A COMPARATIVE STUDY OF SELECTED CANINE SKIN NEOPLASMS

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ABSTRACT

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by Dibakar Panigrahi

A study was made of the canine skin neoplasms diagnosed as transmissible venereal tumor, adnexal carcinoma or basal cell carcinoma in the Department of Pathology over a 10-year period. Of the 459 neoplasms in these groups, 43 were diagnosed as transmissible venereal tumor, 302 as adnexal carcinoma, and 114 as basal cell carcinoma. These neoplasms were compared with regard to breed, sex and age incidences and primary location in the body. Microscopic study was made of 25 transmissible venereal tumors, 25 adnexal carcinomas and 25 basal cell carcinomas. Histochemical studies were done on 22, 17 and 19 neoplasms, respectively, from the 3 groups.

Of the 25 transmissible venereal tumors studied microscopically, only 3 had the typical histologic characteristics described for this neoplasm. Most of the remaining neoplasms in this group histologically resembled the adnexal carcinoma. This discrepancy probably represents a change in the diagnostic criteria used in recent years in evaluating skin neoplasms of dogs. The 3 neoplasms typical of transmissible venereal tumors occurred on the genital organs and in the neighboring area.

The adnexal carcinoma appears to represent a distinct group of neoplasms seen in dogs and is probably sometimes diagnosed as histiocytoma or extragenital venereal tumor. It occurs frequently in the

skin of young dogs. Boxer dogs are commonly affected. The term atypical epithelioma is suggested by the author for this group of neoplasms, for the following reasons:

1. The neoplastic cells appear to originate from the stratum germinativum of the stratified squamous epithelium of the epidermis and other areas, such as lips and oral cavity, and the epithelium of hair follicles.

2. The cells are atypical due to the fact that they are pleomorphic and do not resemble the parent cells from which they originate.

This neoplasm may easily be confused with the transmissible venereal tumor when the latter is present in extragenital sites. No specific criteria could be established in this study to differentiate atypical epithelioma from transmissible venereal tumor. The use of special stains within the limitations of fixed tissues is not very helpful in their differentiation. Microscopic characteristics, history and the area of location of the neoplasm are of some value to the pathologist. More work with histochemistry on fresh tissues, chromosome counts, tissue culture, and electron microscopy is needed to define the nature of the neoplastic cells.

The basal cell carcinoma is microscopically distinct from the transmissible venereal tumor and adnexal carcinoma. The tumor occurred frequently in Cocker Spaniel dogs. Older dogs were affected. These neoplasms were commonly located in the head and neck areas. The occurrence of glycogen in the cytoplasm of the neoplastic cells in some of them is of interest. The average greatest nuclear diameter is significantly greater in basal cell carcinoma than in transmissible venereal tumor or adnexal carcinoma.

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To my uncle

Sri. Baikuntha nath Panigrahi

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INTRODUCTION

Although transmissible venereal tumors of dogs were reported nearly a century ago, the actual cell type involved in the formation of this tumor is not unanimously agreed upon. Probably no neoplasm of dogs has aroused so much interest and confusion among research workers as has this one. Some investigators have suggested the name "histiocytoma" (Mulligan, 1948) based on the histologic characteristics of the tumor, while others do not accept this nomenclature.

A group of tumors occurring primarily in the skin of dogs, with histological characteristics somewhat similar to the transmissible venereal tumor, has been encountered in the Department of Pathology, Michigan State University. These tumors have been considered as originating from the basal cell layer of the epidermis and adnexal structures of the skin and accordingly designated as "adnexal carcinomas". These would have probably been referred to as "extragenital venereal" tumors by some investigators (Smith and Jones, 1966).

Although basal cell carcinomas are quite distinct in their morphologic characteristics, they were included in this study because they can be confused with the transmissible venereal tumors in extragenital locations and with adnexal carcinomas.

The objectives of this study were:

1. To study the morphologic characteristics of the neoplastic cells from the 3 tumor types.

2. To demonstrate any possible differences between these neoplasms by the use of special stains.

3. To summarize the incidence by breed, age, sex and anatomical location.

REVIEW OF LITERATURE

Transmissible Venereal Tumor

The transmissible venereal tumor of dogs is a unique biological entity and has created considerable interest among workers in both veterinary and human medical research. The origin of the neoplastic cells is yet to be ascertained.

Several names, such as "infective sarcomata" (Smith and Washbourn, 1899), "endothelioma" (Beebe and Ewing, 1906), "transmissible lymphosarcoma" (Feldman, 1932), "contagious lymphosarcoma" (DeMonbreun and Goodpasture, 1934), "venereal sarcoma" (Stubbs and Furth, 1934), "neuroblastoma" (Jackson, 1936, 1944), "histiocytoma" (Mulligan, 1948), and "transmissible venereal tumor" (Bloom, Paff, and Noback, 1951) have been applied to this tumor since the first report that appeared nearly a century ago (Novinsky, 1877). Almost all the classifications, however, have been based on the histologic characteristics and general behavior of the tumors. The transmissible nature of this tumor, both by coitus and experimental transplantation, has been well established in numerous experiments. The most important of these experiments have been conducted by Smith and Washbourn (1898), White (1902), Sticker (1904, 1906a), Beebe and Ewing (1906), Beebe (1907), Wade (1908), Stubbs and Furth (1934), DeMonbreun and Goodpasture (1934), Bloom *et al.* (1951), and Karlson and Mann (1952). The tumors were successfully transplanted into fox cubs (Sticker, 1906b; Wade, 1908), but almost all the laboratory animals, including cats and chickens, were refractory to experimental

transfers (Smith and Washbourn, 1898; Sticker, 1904, 1906a; DeMonbreun and Goodpasture, 1934).

Development of immunity to further transplants following spontaneous regression of the transmissible venereal tumors has been reported by most workers in this field. But to the knowledge of the author, no one has explained the mechanism responsible for such reaction. The relatively benign nature of the neoplasm has also been recognized, although a few reports have dealt with its metastasis to lymph nodes, spleen, liver, and eye (Feldman, 1929; Rust, 1949; Karlson and Mann, 1952; Barron, Saunders, Seibold and Heath, 1963). Microorganisms were suspected by some early workers of being responsible for the etiology and transmission of the tumor, but subsequent works proved that it could be transplanted only by viable tumor cells and no organisms were involved.

Shimkin (1955) cited Novinsky (1877) as being the first worker who experimentally transmitted this tumor to puppies in his laboratory. He has been referred to as the "forefather of experimental oncology" (Stewart, Snell, Dunham, and Schlyen, 1959).

Smith and Washbourn (1898, 1899) reported the incidence of this neoplasm in "highly-bred" dogs. In 1 dog they noted metastatic tumor cells in other organs. The authors stated that the malignancy seen in "highly-bred" and ill-nourished dogs might not manifest such characteristics in healthy animals. The tumor they studied had the histologic structure of "round-cell sarcoma" with very little stroma.

"Contagious tumors" were observed by White (1902) in 2 purebred female bulldogs. One of these animals, which had a vaginal growth, also developed a mammary tumor. Histologic examination of tumors from both these sites revealed small, closely packed round cells with delicate

stroma. The author concluded that transmission of these tumors might be an example of transplantation rather than infection.

Beebe and Ewing (1906) observed a number of these neoplasms in New York City. Microscopic examination revealed the arrangement of tumor cells in cords supported by thin strands of connective tissue. They believed that it was a true malignant neoplasm and thought that the transplanted cells actually gave rise to the tumor in the hosts. These writers diagnosed the tumor as an "alveolar sarcoma" or "endothelioma".

Beebe (1907) implanted the tumors into dogs by several methods with some interesting results. He stated, "An immune animal may again become susceptible by any process which would be likely to diminish his general resistance". He did not believe that any microorganism was responsible for either origin or transmission of the transmissible venereal tumors. The neoplasm under his observation had a benign nature in the beginning, but subsequent metastasis caused cachexia and eventually death of the animals.

Several bacterial endotoxins injected into the tumor tissues and at a considerable distance from them in experimental animals caused necrosis and subsequent regression of the tumors until they disappeared (Beebe and Tracy, 1907). However, 1 dog treated with suspensions of "Staphylococcus pyogenes aureus" had no tumor regression.

Whole blood transfusion from spontaneously recovered and naturally immune animals to the tumor-bearing dogs caused complete regression in 7 out of 10 dogs. Only 1 dog died without showing any evidence of regression (Crile and Beebe, 1908). The authors believed the regression was probably due to transfer of some immune material through the blood.

Wade (1908) successfully transplanted the tumors through 8 tumor generations. In 1 case (2nd generation), secondary foci were noted in several visceral organs. He emphasized that the implanted cells stimulated the connective tissue cells of the host through intracellular viruses to give rise to new tumors, although he could not demonstrate the presence of any such microorganisms. This theory, however, has not been accepted by other workers. Wade noted acute interstitial nephritis, which he thought was due to a soluble toxin from the tumor cells, in many of his experimental dogs. This has not been reported by others, possibly because kidneys have not been routinely included in their studies.

Huebner (1922) reported the involvement of both eyes of a 2-year-old male Fox Terrier with metastatic tumors. The dog also had a penile tumor that had been removed previously. Several lymph nodes had metastatic foci. Huebner called this tumor a "small-celled, medullary round cell sarcoma". His attempts to transmit the tumor to other dogs met with no success. It is therefore doubtful that the tumor he was dealing with was a true venereal tumor. However, his diagnosis cannot be ruled out completely, as other workers have not been successful in transplanting the tumors into all dogs they studied.

