Malignant Mammary Tumors in Beagle Dogs Dosed With Investigational Oral Contraceptive Steroids [1, 2, 3]

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ABSTRACT—Of 172 beagle dogs administered investigational oral contraceptive steroids for 2.4–5.2 years, 9 developed malignant mammary tumors. At necropsy their ages varied from 41 to 70 months, with a mean age of 4.9 years. The malignant tumors were observed in 1 dog that received ethynerone plus mestranol at 1.05 mg/kg/day and in 4 dogs that received chlorethynyl norgestrel plus mestranol at 1.05 mg/kg/day. Also, 4 dogs that received anagestone acetate plus mestranol at either 0.44 or 1.10 mg/kg/day developed malignant mammary tumors. Malignant tumors were not seen in 33 dogs administered mestranol at 0.02 and 0.05 mg/kg/day for 7 years or in 18 dogs given ethynerone without mestranol at 1.00 mg/kg/day for 5 years. No malignant tumors were observed in 18 control dogs maintained for 7 years without treatment. Three dogs had single malignant mammary nodules, 3 dogs had 2 malignant nodules, 2 dogs had 4–6 malignant nodules, and 1 dog in the treatment group given high dosages of ethynerone plus mestranol had 14 mammary nodules composed of fibrosarcoma. The malignant tumors were histologically classified as 5 anaplastic carcinomas, 2 solid carcinomas, 1 tubular adenocarcinoma, 1 squamous cell carcinoma, and 1 fibrosarcoma. Most dogs had only 1 histologic type of cancer (8/9 dogs); however, 1 dog had carcinomas of both solid and anaplastic types involving different glands. Metastases were present in 5 dogs and most often involved regional lymph nodes and lung.—JNCI 65: 137–144, 1980.

Although the long-term administration of certain contraceptive steroids to beagle dogs has been shown to produce numerous palpable mammary nodules and tumors, few detailed clinicopathologic descriptions of the individual tumors as to tumor morphology, time of tumor development, and effects of the tumor on the dog have been reported (*1–8*). Recently, 12 adenocarcinomas were reported in groups of dogs treated with megestrol acetate or chlormadinone acetate for 7 years, but details other than their occurrence were not given (*8*). In previous publications we have described an unusual type of mammary adenoma in beagle dogs receiving certain oral contraceptive steroids as well as the clinical and pathologic features of 925 mammary nodules that occurred in 172 beagle dogs dosed with oral contraceptive steroids for 5–7 years (*9, 10*). In this communication we describe the detailed clinical and pathologic aspects of the malignant mammary tumors seen in 9 female beagle dogs administered oral contraceptive steroids.

MATERIALS AND METHODS

In 1968 the Food and Drug Administration initiated a series of long-term experiments on female beagle dogs to assess the potential tumorigenesis of certain contraceptive steroids (*5, 7*). These studies were intended to serve as "positive controls" for similar studies conducted by the pharmaceutical industry on marketed and investigational contraceptive steroids (*11, 12*). The investigational contraceptive steroids have not been marketed for use in humans. All specimens and clinical data for this report were obtained from these Food and Drug Administration studies.

A total of 172 treated and 18 control dogs comprising 12 experimental groups was evaluated (table 1). All dogs were purebred female beagles 10–14 months of age at the initiation of the experiment. The drugs administered were mestranol (methyl ester of ethinyl estradiol), ethynerone, ethynerone plus mestranol, WY-4355 (chlorethynyl norgestrel) plus mestranol, and anagestone acetate plus mestranol in continuing cycles of 21 days of treatment and 7 days without treatment. The duration of treatment was approximately 5 years for dogs administered ethynerone, ethynerone plus mestranol, WY-4355 plus mestranol, and anagestone acetate plus mestranol. The dogs that received mestranol and the control group were observed for approximately 7 years. The progestational compounds are halogenated 19-nortestosterone derivatives (WY-4355 and ethynerone) and a 17-acetoxy derivative of progesterone (anagestone acetate).

All dogs were hysterectomized at about 2 years of age to prevent deaths from pyometritis. Ovaries were left intact. Treatment of 1 dog in the group given anagestone acetate plus mestranol was discontinued during

ABBREVIATIONS USED: AFIP = Armed Forces Institute of Pathology; H & E = hematoxylin and eosin; L and R = left and right mammary glands; WY-4355 = chlorethynyl norgestrel.

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[3] Animals were maintained under the guidelines set forth by the National Research Council.

