and neoplastic, by necessity were destroyed, detailed relationships of vessels and nourishment of tissues could not be examined. Furthermore, although fine vascular channels may have been filled within the tumor by either bronchial or pulmonary arteries at the time of digestion, support for these fine vessels that had been given injections was lost and the most important regions of study destroyed. Evidence for this was obtained by finding fragments of bronchial artery casts in the digestion vat. The gelatin procedure overcame the difficulties encountered in the vinylite technique. Since tissue preservation was maintained, gross and microscopic examination was possible, permitting detailed study of fine vascular relationships to normal and neoplastic tissue. This proved worthwhile since only in the gelatin preparation was evidence obtained for nourishment of tumor cells by pulmonary arteries when they grew in alveolar

lumens. Because artifactual destruction of regions given injections following tissue digestion was avoided, a more definitive statement could be made regarding the absence of any pulmonary artery proliferation. Fine venous channels could also be studied, an area notably lacking in our cast preparation.

Histologic examination of all parts of the lung is available with the gelatin technique. An example of this advantage was demonstrated dramatically when in Dog 6 a primary papillary adenocarcinoma of the lung was found along with a metastatic highly undifferentiated carcinoma from skin in the same lobe. This would have been missed completely if a cast had been made of this lung.

One main disadvantage of the gelatin technique is the partial loss of immediate three-dimensional visualization. However, by examining serial sections a reliable reconstruction of the area under study can be made. Since the sections are 300 mμ thick, this reconstruction procedure is not excessively tedious.

Summary

Dogs with naturally occurring primary and metastatic pulmonary tumors were studied to evaluate this species as a possible model system for study of vascular patterns and supply of pulmonary tumors. Five vinylite corrosion cast specimens and four specimens given injections of gelatin were studied. In-vivo angiography was performed in one dog prior to successful vinylite injection. The bronchial artery proliferated in all cases studied, suggesting this vascular supply to be the primary source of nourishment. Pulmonary arteries did not proliferate, but substantially contributed to the nourishment of the periphery of tumor nodules, and in those cases in which cancerous pneumonia occurred.

Bronchial artery proliferation occurs when the tumor destroys alveolar septums and pulmonary parenchyma. Pulmonary arteries do not proliferate, but supply the tumor by the pre-existing pulmonary arterial capillary bed if the septums are intact as in cancerous pneumonia. Bronchial artery to pulmonary artery anastomosis and venous drainage of tumors were demonstrated.

The dog appears to react to pulmonary neoplasia in a manner nearly identical to man and therefore may have decided merit as a model system in the study of this disease.

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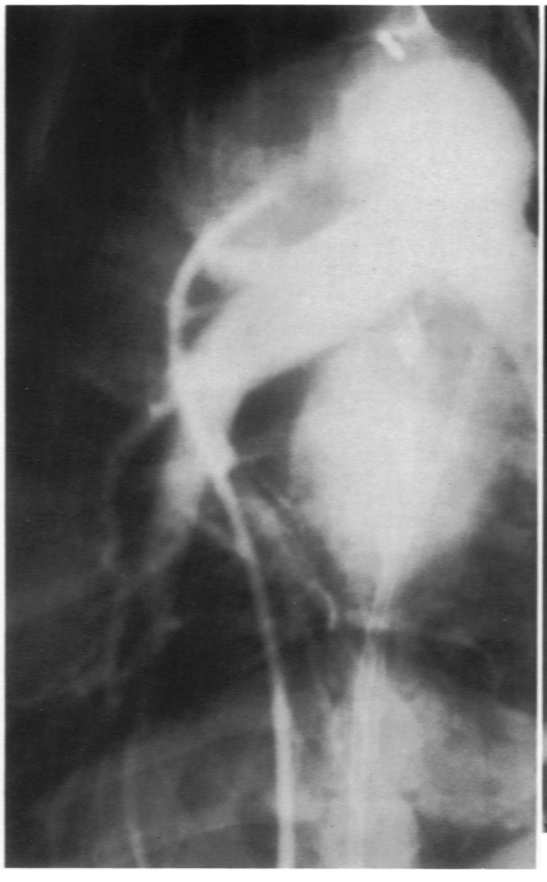
[*Illustrations follow*]

Legends for Figures

Fig 1. Pulmonary artery is displaced and distorted by expanding tumor nodule. No tumor "blush" or evidence of new vessel growth is seen. Pulmonary arteriogram from dog with metastatic transitional cell carcinoma of urinary bladder.