Glass (1923) reported on the incidence of the tumor in the United States and thought that the disease was carried through English Bulldogs to this country and subsequently spread to other dogs.

Attempts to transplant the tumor by vaginal scarification and planting under the rectus fascia of the abdominal wall in 2 dogs were disappointing (Novak and Craig, 1927). Although the tumors were removed from the vagina, their failure to grow in transmission experiments remained unexplained.

Feldman (1929, 1932), in his comprehensive review, called the tumor a "transmissible lymphosarcoma". His diagnosis was based on the cellular morphology, and he believed that the tumor was arising from undifferentiated lymphocytes, although definite proof was lacking. Feldman listed 81 tumors of dogs, of which 7 (8.6%) fell into this classification. All the tumors listed by him were from mongrels. Two tumors from the same dog, 1 from the orbit and another from below the skin of the frontal region, were described as resembling the genital tumors. However, no transmission experiments were done to define the nature of the orbital neoplasm. Microscopic examination revealed the presence of large, irregular cells with fairly granular cytoplasm. Cell nuclei were large, with much chromatin and slightly eccentric nucleoli. Many mitotic figures were seen. Histochemical methods were negative for intercellular reticulum fibers.

Stubbs and Furth (1934) expressed doubts regarding the lymphocytic origin of the cells constituting the venereal sarcoma of dogs. They stated,

> "Evidence for the view that the tumor cells are lymphocytes is wanting. ...The cells in this growth were never seen maturing into typical lymphocytes. For this reason its designation as lymphosarcoma has no basis."

They also thought that the cells were not histiocytes; no phagocytosis was observed in fixed tissues. The term "endothelioma" was not acceptable to them, as the origin of the cells from the endothelium was inapparent.

DeMonbreun and Goodpasture (1934) made significant research contributions. Histochemical investigation revealed the absence of mitochondria, which they thought might have been masked by the large numbers of fat globules that were present in the tumor cells. Their attempts to stain

the cells, by both supravital and intravital methods, were regarded as failures. They postulated that the tumors probably were derived from the cells of the lymphocytic series. In one of their experiments, they noted a destructive action of serum from rabbits immunized with tumor tissue emulsion on the tumor cells in vitro. No tumor could be produced in dogs following inoculation of cells previously exposed to the serum.

An interesting and useful report was presented by Jackson (1936) regarding the various differences between the basal cell tumor and the contagious venereal tumor of dogs, which he thought might be confused with each other when occurring in unusual locations. He observed a striking similarity in the microscopic characteristics of venereal tumors and heart-base tumors of dogs. This led him to suspect a common origin for both the tumors. However, transmission experiments, which might have thrown some light on the apparent differences between them, were not conducted.

Jackson (1944) demonstrated the presence of numerous lipid globules in the tumor cells by a specially devised acetic-carbol-Sudan method. He thought that the globules had been overlooked by previous workers. This supported the observation of DeMonbreun and Goodpasture (1934).

From Ireland, an increased incidence of "infectious venereal granuloma" in Greyhounds was described by Gleason (1947). He noted that the histologic characteristics resembled those of round-cell sarcoma, although the stroma was better developed in venereal granuloma.

Lacroix and Riser (1947) reported 52 "transmissible lymphosarcomas" in dogs, of which 2 had penile locations. The neoplasm was common in Boston and Scottish Terriers, and a common location was the ear. Dogs below 1 year of age had the highest incidence.

Some interesting observations were published by Mulligan (1945, 1948, 1949). In the beginning he suspected some hormonal relationships with the incidence of venereal tumors. Mulligan studied 5 skin tumors of dogs and concluded that they were similar, microscopically, to lymphosarcoma (lymphoblastic type). He did not consider them as extragenital venereal sarcomas because he had never found the latter in hundreds of dogs observed in his laboratory over a period of 8 years. Even though he considered this tumor as lymphosarcoma, subsequently the name "histiocytoma" was suggested by him. He concluded that the previously reported venereal sarcoma may have been a histiocytoma.

The lymphoid nature of the "transmissible lymphosarcoma" was accepted by Rust (1949). Of the 12 tumors he observed, only 1 had metastatic lesions in visceral organs.

Bloom et al. (1951) clarified much of the long-existing confusion regarding the histogenesis of transmissible venereal tumors of dogs. After extensive studies on different aspects of the neoplasm they indicated that the cells were mature end-cells of reticulo-endothelial origin, as they did not grow further in in vitro experiments. Histochemical studies revealed the presence of fine granules of mitochondria, Golgi elements, and moderate amounts of acid phosphatase in the nuclei and traces in the cytoplasm of tumor cells. They also observed a considerable number of lipid droplets in degenerate cells. In intact cells they were present as "bound lipids" (Jackson, 1944). Cytochrome oxidase, peroxidase, phospholipids, glycogen, and PAS-positive materials were absent. The authors disagreed with the use of the term histiocytoma and suggested retaining the name "transmissible venereal tumor".

Reviewing the "transmissible venereal tumor" of dogs, Karlson and Mann (1952) referred to it as a true neoplasm. They did some significant studies of the transplantation of this tumor to other dogs. Using 601 dogs in 62 groups, they transmitted for 40 tumor generations. The highest incidence (95%) was in the 15th generation. However, only 5 dogs from the entire group had metastatic growths. Surprisingly, there was no significant change in the microscopic character of the tumors, even after several passages.

Cotchin (1954) stated that the incidence of "transmissible venereal tumor" in dogs was lower than in previous years. In another report Cotchin (1956) listed 8 instances of the transmissible venereal tumor among 104 genital tumors of dogs, 3 from the vagina and 5 from the male genital organs. In the beginning he expressed doubt regarding its neoplastic nature, but subsequently he believed it to be a true neoplasm.

A high incidence of "venereal sarcoma" in dogs in Indonesia was recorded by Ressang, Titus, Sikar, and Nabib (1958). Their studies of these neoplasms revealed a wide clinical and histologic variation from the European data.

Moulton (1961) gave a comprehensive review regarding the incidence and gross and microscopic characteristics of this tumor. He expressed doubt that the venereal tumors reported in extragenital sites were true venereal tumors, as transmission experiments to prove their identity were lacking. He indicated the possibility of confusing this tumor with the mastocytoma and histiocytoma occurring in the skin.

The first and, to date, the only experiment, to the knowledge of the writer, regarding the nutritional requirements of the "transmissible

venereal sarcoma" cells was published by Prier (1963). He attempted to cultivate the cells in the laboratory on different commercial media. Luxuriant growth with maximum cell yield was achieved on Puck's medium with addition of 20% calf serum. Addition of 50 mg. of cysteine per liter of the medium caused 85% reduction in the cell yield, whereas no detectable growth was noted when this amino acid was added in levels of more than 250 mg. per liter. However, low levels of cystine or cysteine in the base medium were essential for production of viable cells in large numbers. Addition of methionine in levels above 25 mg. per liter of the medium caused degeneration of the cells.

Barron, Saunders, Seibold, and Heath (1963) studied 2 additional transmissible venereal tumors involving eyes of dogs and thought that the tumor might have some affinity for intraocular metastasis. Several other reports also confirm their observation (Huebner, 1922; Feldman, 1929; Rust, 1949; Karlson and Mann, 1952).

A valuable contribution was made by Makino (1963) on chromosome numbers, their distribution, and DNA content in the tumor cells. This was a new approach toward classifying the neoplasm. His observation included 17 tumors obtained from different areas of Japan. He noted morphologic and numerical alterations and structural changes in chromosomes of tumor cells. The chromosome number was 59 instead of the normal 78 (76 autosomes and a pair of sex elements); 17 of them were metacentric and 42 acrocentric. An increased DNA content in the cells was suggested to be due to either increased mitosis or change in total volume of chromosomes.

Weber, Nowell, and Hare (1965) confirmed the observations of Makino (1963) by chromosome counts. Their experiments with an artificially

transplanted "venereal sarcoma" and a primary "venereal sarcoma" with multiple metastases revealed the presence of identical karyotypes in both. However, among the total of 59 chromosomes, 15 metacentric and 44 acrocentric forms were noted as compared to 17 and 42, respectively, in the report by Makino. Both the tumors under their investigation were removed from the genital organs of dogs in the State of Pennsylvania. The authors commented on the histologic characteristics of the tumors and thought they were quite useful in differentiating this tumor from histiocytoma, mastocytoma, and lymphosarcoma.

Referring to this tumor as "histiosarcoma", Runnells, Monlux, and Monlux (1965) stated that the tumor is composed of histiocytes. Confinement of this tumor to the genitalia and occasionally to the skin of young dogs (2 to 6 years of age) was mentioned.

Referring to the "transmissible venereal tumor" of the dog, Smith and Jones (1966) mentioned its frequent location near or on the genitalia. Another tumor with similar histologic characteristics that occurred in the skin other than genitalia of young dogs was described as "extragenital venereal tumor" or "round-cell tumor". According to their observation, this tumor was a different entity from the transmissible venereal tumor. They demonstrated the presence of normal chromosomes, both in number and morphology, in the extragenital venereal tumor cells, in contrast to the reduced number and abnormal morphology of chromosomes in cells of transmissible venereal tumor. This observation is definitely significant but disagrees with the conclusions drawn by Mulligan (1948).

Histiocytoma

Mulligan (1948) suggested the name "histiocytoma" for a group of neoplasms located in the skin and on the genital organs of predominantly young dogs. His diagnosis was based chiefly on the microscopic characteristics of the tumors. Two of 21 tumors were located on the genital organs. Histochemical studies revealed the absence of lipids. Reticulum fibers, which he thought were part of the stroma, were present. To defend his diagnosis, Mulligan (1949) stated,

> "The cutaneous connective tissue is the natural habitat of the histiocyte, not the case with any cell of the lymphoid series. When a neoplasm, not of fibroblastic or of mast cell type, is noted in this tissue, the histiocyte is the logical cell to suspect, whether cutaneous or genital locations are involved. So called venereal lymphosarcoma is apparently histiocytoma."