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the 29th month of the study. Mammary glands were palpated monthly, and size, location, and description of mammary nodules were recorded. The term "mammary nodule" refers to a palpable mass that was later confirmed by histopathologic evaluation to be composed of mammary tissue. At necropsy, tissue specimens were obtained from palpable mammary nodules, normal mammary glands, and all major organs. The specimens were fixed in 10% neutral buffered Formalin, embedded in paraffin, sectioned at 6 μm, and stained with H & E. Selected specimens were stained with Masson's trichrome, acid mucopolysaccharide with and without sialidase, reticulum, tannic acid-phosphomolybdic acid-amido black, periodic acid-Schiff with and without diastase, and phosphotungstic acid-hematoxylin. Multiple sections from each mammary nodule were usually examined histopathologically, including serial or step sections when appropriate. Tumors were classified according to the International Histological Classification of Tumors of Domestic Animals prepared by the World Health Organization (*13*). Criteria for the determination of malignancy were metastasis, permeation of lymphatics, or invasion of surrounding tissue.

RESULTS

During the course of the study, 9 dogs in 4 treatment groups receiving either ethynerone plus mestranol, WY-4355 plus mestranol, or anagestone acetate plus mestranol developed malignant mammary tumors (table 1). Clinical information concerning development of the malignant tumors, numbers of mammary nodules, and time the animal died or was killed are presented in table 2. The morphologic types of tumors, location or distribution of the tumor within the mammary gland, tumor size, and sites of metastases are summarized in table 3. The gross necropsy and histopathologic findings in dogs with malignant mammary tumors are presented by treatment group.

TABLE 1.—*Experimental groups and number of dogs with malignant mammary tumors in groups administered oral contraceptive steroids*

Contraceptive and dosage (mg/kg/day)[a]	No. of dogs with malignant tumors at necropsy	No. of dogs/ group
Control	0	18
Mestranol (0.02)	0	15
Mestranol (0.05)	0	15
Ethynerone (1.00)	0	18
Ethynerone + mestranol (0.084)	0	16
Ethynerone + mestranol (0.42)	0	16
Ethynerone + mestranol (1.05)	1	17
WY-4355 + mestranol (0.084)	0	16
WY-4355 + mestranol (0.42)	0	17
WY-4355 + mestranol (1.05)	4	16
Anagestone acetate + mestranol (0.44)	3	13
Anagestone acetate + mestranol (1.10)	1	13

[a] Ratio of progestin to mestranol was 20:1 for ethynerone plus mestranol and WY-4355 plus mestranol and 10:1 for anagestone acetate plus mestranol. Dosages correspond to 2, 10, or 25 times the proposed human dosage.

Ethynerone Plus Mestranol

Dog #68-544.—At necropsy this dog was emaciated, weighed 12.7 kg, and had generalized alopecia. The median size of the 14 malignant nodules was 31.1 mm, with a range of 2-300 mm (tables 2, 3). Large confluent malignant tumors were found in the L3 area, the result of coalescence of adjacent nodules. All malignant nodules were similar histologically and consisted of a highly cellular fibrosarcomatous proliferation of ovoid and spindle cells associated with a few collagenous and reticular fibers. The sarcomatous cells were often dispersed among benign proliferating tubules, which gave the histologic appearance in some areas of a sarcoma arising in an adenoma (figs. 1, 2). Tumor metastases of identical fibrosarcoma cells were found in the lung, liver, pleura, intercostal muscles, and superficial inguinal lymph nodes (fig. 3). The benign mammary nodules in this dog were histologically classified as 11 simple adenomas and 6 lobular hyperplasias. Other pathologic changes included nephrosclerosis, mild hydronephrosis, leiomyomas of the vagina and urinary bladder, and moderate cystic epithelial hyperplasia of the gallbladder.

WY-4355 Plus Mestranol

Dog #68-462.—This animal was obese and weighed 17.6 kg. Severe subcutaneous edema present in the ventral thorax and mammary region tended to obscure the mammary nodules. Both malignant tumors in this dog were classified histologically as anaplastic carcinoma. Multifocal aggregates of the anaplastic tumor cells were located within the ducts of adjacent nonneoplastic hyperplastic mammary lobules. Diffuse infiltrations of individualized neoplastic cells occurred in the surrounding stroma (fig. 4). Frequently, necrotic and degenerating malignant cells were seen within preexisting ducts accompanied by infiltrating neutrophils. In some areas the masses of tumor cells had obliterated duct walls and infiltrated the surrounding stroma, eliciting a periductal inflammatory response. Lymphatic invasion by malignant cells was common. Tumor cells were large and pleomorphic with hyperchromatic nuclei and eosinophilic cytoplasm that was sometimes granular or vacuolated. Mitotic figures were frequent. Metastases were found in the sublumbar lymph nodes, cervix, the outer wall of the anterior vagina, and the lung (table 3). Lung metastases were extensive and associated with pulmonary thromboembolism. The gallbladder exhibited marked cystic epithelial hyperplasia.