Fig 2. Bronchial vessels are coursing toward the tumor nodule and suggest an in-vivo blood flow via bronchial arteries to this region. Same dog and view as in Fig 1. Hyapaque, 90%, injected via catheter tip placed at aortic arch. Seventh exposure.



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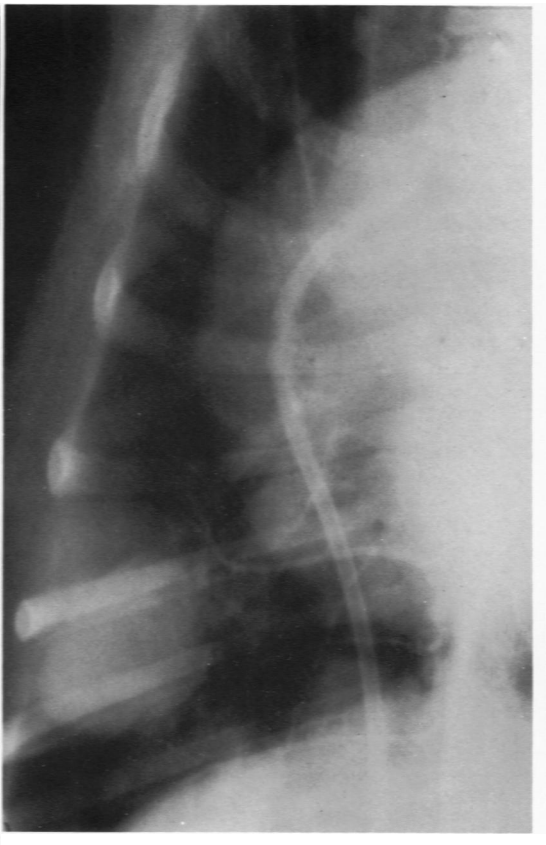

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Fig 3. Cast corrosion specimen of same dog seen in Fig 1 and 2. Proliferating bronchial arteries are projecting into a large tumor that has been digested away. Normal pulmonary architecture has been destroyed completely by tumor. Bronchial arteries are black; pulmonary arteries, red; pulmonary veins, green; tracheobronchial system, white.

Fig 4. A twisted complex of vessels given black injection is evidence for in-vivo supply of this tumor nodule by bronchial artery. Tumor has displaced a large pulmonary artery. Note normal pulmonary architecture except in region of proliferated blood vessels. Intrapulmonary metastasis of undifferentiated primary pulmonary carcinoma. Cast corrosion method.

Fig 5. Entire middle lobe has been invaded by tumor, leaving only a skeleton of the tracheobronchial system intact. Bronchial vessels (black) have proliferated into the tumor mass. The pulmonary artery (red) is occluded by either tumor obstruction or artifact. Primary undifferentiated carcinoma. Cast corrosion method.

Fig 6. Intrapulmonary metastasis of primary undifferentiated tumor supplied by a bronchial artery arising alongside a bronchus. Gelatin method: red, systemic injection; blue, pulmonary artery.

Fig 7. Tumor nodule given injection of blue gelatin, suggesting possible pulmonary artery supply. Intrapulmonary metastasis of primary undifferentiated carcinoma. Gelatin technique. Gross specimen.