The human counterpart of histiocytoma was mentioned as "xanthoma" (Mulligan, 1961). The author also suggested deleting the term "venereal sarcoma".

Ottosen (1949) studied several skin neoplasms in dogs. His study included "histiocytic sarcomata" in 10 dogs. Almost all the tumors were recorded in older dogs (about 10 years of age). This statement does not agree with that of Mulligan (1948), in which the latter noted an increased tumor incidence in younger dogs (up to 5 years of age). Ottosen noted giant cells in the tumors, an observation which has not been mentioned in other reports. Also, there were numerous fat globules of uniform size in the tumor cells. This again contradicts the original report of Mulligan in which he mentioned the absence of fat globules in the tumor cells. It seems that Ottosen probably dealt with a group of tumors quite different from the histiocytomas originally reported.

Bloom et al. (1951) conducted studies of these tumors and suggested eliminating the term histiocytoma, as the tumor cells were not histiocytes.

Head (1953) reported the incidence of "histiocytic cell sarcoma" in young dogs. There were more male than female dogs affected. Although the author did not observe any metastatic lesions, 7 tumors recurred following attempted excision. However, no gross or microscopic descriptions of the tumors were listed.

Howard and Nielsen (1965) listed some interesting findings in their survey of tumors from Boxers and other dogs. In their study the incidence of histiocytoma in the skin of the Boxer was nearly 4 times as high as encountered in other breeds.

Basal Cell Carcinoma

The basal cell carcinoma was first reported by Jacob (1827). This neoplasm has also been referred to as "hair-matrix tumor" (Haythorn, 1931), "adnexal carcinoma" (Foot, 1947), "basal cell epithelioma", "basalioma" (Nielsen and Cole, 1960), "basal cell tumor" (Moulton, 1961), and "rodent ulcer" (Runnells et al., 1965).

The origin of the tumor cells has created controversies among different workers. Owen (1930) stated,

> "The term basal cell carcinoma is used to designate carcinoma arising from the basal cell layer of the epidermis and retaining to a large extent the characteristics of basal cells."

She also suggested the use of thin tissue sections (2 to 3 microns) for demonstration of intercellular bridges in the tumors.

Multicentric origin of the tumors from the basal cells of epidermis and skin adnexa has been reported by several authors (Haythorn, 1931; Foot, 1947; Mulligan, 1949; Moulton, 1961; Smith and Jones, 1966). The

benign nature of this neoplasm has also been recognized, although Head (1953) included 2 tumors that had metastatic foci.

Using several special staining techniques, Haythorn (1931) noted the presence of rudimentary hair shafts in the tumors. He postulated the origin of these tumors from the hair-matrix.

Feldman (1932) reported 2 cases of "so-called basal-cell carcinoma" of dogs. The tumors grew slowly and intercellular bridges were noted by him in one of the sections.

Jackson (1936) emphasized the resemblance of the tumor cells to the basal cells of stratified squamous epithelium and its derivatives and anticipated the possibility of confusion of this tumor with "contagious venereal tumor" of the dog when occurring in unusual locations. Microscopic examinations of 8 tumors from dogs revealed the uniform appearance of tumor cells. Two chief patterns of cellular arrangements were described: (1) groups of cells with their long axes perpendicular to the cell columns and (2) groups of cells forming alveolar structures.

Extensive studies of the basal cell carcinomas of man revealed their origin from the specialized basal cells of the dermal adnexa rather than from basal cells of the stratified squamous epithelium (Foot, 1947). Foot suggested the term "adnexal carcinoma" for these tumors.

Mulligan (1948) listed "basal cell carcinomas" which originated in the hair follicles. The cells resembled the epidermal basal cells, although their continuity with the latter was not apparent. Mulligan (1949) described "basal cell (hair follicle) carcinomas" and believed that their origin was from hair follicles, epidermis, and lining epithelium of epidermal inclusion cysts. Formation of structures

resembling hair follicles by the tumor cells was also noted.

A statistical review of 47 "basal cell and sweat gland" tumors from a total of 510 tumors of skin, subcutaneous tissue, and associated structures was presented by Cotchin (1951). In another report Cotchin (1954) included 51 "basal cell and glandular" tumors of dogs, although there was no mention of the actual basal cell types he studied. No gross or microscopic descriptions of these tumors were given.

Head (1953) examined tumors from dogs and divided them into 2 groups: (1) superficial origin and (2) subepidermal origin. Two of the 28 tumors in the first group metastasized to the regional lymph nodes and 1 to the lungs. The second group of 21 tumors had the characteristic Medusa head appearance histologically and did not manifest any metastases, although 3 recurred after attempted removal. Frequent occurrence of the tumors of both groups in male dogs was indicated.

Histologic studies of "basal cell epitheliomas" by Goltz, Fusaro and Jarvis (1959) with special staining techniques revealed the absence of glycogen in most of the tumors. However, a few tumors that differentiated to form "adenoid or trichoepitheliomatous" structures had glycogen in them.

Nielsen and Cole (1960) did a comprehensive review of the tumors in dogs. In their study the term "basal cell tumor" was used to designate the epithelial tumors that did not have any differentiation. They studied 33 tumors, of which 26 were located in the head and neck area. Three tumors recurred due to incomplete excision. However, no metastasis was observed by the authors. There were 2 histologic patterns of cells: (1) ribbon type and (2) solid type, the former being more common. The solid type was more malignant than the ribbon type.

Thrasher (1961) reported 2 primary "basal cell carcinomas," 1 in the skin and another from the mammary gland. However, their microscopic characteristics were not described.

Sams, Smith, and Finlayson (1963) demonstrated delicate unbranched argyrophilic fibers in the human "basal cell epitheliomas". Most of the fibers disappeared on treatment with 0.5 M acetic acid solution, revealing their collagenous rather than true reticular nature. The presence of large amounts of acid mucopolysaccharides was noted by them especially in the stroma having numerous fibroblasts, although their presence in the intercellular spaces was variable. Small amounts of neutral polysaccharides were also seen within the cell nests. An increase in the number of mast cells was noted in the stroma surrounding the tumors.

MATERIALS AND METHODS

The tissues used for this study were assembled from the collection of the Department of Pathology, Michigan State University. They had been sent to the department by several private practitioners and the Small Animal Clinic, Michigan State University, for histopathologic diagnosis. The instances in which these neoplastic conditions were diagnosed (from June 1956 to December 1965) were recorded, along with the available data, in the form of tables under the following headings: breed, age, sex, location in the body, and degree of malignancy and metastasis, if any. All the tissues had been fixed in 10% formalin and embedded in paraffin.

Hematoxylin and eosin (H & E) stained sections were examined microscopically. Those sections having heavy inflammatory processes and post-mortem changes due to improper fixation were considered to be unfit for study and hence were discarded. Available paraffin blocks for the selected slides were collected, and sections were cut at a thickness of 6 microns.

Sections were stained with Gomori's one-step trichrome stain and periodic acid-Schiff (PAS) with and without diastase digestion (Armed Forces Institute of Pathology, 1960). Another stain, outlined by Gurr (1962), was used to demonstrate the presence of reticulum fibers in the tumors. May-Grunwald-Giemsa stain (Armed Forces Institute of Pathology, 1960) was applied to the tissues to stain mast cells and to eliminate mast cell tumors if any had been inadvertently included.

All the sections were examined for ulceration, necrosis, amount and type of stroma, inflammatory cells, hyperchromatism, size and shape of nuclei, and morphology of neoplastic cells.

Measurement of the size of the nuclei in their longest diameter was done with an ocular micrometer fitted to the microscope and calibrated by stage micrometer. Ten representative neoplasms from each group were included and 100 nuclei from each section were measured. The results were statistically evaluated for any difference in size of the nuclei between these 3 groups.

An average of 5 high-power fields (hpf) was examined to enumerate the average number of mitoses present.

Photomicrographs were taken from representative sections. The description of these neoplasms, by Moulton (1961), was accepted as a guide during the entire study.

RESULTS

Transmissible Venereal Tumor

Breed incidence. Of 43 transmissible venereal tumors diagnosed, information regarding breed affected was available in 40 cases. Of these, 10 (25%) were Boxers, 6 (15%) were Boston Terriers, and 4 (10%) were Cocker Spaniels. This is recorded in TABLE 1.

Sex distribution. Of 36 dogs for which information was available, 18 tumors were recorded in male dogs and 18 in females.

Age distribution. The range in age of affected animals was 4 months to 13 years; the mean age was 5 years.

Primary location. Among 43 recorded instances, only 2 tumors were removed from the male genitalia. One was from the glans penis and the other from the inside of the prepuce. Two tumors, 1 each from the tongue and lip areas, were also reported. Others were from the skin on various parts of the body.

Metastases. Although data from referring veterinary practitioners may have been deficient, metastatic foci were reported in the mandibular and sublingual lymph nodes in 1 instance. Five dogs from the entire group reportedly had multiple skin lesions.

TABLE 1. Breed and sex incidence of 3 skin tumor types.