Dog #68-548.—At necropsy this dog exhibited extensive alopecia. Subcutaneous edema was present on the flanks and hind limbs. The two caudal pairs of mammary glands were indurated with focal abscesses,

TABLE 2.—*Number of mammary nodules, number of malignant neoplasms, month of onset, and time of death or killing of beagle dogs administered oral contraceptive steroids*

Animal No.	Contraceptive and dosage (mg/kg/day)	No. of nodules at necropsy	No. of malignant nodules	Treatment month that	
				Malignant nodule(s) were detected	Dog was killed (K) or died (D)[a]
68-544	Ethynerone + mestranol[b] (1.05)	31	14	11–33	53-K
68-462	WY-4355 + mestranol (1.05)	2	2	38, 40	47-K
68-548	"	1	1	25	29-D
68-556	"	18	1	24	53-K
68-458	"	18	2	21, 28	49-D
67-605	Anagestone acetate + mestranol (0.44)	20	4	37–40	62-K
67-667	"	3	1	38	41-D
68-112[c]	"	20	6	28–37	41-D
67-709	Anagestone acetate + mestranol (1.10)	14	2	26, 50	58-K

[a] Dogs were placed on test at 10–14 mo of age.
[b] The ratio of progestin to mestranol in the ethynerone and WY-4355 groups was 20:1 and 10:1 for anagestone acetate.
[c] Drug was withdrawn during 28th mo of treatment.

and a single large tumorous mass involved the entire L4-L5 mammary area. Histologically, the mass consisted of pleomorphic anaplastic carcinoma cells in both nodular and diffuse accumulations with reactive fibrosis and subacute mastitis characterized by large abscesses. The malignant cells were similar to those described in dog #68-462. Although invasion of local lymphatics was widespread, metastases were not found. The causes of death were extensive pulmonary thromboembolism and suppurative pneumonia possibly secondary to the severe mastitis.

Dog #68-556.—An ulcerated area was seen in mammary gland R5, the site of the malignant tumor. The tumor was a well-differentiated squamous cell carcinoma with abundant prickle cells and keratinization (figs. 5, 6). It appeared to arise from the teat ducts or cisternae at R5. Although the neoplasm extensively invaded surrounding tissues and lymphatics, metastases were not demonstrated. The benign mammary nodules seen in this dog included 11 simple adenomas, 3 complex adenomas, 1 benign mixed tumor, and 2 lobular hyperplasias. The gallbladder exhibited marked cystic hyperplasia.

Dog #68-458.—Due to extensive autolysis, only the mammary glands were available for histopathologic examination (tables 2, 3). The malignant nodules were similar histologically and consisted of multifocal involvement of hyperplastic mammary lobules by diffuse and nodular collections of large, pleomorphic, anaplastic epithelial cells. Malignant cells were found principally in terminal ducts and alveoli, the largest collections being intraductal. Carcinoma cells had numerous mitotic figures, and lymphatic and blood vessel invasion was widespread. Other nodules in this dog included 12 complex adenomas, 1 simple adenoma, and 3 lobular hyperplasias.

TABLE 3.—*Diagnosis, location, and size of malignant neoplasms and sites of metastases in beagle dogs administered oral contraceptive steroids*

Animal No.	Contraceptive and dosage (mg/kg/day)	Diagnosis	Location in mammary gland	Tumor size, mm	Sites of metastases	
					Regional lymph node	Other
68-544	Ethynerone + mestranol[a] (1.05)	Fibrosarcoma	L3-L5 R3-R5	2–300	+	Lung, liver, pleura, muscle
68-462	WY-4355 + mestranol (1.05)	Anaplastic carcinoma	R5,L1	2, 30	+	Lung, vagina, cervix
68-548	"	Anaplastic carcinoma	L4,L5	240	–	
68-556	"	Squamous cell carcinoma	R5	40	–	
68-458	"	Anaplastic carcinoma	R2	9, 15	–	Not examined
67-605	Anagestone acetate + mestranol (0.44)	Solid and anaplastic carcinoma	R3,L1, L2,L5	5–160	–	
67-667	"	Tubular adenocarcinoma	L1-L2	100	+	Lung, adrenal gland, bone
68-112[b]	"	Anaplastic carcinoma	L3-L4 R4-R5	Fn *c*	+	Bone, vagina, lung
67-709	Anagestone acetate + mestranol (1.10)	Solid carcinoma	L-5	Fn *c*	+	Lung

[a] The ratio of progestin to mestranol in the ethynerone and WY-4355 groups was 20:1 and 10:1 for anagestone acetate.
[b] Drug was withdrawn during 28th mo of treatment.
[c] Final size was not recorded due to extensive swelling and inflammation in region of carcinomas.