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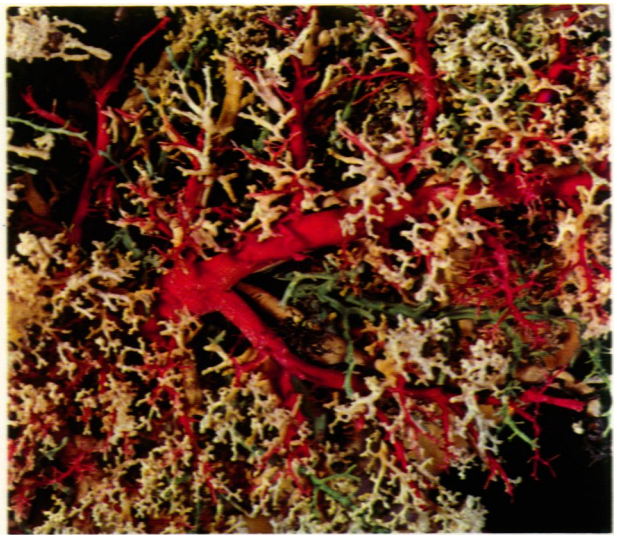

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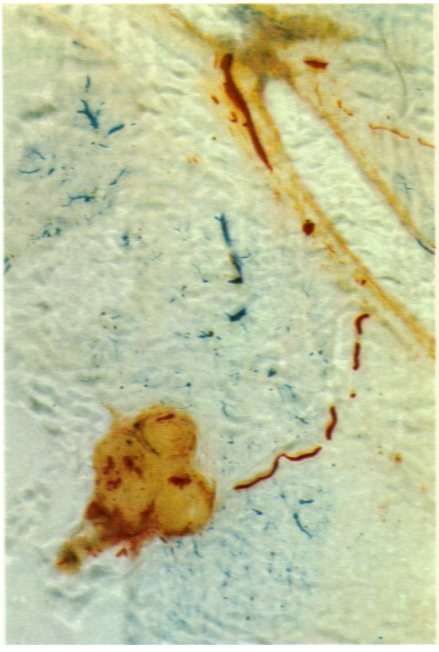

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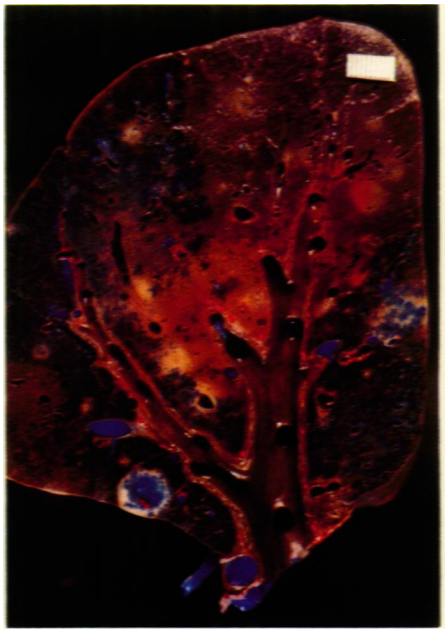