Breed	Transmissible Venereal Tumor				Adnexal Carcinoma				Basal Cell Carcinoma			
	Male	Female	Sex Not Known	Total	Male	Female	Sex Not Known	Total	Male	Female	Sex Not Known	Total
Afghan	-	-	-	-	1	-	-	1	-	-	-	-
Airedale Terrier	-	-	-	-	-	-	-	-	2	-	-	2
Basenji	-	-	-	-	1	-	-	1	-	-	-	-
Basset Hound	-	-	-	-	-	1	-	1	-	-	-	-
Beagle	1	-	-	1	8	1	1	10	-	-	1	1
Bloodhound	-	-	-	-	-	2	-	2	-	-	-	-
Borzoi	-	-	-	-	-	1	-	1	-	-	-	-
Boston Terrier	3	3	-	6	6	5	3	14	2	-	1	3
Bouvier des Flanders	-	-	-	-	1	-	-	1	-	-	-	-
Boxer	2	7	1	10	31	18	9	58	5	1	1	7
Brittany Spaniel	1	-	-	1	2	1	-	3	-	1	-	1
Bulldog	1	-	-	1	1	-	-	1	-	-	-	-
Cairn Terrier	-	-	-	-	3	1	-	4	-	-	-	-
Chihuahua	-	-	-	-	1	3	-	4	-	-	-	-
Chow Chow	-	-	-	-	-	-	-	-	1	1	-	2
Cocker Spaniel	1	2	1	4	9	15	6	30	5	9	2	16
Collie	1	-	-	1	1	3	1	5	10	3	2	15
Dachshund	1	1	-	2	10	16	2	28	-	1	1	2
Dalmatian	-	-	-	-	1	-	1	2	-	-	-	-
Doberman Pinscher	-	-	-	-	1	2	-	3	-	-	1	1
English Setter	-	-	1	1	1	-	-	1	-	1	-	1
Fox Terrier	-	-	-	-	1	1	-	2	-	1	-	1
German Shepherd	-	-	-	-	4	5	-	9	1	1	1	3
Golden Retriever	-	-	-	-	2	-	-	2	-	-	-	-
Great Dane	-	-	-	-	-	2	1	3	-	-	-	-
Irish Setter	-	-	-	-	2	1	-	3	-	1	-	1
Italian Greyhound	-	-	-	-	-	2	-	2	-	-	-	-

TABLE 1--continued

Breed	Transmissible Venereal Tumor				Adnexal Carcinoma				Basal Cell Carcinoma			
	Male	Female	Sex Not Known	Total	Male	Female	Sex Not Known	Total	Male	Female	Sex Not Known	Total
Kerry Blue Terrier	-	-	-	-	1	-	-	1	2	4	1	7
Labrador Retriever	-	1	-	1	1	2	-	3	-	-	-	-
Maltese Terrier	-	-	-	-	-	-	-	-	-	-	1	1
Manchester Terrier	-	-	-	-	3	3	-	6	-	1	-	1
Miniature Pinscher	1	-	-	1	-	-	-	-	-	-	-	-
Mixed	4	1	2	7	7	17	5	29	7	8	1	16
Old English Sheepdog	-	-	-	-	-	-	-	-	2	-	-	2
Pekingese	-	1	-	1	-	-	-	-	-	-	-	-
Pointer	-	-	-	-	1	2	-	3	-	-	-	-
Poodle	1	-	-	1	9	2	2	13	7	1	1	9
Pug	-	-	-	-	-	1	-	1	-	-	-	-
Retriever (unspec.)	-	-	-	-	2	-	-	2	-	-	-	-
Schnauzer	-	-	-	-	3	3	-	6	-	-	-	-
Scottish Terrier	-	1	-	1	9	4	3	16	-	2	-	2
Setter (unspecified)	-	-	-	-	-	1	-	1	2	2	-	4
Shetland Sheepdog	-	-	-	-	1	-	-	1	-	-	-	-
Siberian Husky	-	-	-	-	1	-	-	1	-	-	-	-
Spitz	-	-	-	-	-	-	1	1	1	-	-	1
Springer Spaniel	1	-	-	1	1	-	1	2	1	3	-	4
St. Bernard	-	-	-	-	-	-	-	-	1	-	-	1
Weimaraner	-	-	-	-	3	1	-	4	2	-	-	2
Welsh Terrier	-	-	-	-	-	2	-	2	-	-	-	-
Whippet	-	-	-	-	1	-	-	1	-	-	-	-
Yorkshire Terrier	-	-	-	-	-	1	-	1	-	-	-	-
No breed reported	-	1	2	3	3	3	11	17	2	5	1	8

Microscopic characteristics. Tissue sections from 25 different dogs were studied microscopically. The characteristics are recorded in TABLE 2.

Three tumors, 2 from the penis and prepuce, respectively, and another from the perianal region, had somewhat different histologic characteristics than others listed in this group. The histologic characteristics are given below.

In 2 of these neoplasms the cells were arranged in sheets (Figure 1) which occasionally were interspaced with lymphatics. All 3 tumors were vascular. The neoplastic cells were spherical or oval and more or less uniform in shape (Figure 2). Cytoplasm was moderate in amount and slightly granular. Nuclei were round, oval or occasionally slightly indented and hyperchromatic (Figure 3). Solitary nucleoli were centrally or eccentrically placed. Three to 9 mitoses were seen in some high-power fields (hpf); the average number was 4. Inflammatory cells, mostly lymphocytes, with a few plasma cells, neutrophils and occasional eosinophils, were present. The nuclei were measured in 2 sections and varied in diameter from 4.7 to 9.2 microns, with an average of 6.8 microns.

Only 1 neoplasm from this group of 3 was studied with special stains. Reticulum fibers were scanty in some areas (Figure 4) and moderate in amount in other areas (Figure 5). The supporting stroma was scanty. Occasional periodic acid-Schiff (PAS)-positive granules were seen in some cells. Mast cells were rarely seen in the section stained with Giemsa stain.

Four neoplasms from the remaining 22 were very undifferentiated, with cells which were more pleomorphic than the transmissible venereal tumor cells described earlier. Nuclei were hyperchromatic and pleomorphic. Giant cells were seen in 2 neoplasms (Figures 6 and 7). One section

TABLE 2. Microscopic characteristics of transmissible venereal tumor, adnexal carcinoma, and basal cell carcinoma.

Path. No.	Shape of cell	Shape of nuclei	Staining reaction of nuclei	Mitoses /hpf	Degree of anaplasia	Ulceration	Inflammatory cells	Probable origin of the neoplastic cells
				Transmissible Venereal Tumor				
A261	round to oval, uniform	round to oval; some slightly indented	hyperchromatic	0 - 2	none	yes	+	unknown
B4770	pleomorphic	oval to pleomorphic	hyperchromatic	0 - 2	none	yes	+	epidermis
B6315	pleomorphic	oval to pleomorphic; some giant forms	vesicular	2 - 5	+	none	+	unknown
C819	pleomorphic	oval to pleomorphic	slightly hyperchromatic	2 - 5	none	none	+	hair follicles
C4041	fairly uniform	oval to round to slightly pleomorphic	hyperchromatic	2 - 8 (av. 5)	none	none	-	hair follicles
C6147	pleomorphic	oval to elongated to pleomorphic	slightly hyperchromatic	2 - 4	none	yes	+	epidermis
D373	fairly uniform to slightly pleomorphic	oval to round to slightly pleomorphic; some giant forms	normochromatic	0 - 4 (av. 2)	none	yes	+	hair follicles and epidermis

TABLE 2--continued

Path. No.	Shape of cell	Shape of nuclei	Staining reaction of nuclei	Mitoses /hpf	Degree of anaplasia	Ulceration	Inflam-matory cells	Probable origin of the neo-plastic cells
D948	fairly uni-form	oval to round to slightly pleomorphic; some bilobed	vesicular	3 - 16 (av. 8)	+	yes	+	hair folli-cles
D1350	pleomorphic	oval to pleo-morphic; some giant forms	hyperchromatic	2 - 8 (av. 5)	none	none	-	hair folli-cles
D1356	slightly pleomorphic	oval to slight-ly pleomorphic	normochromatic to vesicular	1 - 5 (av. 2)	none	none	+	unknown
D4060	pleomoprhic	oval to pleo-morphic	vesicular	3 - 10 (av. 4)	+	yes	+	hair folli-cles
D4067	slightly pleomorphic	oval to round to slightly pleomorphic	normochromatic to vesicular	2 - 6 (av. 3)	none	none	+	hair folli-cles
D4068	pleomoprhic	round to oval to pleomorphic	hyperchromatic	5 - 12 (av. 6)	++	yes	+	unknown
D4656	pleomorphic	oval to round to slightly pleomorphic	slightly hyper-chromatic to vesicular	3 - 7 (av. 4)	none	yes	+	epidermis; hair folli-cles
D5515	pleomorphic	oval to pleo-morphic	vesicular	2 - 8 (av. 5)	+	yes	+	epidermis

TABLE 2--continued

Path. No.	Shape of cell	Shape of nuclei	Staining reaction of nuclei	Mitoses /hpf	Degree of anaplasia	Ulceration	Inflammatory cells	Probable origin of the neoplastic cells
D5696	pleomorphic	round to oval to slightly pleomorphic	hyperchromatic	5 - 12 (av. 6)	++	yes	+	unknown
D6004	pleomorphic	oval to pleomorphic; some bilobed	vesicular	4 - 8 (av. 6)	+	yes	+	epidermis
D6051	uniform spherical to oval	oval to round	hyperchromatic	3 - 7 (av. 4)	none	yes	+	unknown
E2442	pleomorphic	round to oval to pleomorphic	normochromatic	1 - 6 (av. 4)	none	yes	+	hair follicles
E2495	slightly pleomorphic	oval to round; some indented	hyperchromatic	2 - 5	none	none	-	hair follicles
E3187	fairly uniform	oval to pleomorphic	normochromatic	1 - 3	none	yes	+	hair follicles
E3260	fairly uniform	oval to round to slightly pleomorphic	hyperchromatic	3 - 5	none	none	+	unknown
G2613	pleomorphic	round to oval to slightly pleomorphic	hyperchromatic	2 - 4	none	yes	+	hair follicles