Anagestone Acetate Plus Mestranol

Dog #67-605.—The tumors in the R3 and L5 mammary glands were solid carcinomas. The carcinoma in R3 had small areas of tubular and papillary differentiation. Clusters of malignant cells with squamous features infiltrated the periphery of the mass. The carcinoma at L5 was composed of cords and clusters of epithelial cells that exhibited squamous metaplasia with little tubular differentiation. Stromal invasion occurred in a few areas. The malignant tumors in the L1 and L2 areas were anaplastic carcinomas (fig. 7). Metastasis could not be demonstrated. Benign mammary lesions consisted of 11 complex adenomas, 1 benign mixed tumor, 4 lobular hyperplasias, and 1 intraductal hyperplasia. Mild membranoproliferative glomerulonephritis was observed in the kidneys.

Dog #67-667.—Severe mastitis with fistulous tracts was found in the L1-L2 region. Histopathology revealed a simple tubular adenocarcinoma at this site. Tumor cells were arranged in tubular and solid patterns, sometimes associated with reactive fibrosis (fig. 8). The neoplastic cells were widely infiltrative causing ulceration of the overlying skin. In some areas, tumor cells infiltrated as small nests or individual cells. In a few instances, tumor cells appeared spindle shaped or squamous. The overall histologic pattern suggested that the carcinoma was arising in multiple lobules with centripetal spread from affected ducts. Metastases from this neoplasm involved the right superficial inguinal lymph node, lung, adrenal gland, and sternal bone marrow. The other nodules seen in this dog were 2 lobular hyperplasias.

Dog #68-112.—Drug administration for this dog was stopped after 28 months of treatment. A large effacing malignant mass was present in the L3-L4 area at the site where several smaller nodules had been palpated clinically beginning at approximately the 28th treatment month. The extensive chronic inflammatory process in this area prevented identification of the original mammary nodules at necropsy. Representative histologic sections taken through the L3-L4 area revealed hyperplastic lobules and multifocal areas of anaplastic carcinoma with reactive fibrosis and chronic mastitis. Lymphatic invasion by neoplastic cells was frequent. The cancer appeared to originate from mammary ducts. In addition, discrete foci of malignant epithelial cells were present in lymphatics adjacent to 4 benign tumors or hyperplasias located in the R4-R5 area and adjacent to a single benign tumor anterior to the large anaplastic carcinomatous mass involving L3 and L4. Serial sections of selected nodules with the carcinomatous lymphatic involvement revealed no areas to suggest that the malignant cells originated in the adjacent benign tumors or hyperplasias. Metastases were found in the left inguinal lymph node, lung, vagina, and bone marrow (fig. 9). Tumor metastases in the vaginal wall consisted of isolated areas of lymphatic invasion without parenchymal involvement. The remaining benign nodules in this dog consisted of 14 mixed tumors. Other significant lesions were severe vegetative endocarditis, hydrothorax, and extensive pulmonary consolidation due to severe chronic congestion, pneumonia, and tumor metastasis.

Dog #67-709.—The malignant nodules in this dog were located anterior and posterior to L5 (tables 2, 3). The posterior and larger tumor was predominantly a solid carcinoma composed of pleomorphic epithelial cells, which in some areas were spindle shaped (fig. 10). Smaller areas of tubular differentiation were also seen (fig. 11). Large areas of the intervening stroma were sclerotic. Some malignant cells contained vacuoles or eosinophilic secretory globules. The other malignant nodule, which was smaller and located anterior to the one just described, consisted of small foci of solid carcinoma with multiple areas of the gland showing lobular hyperplasia. Many of the malignant cells were within lymphatics and occurred as solid groups rather than as individual cells. Metastases were present in the lung and inguinal lymph nodes (fig. 12). The inguinal lymph nodes on the right side opposite the malignant neoplasms had large areas of metastatic carcinoma resembling the solid carcinoma in L5. The benign mammary nodules in this dog were 4 simple adenomas, 1 complex adenoma, 1 mixed tumor, and 6 lobular and intraductal hyperplasias. Additional pathologic changes in this dog consisted of severe chronic cholecystitis, pulmonary thrombosis and infarction, and diabetes mellitus associated with pancreatic islet cell vacuolation and bilateral lenticular cataracts.