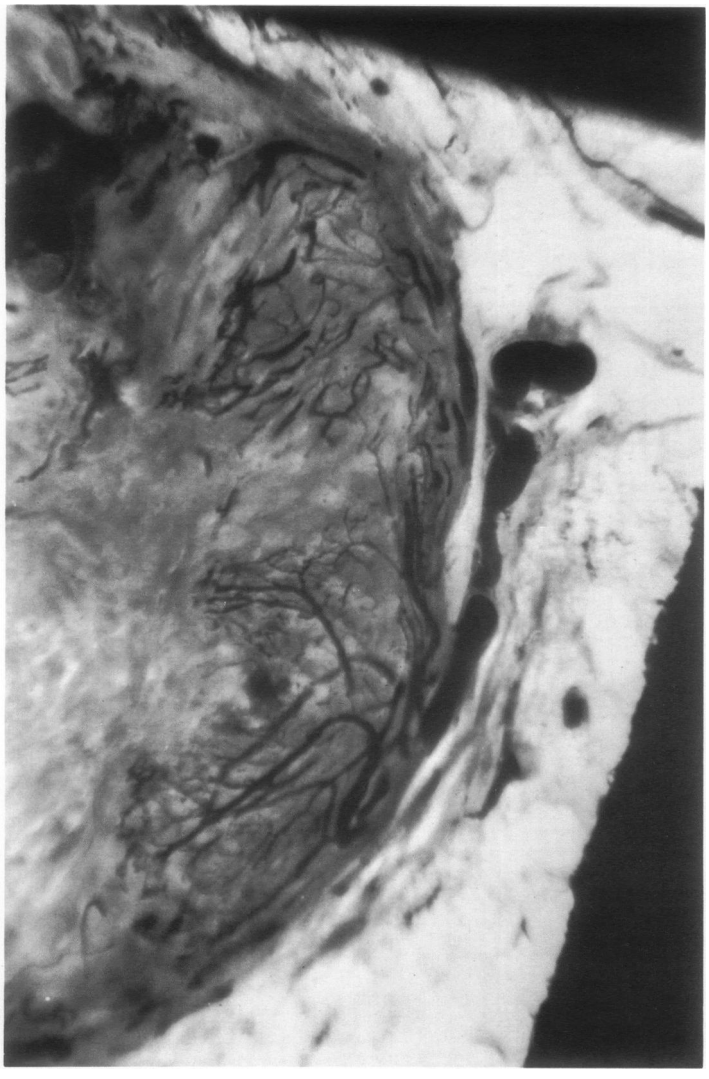
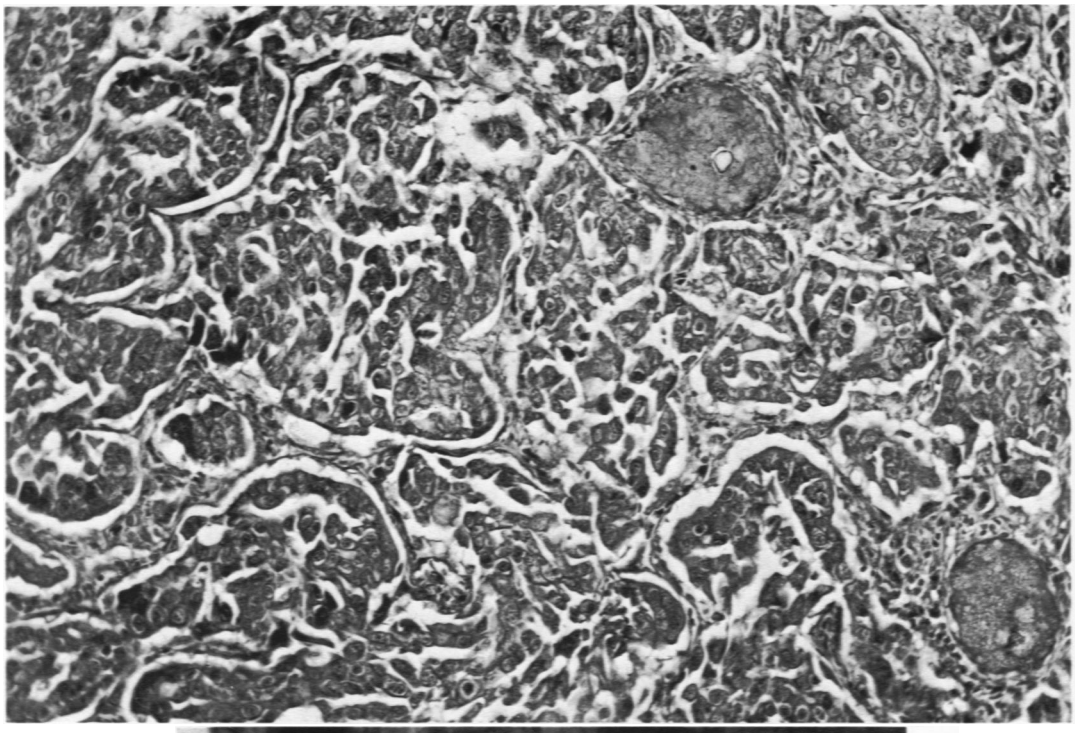


Fig 8. Edge of tumor nodule supplied by a bronchial artery as viewed through a dissecting microscope. Vessel has proliferated into tumor nodule, suggesting this source to be its main vascular supply. Gelatin technique. × 5.