TABLE 2--continued

Path. No.	Shape of cell	Shape of nuclei	Staining reaction of nuclei	Mitoses /hpf	Degree of anaplasia	Ulceration	Inflammatory cells	Probable origin of the neoplastic cells
H3468	fairly uniform	oval to slightly pleomorphic	hyperchromatic	1 - 7 (av. 2)	none	yes	+	unknown
J2161	fairly uniform	round to oval	hyperchromatic	3 - 9 (av. 4)	none	yes	+	unknown
			Adnexal Carcinoma					
A1820	pleomorphic	oval to elongated; slightly indented; some bilobed	slightly hyperchromatic	1 - 4	none	yes	+	hair follicles
D3311	fairly uniform	oval to round; some indented	slightly hyperchromatic to vesicular	3 - 8 (av. 4)	none	none	+	hair follicles
D4364	pleomorphic	oval to pleomorphic	hyperchromatic to vesicular	2 - 5	+	yes	+	hair follicles
D4376	fairly uniform	spherical to oval; indented	slightly hyperchromatic to vesicular	2 - 5	+	yes	+	epidermis; hair follicles
D5698	pleomorphic	oval to round to pleomorphic	hyperchromatic to vesicular	2 - 6 (av. 3)	++	yes	+	unknown

TABLE 2--continued

Path. No.	Shape of cell	Shape of nuclei	Staining reaction of nuclei	Mitoses /hpf	Degree of anaplasia	Ulceration	Inflam-matory cells	Probable origin of the neo-plastic cells
E226	pleomorphic	oval to round to pleomorphic	slightly hyper-chromatic	0 - 3	none	yes	+	epidermis
E1017	pleomorphic	oval to pleo-morphic	normochromatic	3 - 8 (av. 5)	none	yes	+	hair folli-cle and epidermis
E1021	pleomorphic	oval to elonga-ted to pleo-morphic	hyperchromatic	3 - 13 (av. 6)	none	yes	+	hair folli-cle
E3993	fairly uni-form	spherical to oval to pleo-morphic	normochromatic	0 - 2	none	yes	+	epidermis
E4707	pleomorphic	oval to pleo-morphic; some indented	slightly vesicular	1 - 4	none	yes	+	epidermis and hair follicle
F4676	slightly pleomorphic	round to oval to pleomorphic	slightly hyper-chromatic to vesicular	2 - 8 (av. 3)	none	yes	+	epidermis and hair follicle
F5337	pleomorphic	oval to slight-ly pleomorphic	hyperchromatic	4 - 11 (av. 5)	+	yes	+	str. sq. epi.
F5818	pleomorphic	oval to pleo-morphic	hyperchromatic	4 - 15 (av. 7)	+	yes	+	epidermis

TABLE 2--continued

Path. No.	Shape of cell	Shape of nuclei	Staining reaction of nuclei	Mitoses /hpf	Degree of anaplasia	Ulceration	Inflammatory cells	Probable origin of the neoplastic cells
G601	fairly uniform	oval to round to slightly pleomorphic	hyperchromatic	0 - 2	none	yes	+	hair follicles
G604	pleomorphic	oval to elongated to pleomorphic	hyperchromatic to vesicular	0 - 2	++	none	+	unknown
G3056	pleomorphic	oval to pleomorphic	hyperchromatic to vesicular	2 - 6 (av. 3)	none	yes	+	hair follicles
G3163	fairly uniform	round to oval to slightly pleomorphic	slightly hyperchromatic	1 - 3	none	yes	+	hair follicle
G3640	pleomorphic	oval to pleomorphic	hyperchromatic	1 - 4	++	none	+	unknown
G4539	slightly pleomorphic	round to oval to pleomorphic	hyperchromatic	2 - 5	none	none	+	epidermis; hair follicle
H2824	pleomorphic	oval to pleomorphic	hyperchromatic to uniform	1 - 6 (av. 4)	none	yes	+	hair follicle; epidermis
H4732	slightly pleomorphic	oval to pleomorphic; some bilobed	normochromatic	1 - 3	none	yes	+	hair follicles

TABLE 2--continued

Path. No.	Shape of cell	Shape of nuclei	Staining reaction of nuclei	Mitoses /hpf	Degree of anaplasia	Ulceration	Inflammatory cells	Probable origin of the neoplastic cells
H5332	fairly uniform	oval to round to pleomorphic	vesicular	2 - 7 (av. 3)	none	yes	+	str. sq. epi. (lip)
H5449	pleomorphic	oval to pleomorphic	slightly hyperchromatic to vesicular	1 - 2	+	none	+	str. sq. epi.
H5514	fairly uniform	spherical to oval; some indented and bilobed	slightly hyperchromatic	2 - 10 (av. 3)	none	yes	+	epidermis; hair follicle
067-410	pleomorphic	oval to pleomorphic	normochromatic	1 - 11 (av. 6)	+	yes	+	str. sq. epi.
			Basal Cell Carcinoma					
A894	uniform	round to oval to cylindrical	hyperchromatic	0 - 2	none	none	-	unknown
B6734	uniform	round to oval to cylindrical	hyperchromatic	0 - 2	none	none	-	unknown
D1081	uniform	round to oval to cylindrical	hyperchromatic	0 - 3	none	none	-	unknown
E1882	fairly uniform	oval to round to cylindrical	hyperchromatic	0 - 3	none	none	-	hair follicle

TABLE 2--continued

Path. No.	Shape of cell	Shape of nuclei	Staining reaction of nuclei	Mitoses /hpf	Degree of anaplasia	Ulceration	Inflammatory cells	Probable origin of the neoplastic cells
E2965	fairly uniform	oval to round to cylindrical	hyperchromatic	2 - 5	none	none	+	unknown
E3011	uniform	oval to round to cylindrical	hyperchromatic	0 - 5 (av. 2)	none	none	+	unknown
E3870	slightly pleomorphic	oval to elongated to pleomorphic	hyperchromatic	0 - 1	none	none	+	unknown
F2532	uniform	oval to cylindrical	hyperchromatic	0 - 3	none	none	+	unknown
F3396	slightly pleomorphic	oval to cylindrical to pleomorphic	hyperchromatic	1 - 6 (av. 2)	none	none	-	unknown
F3850	fairly uniform	oval to round to slightly pleomorphic	slightly vesicular	0 - 2	none	none	+	unknown
F4435	slightly pleomorphic	oval to cylindrical to pleomorphic	hyperchromatic	0 - 1	+	none	+	epidermis
F5072	uniform	oval to round to cylindrical	normochromatic	0 - 3	none	none	-	unknown

TABLE 2--continued

Path. No.	Shape of cell	Shape of nuclei	Staining reaction of nuclei	Mitoses /hpf	Degree of anaplasia	Ulceration	Inflammatory cells	Probable origin of the neoplastic cells
G146	uniform	oval to cylindrical	hyperchromatic	0 - 1	none	none	+	unknown
G319	uniform	oval to cylindrical	hyperchromatic	0 - 4 (av. 2)	none	yes	+	basal layer of epidermis
G1118	uniform	oval to cylindrical	hyperchromatic	0 - 1	none	none	+	unknown
G3208	uniform	oval to cylindrical	hyperchromatic	1 - 4	none	none	+	unknown
G3884	uniform	oval to cylindrical	vesicular	0 - 2	none	yes	+	unknown
H4186	fairly uniform	oval to cylindrical to slightly pleomorphic	slightly vesicular	2 - 4	none	none	+	unknown
H5376	uniform	oval to cylindrical	slightly hyperchromatic	2 - 5	none	none	+	unknown
J42	uniform	oval to cylindrical	hyperchromatic to vesicular	0 - 3	none	none	+	unknown
J4756	uniform	oval to round to cylindrical	hyperchromatic	1 - 3	none	none	+	unknown

TABLE 2--continued

Path. No.	Shape of cell	Shape of nuclei	Staining reaction of nuclei	Mitoses /hpf	Degree of anaplasia	Ulceration	Inflammatory cells	Probable origin of the neoplastic cells
J5946	uniform	oval to round to cylindrical	hyperchromatic	1 - 4	none	yes	+	unknown
J6485	slightly pleomorphic	oval to round to slightly pleomorphic	hyperchromatic to vesicular	2 - 5	none	none	+	unknown
058-935	uniform	oval to cylindrical	hyperchromatic	1 - 3	none	none	+	unknown
059-379	uniform	oval to cylindrical	hyperchromatic	2 - 8 (av. 4)	none	none	+	unknown

Abbreviations:

Degree of anaplasia: + slight, ++ moderate
Mitoses: hpf high power field, av. average
Inflammatory cells: + present, - absent
Str. sq. epi.: stratified squamous epithelium