DISCUSSION

The occurrence of malignant tumors in these dogs appears to be drug related, inasmuch as 6 of 9 dogs that developed malignant tumors were in the groups given high dosages of ethynerone plus mestranol, WY-4355 plus mestranol, and anagestone acetate plus mestranol. The 3 remaining dogs with malignant tumors were in the group administered 0.44 mg anagestone acetate plus mestranol/kg/day. A similar association between the groups given higher dosages and the incidence of malignant tumors was noted in dogs treated with other contraceptive steroids such as chlormadinone and megestrol acetate (*8*). As we reported previously, the dogs with malignant tumors were in treatment groups that had the largest numbers of benign tumors and hyperplasias (*10*). The administration of ethynerone without mestranol to 18 dogs at 1.00 mg/kg/day for 5 years produced no malignant tumors and few benign tumors or hyperplasias. Malignant tumors were not found in 30 dogs administered the estrogen compound mestranol at 0.02 and 0.05 mg/kg/day for 7 years or in 18 control dogs maintained for 7 years without treatment.

Considerable variation was found among these dogs regarding the total numbers of malignant mammary nodules present at necropsy; the range was from 1 to 14. Primary mammary tumors may be multiple in 25-44% of bitches; however, this tendency is apparently

more common in benign rather than malignant tumors (*14-16*). In this study, 6 dogs had 1-2 malignant nodules, whereas 2 dogs had 4-6 nodules. A single dog (#68-544) in the group given high dosages of ethynerone plus mestranol had 14 nodular masses composed of fibrosarcoma in R3-R5 and L3-L5 glands. Although many of these 14 nodules appeared as distinct masses suggesting multifocal origin, it was impossible to exclude the possibility of metastasis, especially because these tumors involved adjacent mammary glands where lymphatic interconnections are known to exist.

Malignant tumors comprised 33 of 124 or 27% of the proliferative mammary lesions in these 9 dogs. A considerable spectrum of benign lesions also existed consisting of lobular or intrductal hyperplasias, simple adenomas, complex adenomas, and benign mixed tumors. However, no statistical evidence was found by the use of linear regression of an association between the frequency of specific morphologic types of benign tumors and hyperplasias in these dogs and the occurrence of malignant tumors.

In surveys of spontaneous tumors, the mean age of dogs with malignant mammary tumors is usually reported to be around 9-11 years (*17-19*). The ages of these dogs at death or when they were killed were considerably younger (3.4-5.8 yr). When the first malignant nodules were palpated, the ages of the dogs ranged from 23 to 50 months. These earliest palpable lesions probably existed much earlier as occult foci. In attempts to identify preneoplastic lesions, some investigators, using wholemount subgross techniques (*20, 21*), studied the canine mammary gland in dogs 3-12 years of age. They reported that hyperplastic alveolar nodules were the most frequent lesions, the occurrence of which increased greatly with age. After studying the morphologic continuum of these mammary lesions, the authors suggested that the hyperplastic alveolar nodules were preneoplastic. In a previous publication we found evidence to support the above view that mammary hyperplasias probably precede and, in some instances, may progress to benign tumors (*10*). In the present study the clinical and morphologic data neither suggest nor deny that an unequivocal morphologic continuum exists between the development of benign tumors and malignant tumors. In several instances serial sections of benign nodules containing malignant cells suggested infiltration by cells rather than malignant transformation of benign lesions. Other investigators suggested that malignant mammary tumors in the bitch may arise independently of the benign tumors (*8*). Conclusive proof of the neoplastic or malignant potential of these early lesions in the canine mammary gland awaits further studies because no cytologic marker or other marker has been found to predict the biologic potential of the presumptively preneoplastic lesions either in the dog or in other species (*20*).

In this study carcinomas were the most frequent type of malignant tumor observed (8/9 dogs), whereas multiple sarcomas were found in 1 dog. Although the carcinomas were no different morphologically from those seen in published surveys, their frequencies were somewhat different. In reports (*22-24*) in which the World Health Organization tumor classification system was used as in the present study, adenocarcinomas (tubular and papillary) and solid carcinomas were the most common histologic types of malignant tumor. In this study, however, anaplastic carcinoma was most frequent and made up 63% (5/8 dogs) of the morphologic types of carcinoma seen in this small group of dogs (*22-24*).

Morphologically, the anaplastic carcinomas in this series corresponded to previous descriptions (*23, 25*). These tumors are highly invasive with extensive lymphatic invasion, stromal fibrosis, and associated inflammation. Some of these anaplastic carcinomas did not present clinically as discrete nodules but rather as an extensive swelling throughout several glands, which suggested mastitis rather than neoplasia. In some instances the inflammatory reaction appeared to be secondary to the extensive lymphatic invasion by the tumor and resembled the "lymphangitic carcinosis" described by some authors (*26*). Anaplastic carcinomas are highly lethal, and survival is relatively short compared to survival from other types of canine mammary carcinomas (*22-24*). In 1 dog (#68-462) anaplastic carcinomas appeared to be multifocal in origin because both nodules were palpated originally at about the same time and were in mammary glands R5 and L1. Therefore, that these tumors could represent invasion from a single primary focus is unlikely.