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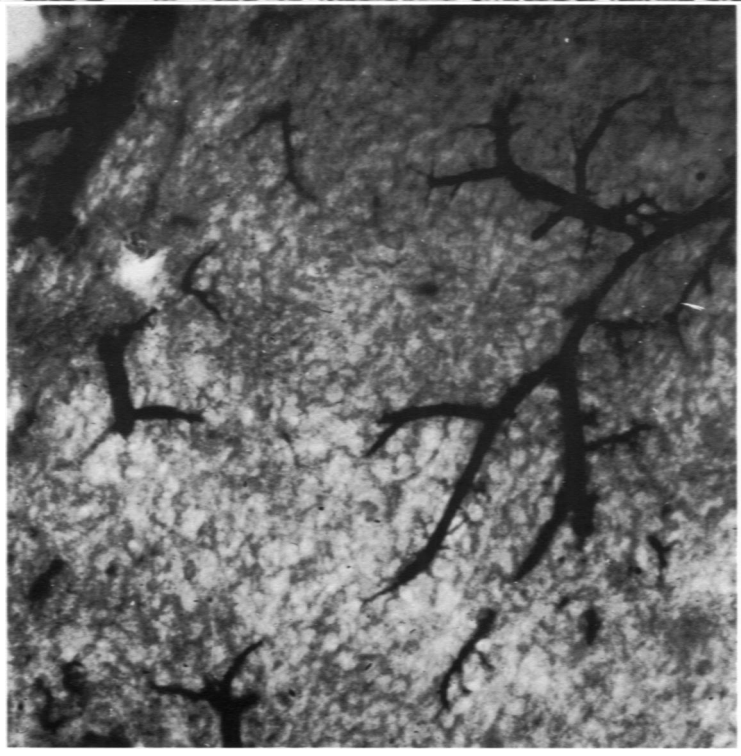

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Fig 9. Tumor growing within alveolar lumens without destruction of septums. Tumor is obtaining nourishment via diffusion across existing pulmonary artery capillaries. Primary undifferentiated carcinoma. Gelatin technique. EVG. × 104.

Fig 10. Tumor growing within alveoli with no evidence of pulmonary artery proliferation. Undifferentiated carcinoma. Gelatin technique. 250 mμ section. × 5.

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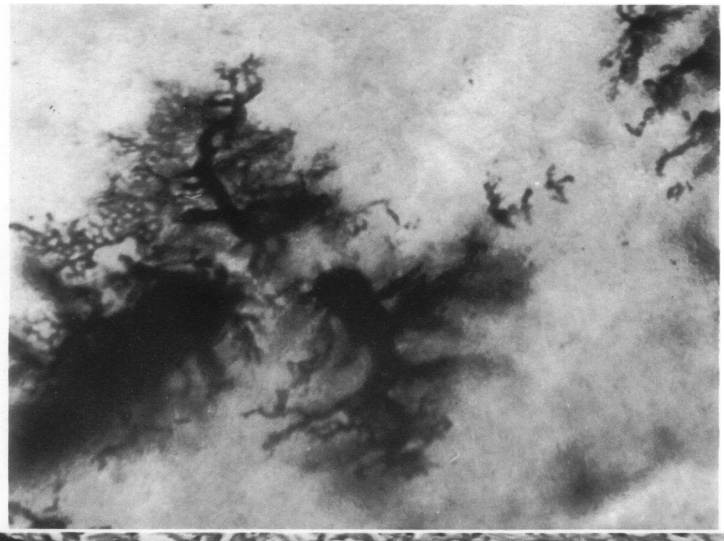