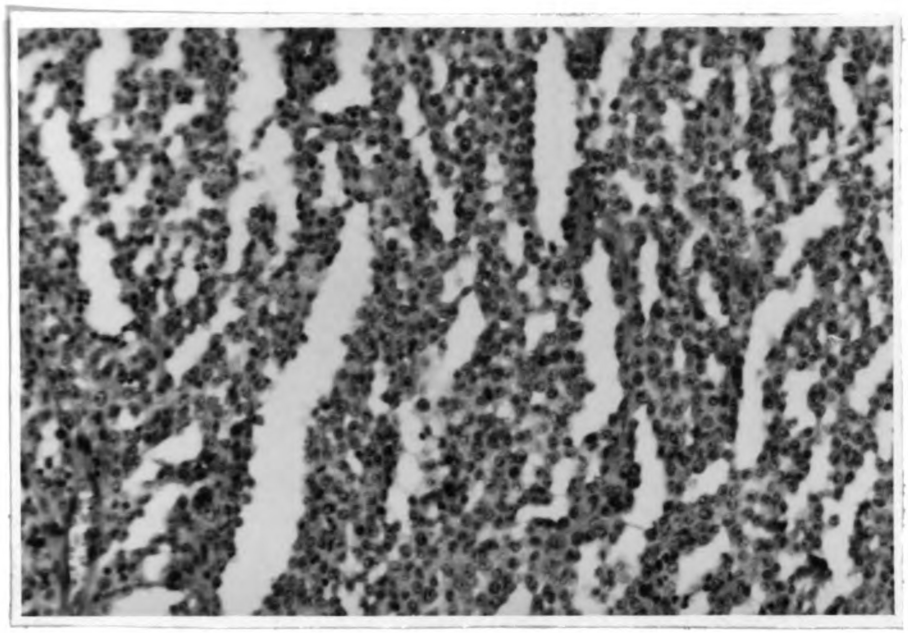

Figure 1. Transmissible venereal tumor. Uniform appearing neoplastic cells arranged in sheets. The supporting stroma is scanty. Hematoxylin and eosin. x 188.

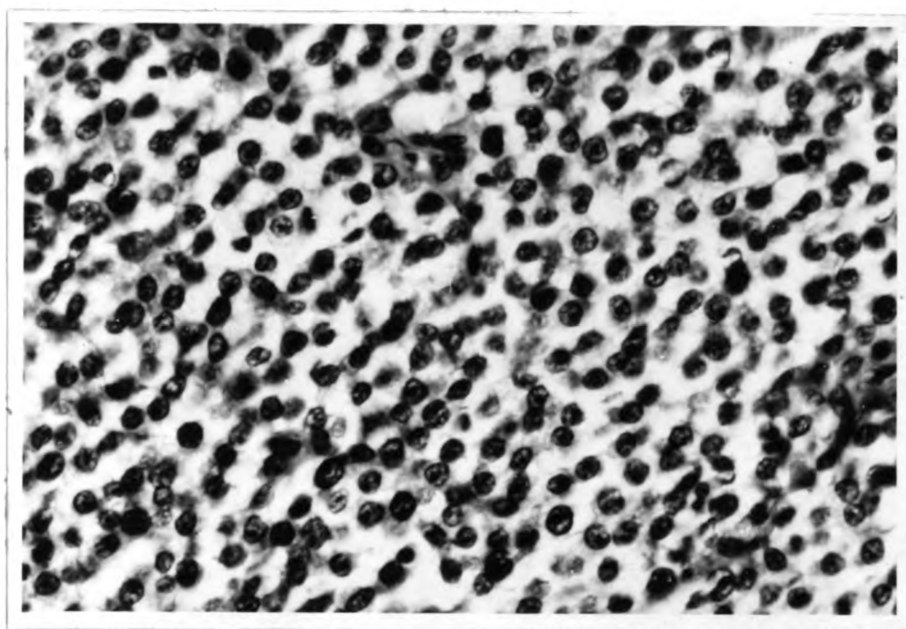

Figure 2. Transmissible venereal tumor. Note uniformity in the shape of the neoplastic cells and hyperchromatism of cell nuclei. Hematoxylin and eosin. x 469.

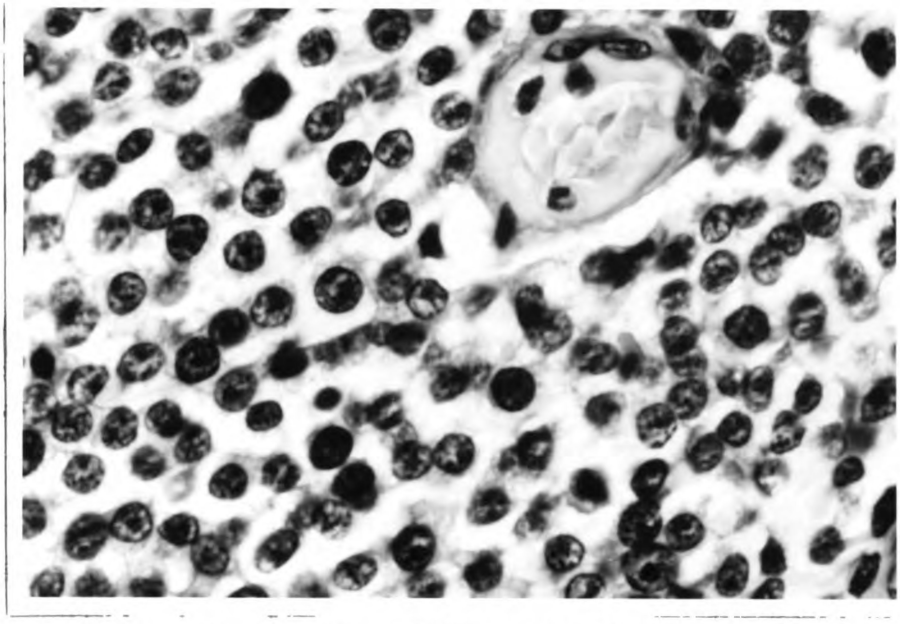

Figure 3. Transmissible venereal tumor. Higher magnification of Figure 2. Note hyperchromatism. Hematoxylin and eosin. x 750.

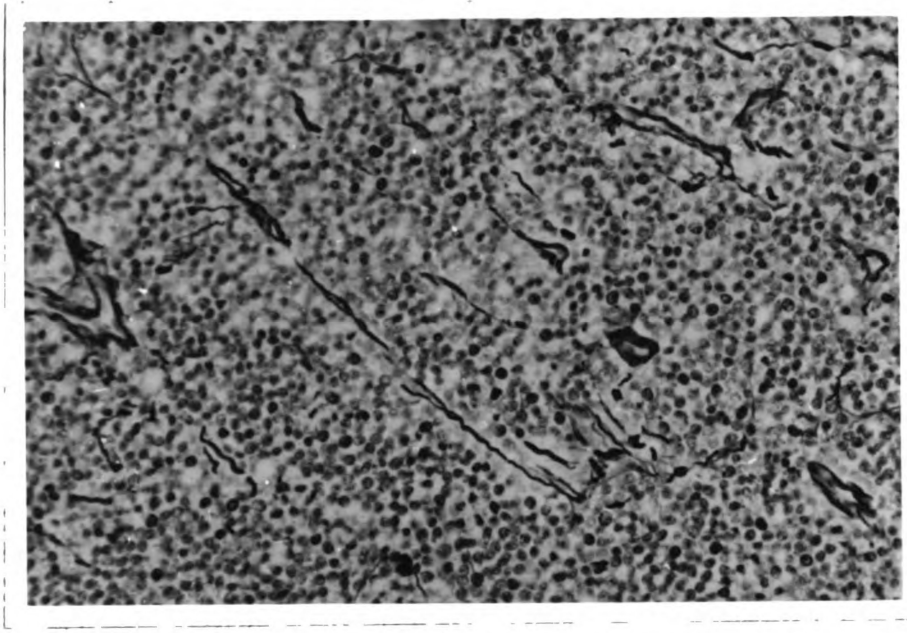

Figure 4. Transmissible venereal tumor, with very small amount of reticulum, mostly around capillaries. Lithium Silver. x 188.

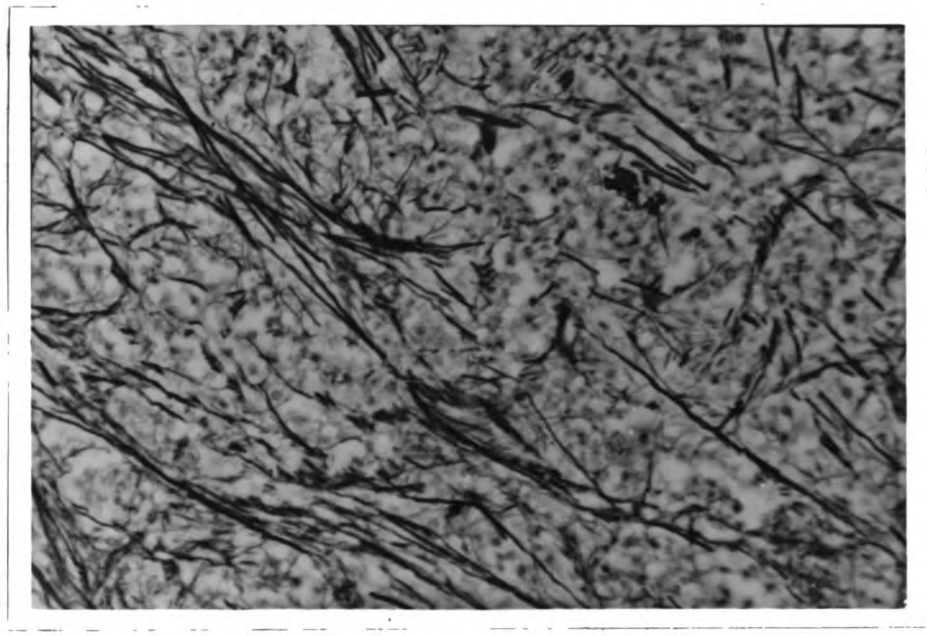

Figure 5. Transmissible venereal tumor, with moderate amount of reticulum distributed irregularly in another area of the same section as Figure 4. Lithium Silver. x 188.