In the present study we found that the clinical behavior of a nodule as related to size, growth characteristics, or clinical appearance was not necessarily a reliable means to determine if a nodule was likely to be malignant or benign. Not all of the malignant tumors showed a progressive growth pattern or attained a large size. After initial growth and palpation, certain nodules remained static for long periods until necropsy.

The finding of bone metastases in 2 dogs having either tubular or anaplastic carcinomas is of interest inasmuch as bone metastases are reported to be rare in canine mammary cancer (*26, 27*). Certain authors have concluded that these bone metastases of canine malignant mammary tumors are probably underreported because of the difficulties involved in conducting detailed evaluations of the skeletal system (*15, 18*). In support of this conclusion, a recent report from a large clinical center states that mammary tumors are the most frequent source of osseous metastases in dogs with metastatic bone tumors; however, the numbers of affected dogs were not given (*28*). Regional lymph node and lung metastases were common in this group of dogs and are likewise frequent in spontaneous tumor surveys (*18, 25*).

These studies demonstrate that the long-term administration of the investigational contraceptive steroids—anagestone acetate and WY-4355 combined with mestranol at 10-25 times the intended human dosage—induces a high incidence of malignant mammary tumors

in the dog. Because only 1 of 26 dogs administered ethynerone and mestranol at mid-dosage and high-dosage levels developed malignant tumors, the carcinogenic action of ethynerone plus mestranol is less clear. Inasmuch as a high incidence of spontaneously occurring malignant mammary tumors is common in the dog, we are uncertain whether these steroid compounds are true carcinogens or act as promoters, accelerating the development of spontaneous mammary tumors at an early age in the dog's life.