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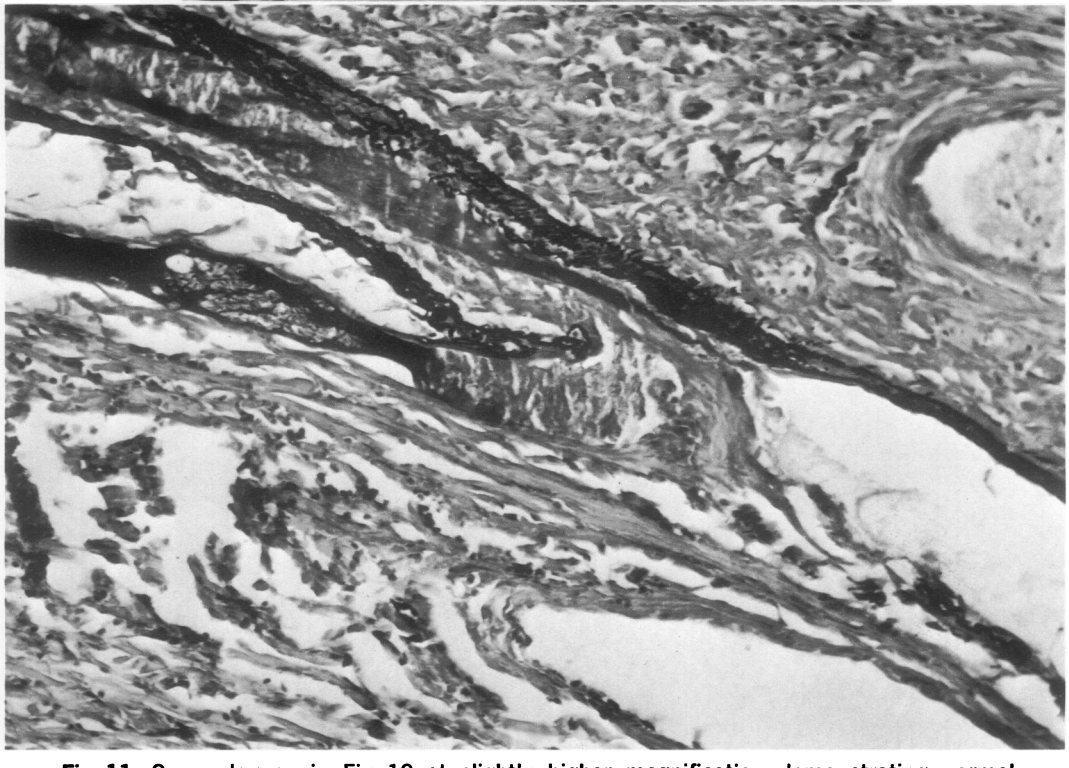
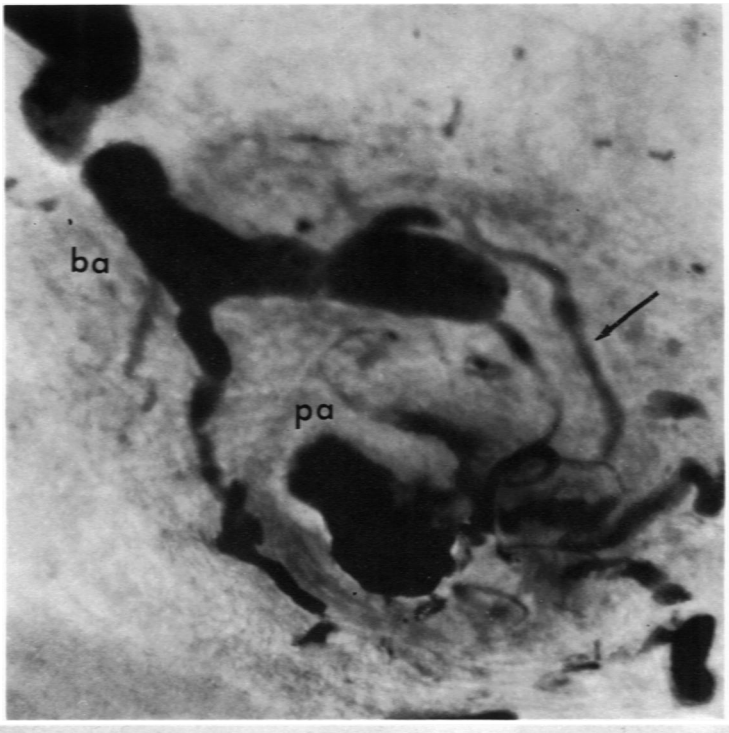


Fig 11. Same dog as in Fig 10 at slightly higher magnification demonstrating normal alveolar capillaries. Tumor occupies all areas in field. Pulmonary arteries have not proliferated, but some branches are distorted. Gelatin technique. 250 mμ section.