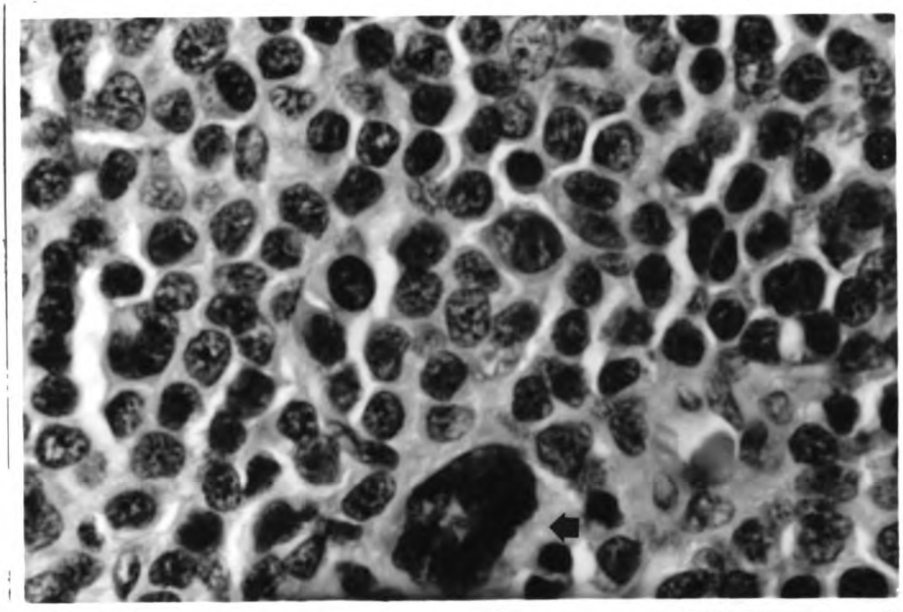

Figure 6. Unclassified neoplasm. Note variation in shape of nuclei and hyperchromatism. One giant cell seen (arrow). Nucleoli are not prominent. Hematoxylin and eosin. x 750.

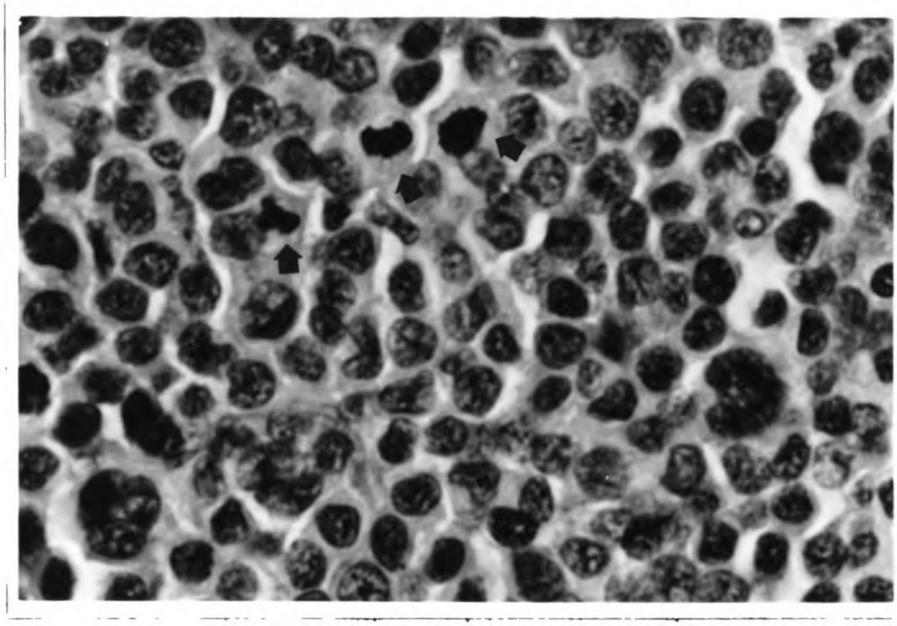

Figure 7. Unclassified neoplasm, same as Figure 6. Note presence of mitotic figures in large numbers (arrows). Hematoxylin and eosin. x 750.

with giant cells had a few prominent eosinophilic nucleoli resembling Reed-Sternberg cells of Hodgkin's disease, but there was no history of lymph node involvement.

Using a special stain, small to moderate amount of reticulum was noted in these neoplasms. The fibers were irregularly distributed. With PAS stain, occasional pink-staining granules were seen in 1 section. Mast cells were rarely seen.

One neoplasm from the remaining 18 was diagnosed as mastocytoma. The neoplastic cells were predominantly mast cells.

The remaining 17 neoplasms were located on various parts of the body. There were various degrees of ulceration and necrosis and hyperplasia of the overlying stratified squamous epithelium. In most cases, some evidence of origin of the neoplastic cells from the stratified squamous epithelium of the skin and other areas and epithelium of the hair follicles was seen. Inflammatory cells, predominantly lymphocytes, with neutrophils and occasionally eosinophils, were also noted. The supporting stroma varied from scanty to moderate in amount and was hyalinized in some cases. Invasion of the dermis and subcutaneous fat by the neoplastic cells was evident in most cases.

Shape of the cells. The cells were spherical, oval or pleomorphic. The amount of cytoplasm was moderate and the cell outlines were fused in most cases. The cytoplasm was pale and slightly granular in appearance. Anaplastic forms were encountered occasionally.

Shape and size of the nuclei. The nuclei were spherical, oval, indented, occasionally bilobed or pleomorphic, the former 2 types being more common. The nuclear chromatin was normal or slightly increased in

amount. Occasionally vesicular forms were also noted. Some nuclei resembled the nuclei of lymphoblasts or were slightly larger. When the average nuclear size for 100 cells in each of 10 neoplasms was determined, these averages varied from 7.1 to 8.7, with a mean value of 7.9 microns.

Nucleoli. Both eosinophilic and basophilic forms were present. Some were located in the centers of the nuclei, whereas others were eccentrically placed.

Mitoses. The number of mitoses varied from 0 to 16 per hpf. The average number was 3 to 4.

Histochemical characteristics. Twenty-two neoplasms from this group of 25 were studied with special stains. The characteristics are recorded in TABLE 3.

Reticulum. Reticulum was present in small, moderate or large amounts and its fibers were distributed irregularly between and around groups of neoplastic cells. There was no indication that these fibers were formed by the neoplastic cells. These fibers were thin, wavy, straight or branched.

PAS reaction. PAS-positive granules were seen in the cytoplasm of cells occasionally but inconsistently.

Giemsa stain. A few mast cells were seen in the cellular areas of almost all the neoplasms studied.

TABLE 3. Histochemical characteristics of transmissible venereal tumor, adnexal carcinoma, and basal cell carcinoma.

Path. No.	Amount of stroma	Amount of reticulum present	Amount of PAS-positive material in the neoplastic cells	Amount of PAS-positive material after diastase digestion	Mast cells
			Transmissible Venereal Tumor		
A261	++	small to moderate	occasional granules	*	++++
B4770	+	moderate to large	occasional granules	*	+
B6315	+	moderate	none	*	none
C819	+	moderate to large	occasional granules	*	+
C4041	++	*	*	*	*
C6147	++ (hyalinized)	moderate	none	*	+
D373	+	small to moderate	none	*	+
D948	+	small	none	*	+
D1350	++	small to moderate	none	*	(+)
D1356	++	large	occasional granules	*	+
D4060	++ (hyalinized)	small to moderate	none	*	+
D4067	+	small	occasional granules	*	+

Abbreviations:

Stroma: + scanty, ++ moderate, +++ large

Mast cells: (+) rare, + a few, ++ moderate, +++ large, ++++ mastocytoma

* not studied

TABLE 3--continued

Path. No.	Amount of stroma	Amount of reticulum present	Amount of PAS-positive material in the neoplastic cells	Amount of PAS-positive material after diastase digestion	Mast cells
D4068	+ (edematous)	moderate	none	*	(+)
D4656	+ (hyalinized)	moderate	none	*	+
D5515	+ (hyalinized)	small to moderate	occasional granules	*	++
D5696	+	small to moderate	none	*	(+)
D6004	+	large	small	*	+
D6051	+	small to moderate	occasional granules	*	(+)
E2442	+	moderate	none	*	+
E2495	++ (hyalinized)	moderate	none	*	+
E3187	++ (hyalinized)	small to moderate	small	*	(+)
E3260	+	*	*	*	*
G2613	+ (hyalinized)	large	occasional granules	*	+
H3468	++ (hyalinized)	moderate	none	*	+
J2161	+	*	*	*	*

TABLE 3--continued

Path. No.	Amount of stroma	Amount of reticulum present	Amount of PAS-positive material in the neoplastic cells	Amount of PAS-positive material after diastase digestion	Mast cells
			Adnexal Carcinoma		
A1820	+ (edematous)	*	*	*	*
D3311	+ (hyalinized)	large	none	*	+
D4364	+ (hyalinized)	*	*	*	(+)
D4376	+ (hyalinized)	moderate to large	none	*	+
D5698	++ (edematous)	small to moderate	none	*	+
E226	++	moderate to large	none	*	(+)
E1017	+	moderate to large	occasional granules	*	+
E1021	+ (hyalinized)	moderate to large	none	*	+
E3993	+	*	*	*	*
E4707	+ (hyalinized)	small	none	*	(+)
F4676	+ (hyalinized)	moderate	small	*	+
F5337	++	*	*	*	+