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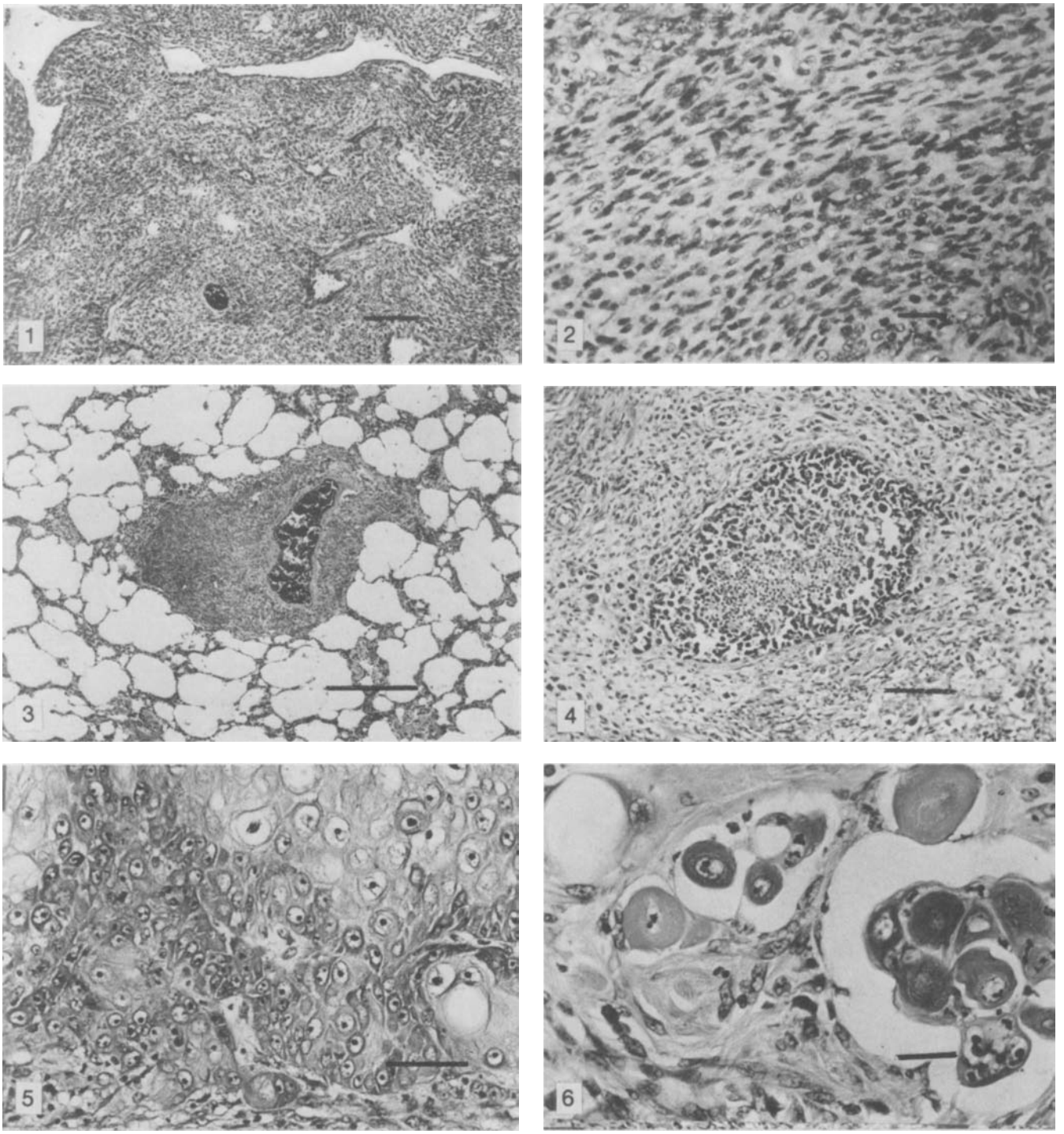
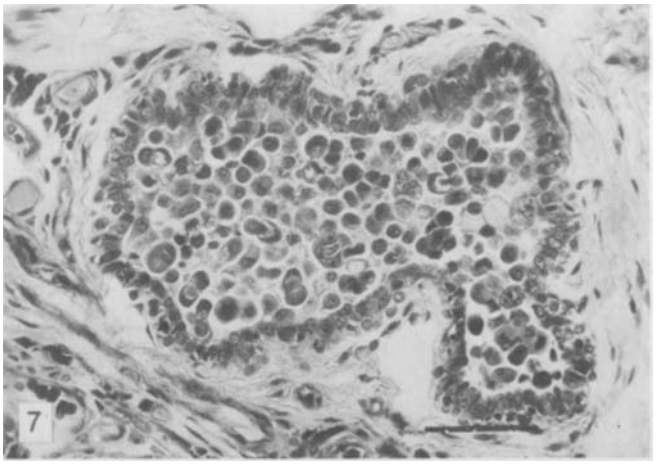
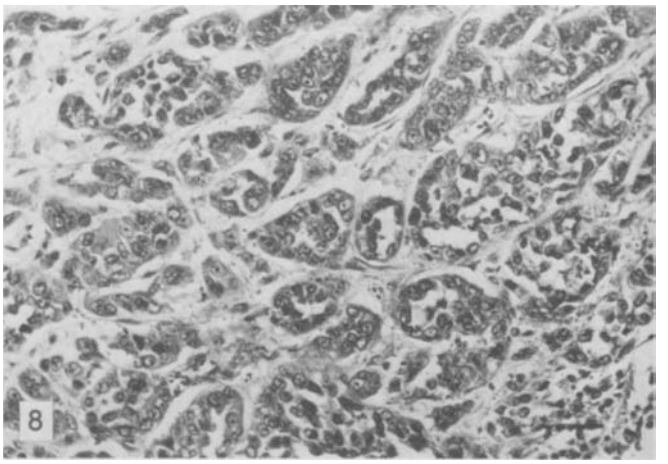
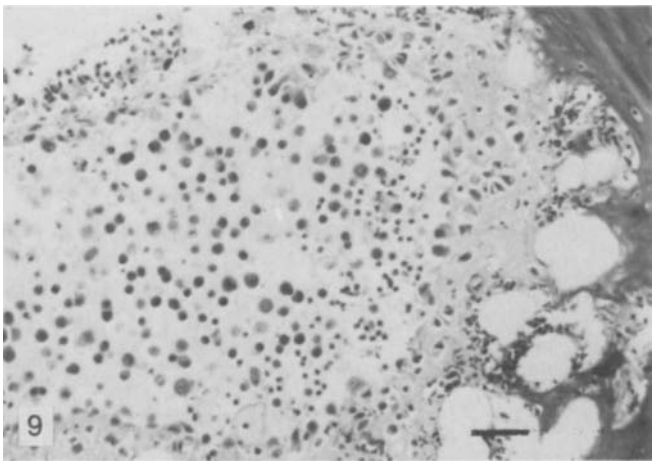
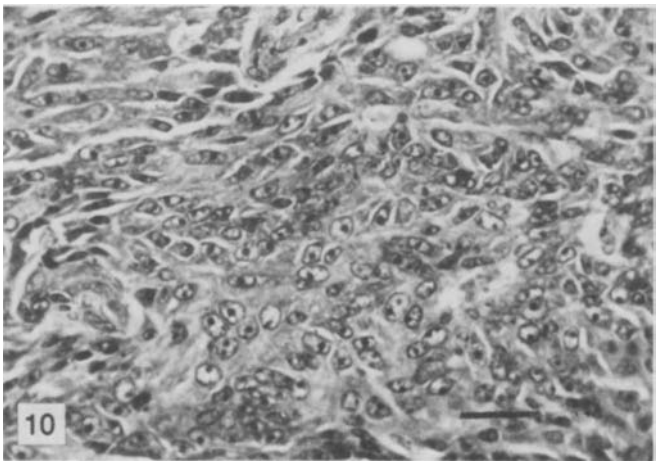