Fig 12. Bronchial artery to pulmonary artery anastomosis in tumor nodule. Gelatin technique. 7 mμ section. EVG. × 104.



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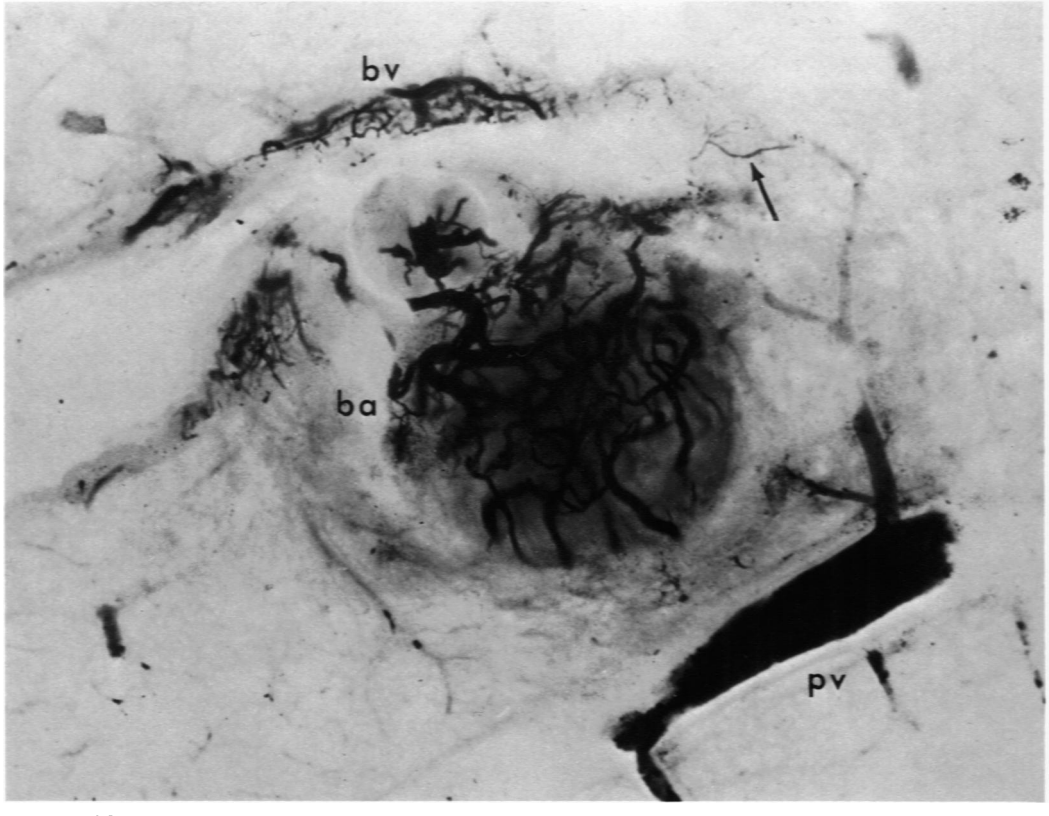

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Fig 13. Bronchial artery to pulmonary artery anastomosis within tumor nodule. Bronchial artery (*ba*); pulmonary artery (*pa*); anastomotic connections (*arrows*).

Fig 14. Tumor nodule supplied by a bronchial artery (*ba*). Venous drainage is via a pulmonary vein (*pv*) and bronchial veins (*bv*) located along the wall of a bronchus. There is an anastomosis between the pulmonary and the bronchial veins (*arrow*).