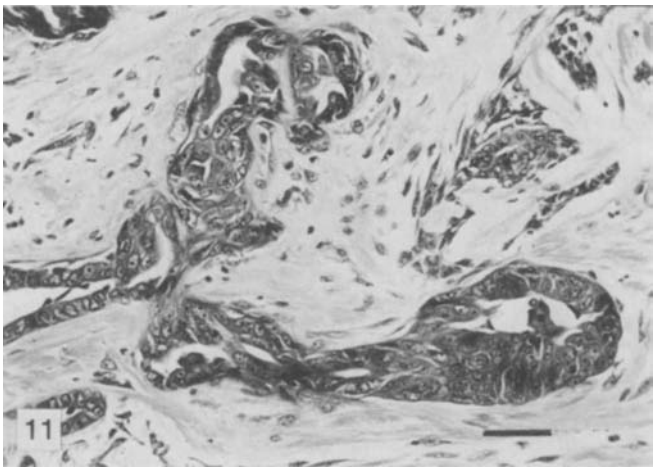
FIGURE 1.—Proliferation of fibrosarcoma cells around mammary ducts in dog #68-544; AFIP #76-7010. H & E. *Bar*=50 μm. × 180
FIGURE 2.—Higher magnification of the fibrosarcoma in dog #68-544; AFIP #77-3089. H & E. *Bar*=25 μm. × 305
FIGURE 3.—Pulmonary metastasis of mammary fibrosarcoma in dog #68-544; AFIP #77-3984. H & E. *Bar*=100 μm. × 145
FIGURE 4.—Anaplastic carcinoma from dog #68-462. Mammary duct is filled with malignant cells that have also infiltrated the surrounding stroma. AFIP #77-3988. H & E. *Bar*=100 μm. × 115
FIGURE 5.—Squamous cell carcinoma in mammary gland of dog #68-556; AFIP #77-2943. H & E. *Bar*=50 μm. × 210
FIGURE 6.—Mammary lymphatic invasion by neoplastic squamous cells in dog #68-556; AFIP #75-11983. H & E. *Bar*=25 μm. × 395











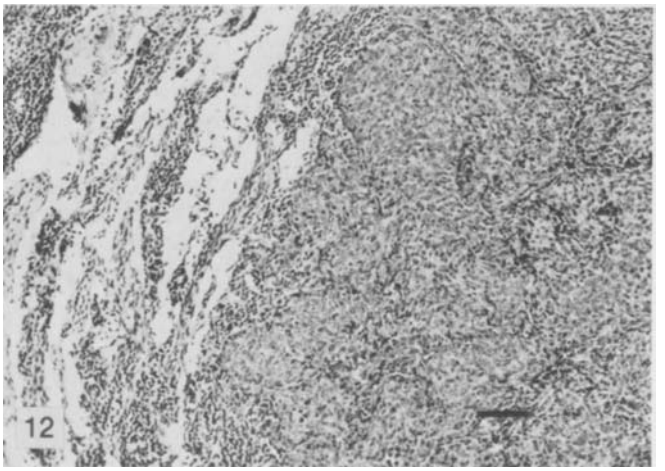


FIGURE 7.—Anaplastic carcinoma in dog #67-605. Pleomorphic epithelial cells in a large mammary duct. AFIP #76-6491. H & E. *Bar*=50 μm. × 305

FIGURE 8.—Tubular adenocarcinoma in dog #67-667; AFIP #77-3092. H & E. *Bar*=50 μm. × 165

FIGURE 9.—Metastatic anaplastic mammary tumor in bone marrow of dog #68-112; AFIP #77-9530. H & E. *Bar*=50 μm. × 160

FIGURE 10.—Solid carcinoma in mammary gland of dog #67-709; AFIP #77-3436. H & E. *Bar*=25 μm. × 350

FIGURE 11.—Area of tubular differentiation in solid carcinoma illustrated in fig. 10. AFIP #77-3994. H & E. *Bar*=50 μm. × 180

FIGURE 12.—Tumor metastasis in cortex of lymph node of dog #67-709. Solid mass of tumor cells is at *right*. AFIP #77-4000. H & E. *Bar*=100 μm. × 70