TABLE 3--continued

Path. No.	Amount of stroma	Amount of reticulum present	Amount of PAS-positive material in the neoplastic cells	Amount of PAS-positive material after diastase digestion	Mast cells
F5818	++ (hyalinized)	moderate	none	*	++
G601	+	*	*	*	*
G604	+ (hyalinized)	moderate to large	none	*	+
G3056	+ (hyalinized)	moderate to large	occasional granules	*	+
G3163	+ (hyalinized)	moderate	none	*	+
G3640	+	large	none	*	+
G4539	+ (hyalinized)	*	*	*	*
H2824	+ (hyalinized)	small	none	*	+
H4732	+ (hyalinized)	small to moderate	small	*	+
H5332	+++	large	none	*	(+)
H5449	+	small to moderate	none	*	(+)
H5514	+ (hyalinized)	*	*	*	*
067-410	+	*	*	*	*

TABLE 3--continued

Path. No.	Amount of stroma	Amount of reticulum present	Amount of PAS-positive material in the neoplastic cells	Amount of PAS-positive material after diastase digestion	Mast cells
			Basal Cell Carcinoma		
A894	++	*	*	*	(+)
B6734	+++ (edematous)	moderate	small to moderate	traces	++
D1081	++	large	large	very small	+
E1882	+++	moderate	large	none	++
E2965	++ (edematous)	moderate	small	none	+
E3011	+++ (edematous & hyalinized)	moderate to large	moderate	none	++
E3870	+++ (edematous & hyalinized)	small	small	none	+
F2532	++ (edematous & hyalinized)	*	*	*	+
F3396	++ (hyalinized)	moderate	small	none	+
F3850	++	large	occasional granules	none	+
F4435	+++	*	*	*	+

TABLE 3--continued

Path. No.	Amount of stroma	Amount of reticulum present	Amount of PAS-positive material in the neoplastic cells	Amount of PAS-positive material after diastase digestion	Mast cells
F5072	++ (edematous & hyalinized)	moderate	large	traces	(+)
G146	++ (edematous)	very small	small	none	+
G319	++ (edematous)	*	*	*	*
G1118	+++ (edematous & hyalinized)	moderate	none	none	+
G3208	+++ (hyalinized)	moderate	moderate	traces	+
G3884	+++ (edematous & hyalinized)	small	large	traces	+
H4186	++	moderate	moderate	traces	(+)
H5376	++	moderate	moderate	traces	(+)
J42	+++ (edematous & hyalinized)	moderate to large	traces	none	+
J4756	++ (edematous & hyalinized)	*	*	*	+

TABLE 3--continued

Path. No.	Amount of stroma	Amount of reticulum present	Amount of PAS-positive material in the neoplastic cells	Amount of PAS-positive material after diastase digestion	Mast cells
J5946	+++ (edematous & hyalinized)	moderate	moderate	none	+
J6485	+++ (edematous & hyalinized)	small to moderate	moderate to large	none	+
058-935	++ (hyalinized)	small to moderate	small	none	+
059-379	++ (edematous)	*	*	*	(+)

Adnexal Carcinoma

Breed incidence. Adnexal carcinomas were encountered quite frequently in the dog population. Almost all the breeds were affected (TABLE 1). Of 285 cases for which information was available, 58 were Boxers (20%), 30 were Cocker Spaniels (11%), and 28 were Dachshunds (10%).

Sex distribution. Although the difference was not remarkable, 133 neoplasms were recorded in male dogs, as against 122 from females, the ratio being 1.1:1.

Age distribution. Among 302 tumors recorded, information regarding age was available in 276 cases. The minimum age at which the condition appeared was 2.6 months. Instances were also recroded in dogs as old as 15 years, and the average age was 4 years.

Primary location. These neoplasms were located on the skin around the ears, lips, forelegs (mostly the elbow region), hind legs, foot pads, eyes, and toes in decreasing order of incidence. Two neoplasms were removed from the prepuce and 3 from the tongue.

Metastases. Primarily the cells invaded the dermis and subcutaneous tissue (Figure 8). Three instances in which metastases occurred to the skeletal muscles and visceral organs were reported. Out of these, only 1 was found to have metastasized to visceral organs; this was confirmed by histologic examination. One dog was reported as having miliary skin tumors in several parts of the body. Although some of the neoplasms listed in this group had histologic evidence of malignancy, there was no evidence that they had metastasized. Only 1 was reported

to have recurred following attempted surgical removal.

Microscopic characteristics. Adnexal carcinomas from 25 dogs were examined histologically. The characteristics are recorded in TABLE 2.

Two neoplasms, on re-evaluation, were diagnosed as adenocarcinomas of sebaceous gland origin and sweat gland origin, respectively. Foam cells were seen in the former neoplasm.

Epidermal ulceration and necrosis (Figure 9) with inflammatory reactions were noted in most of the neoplasms studied under this group. Inflammatory cells, predominantly lymphocytes, neutrophils, a few plasma cells and occasional eosinophils were seen. Hyperplasia of the stratified squamous epithelium overlying the neoplasm was a common feature. In some cases the epithelium was very thin.

In most cases, the neoplastic cells appeared to originate either from the hyperplastic stratified squamous epithelium (Figures 10, 11, and 12) or from the epithelium of the hair follicles (Figures 13 and 14). Occasionally their origin from both the structures was also evident. Some neoplasms, although histologically similar to others in this group, did not have any evidence of origin from the stratified squamous epithelium or from hair follicles. The neoplastic cells immediately under the epidermis were loosely arranged and were separated by edema in some instances (Figure 15). The cytoplasmic outlines of the neoplastic cells were clearly seen in these areas. The cell outlines in deeper areas of the dermis were fused and indistinct.

Shape of cells. The neoplastic cells of adnexal carcinoma were oval, polyhedral or pleomorphic in some instances. Seven neoplasms from this group had evidence of anaplasia. The cytoplasm of the neoplastic cells was pale and granular.

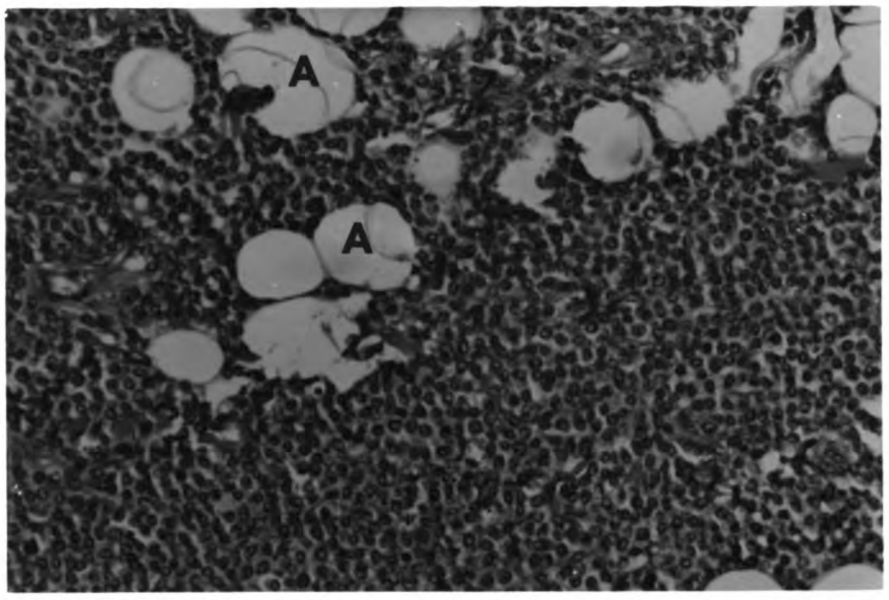

Figure 8. Adnexal carcinoma. Neoplastic cells have invaded the subcutaneous fat and clustered around fat vacuoles (A). Hematoxylin and eosin. x 188.

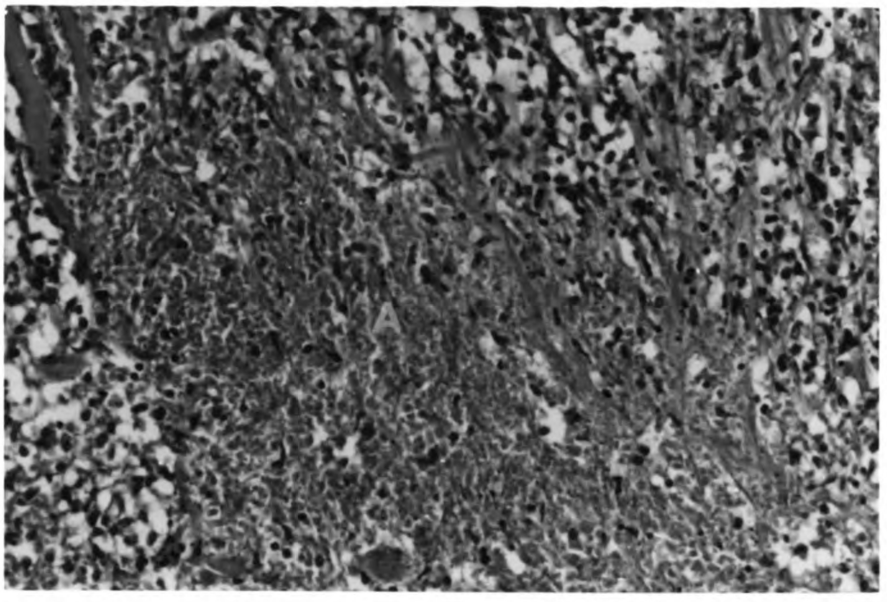

Figure 9. Adnexal carcinoma. Note necrosis in the cellular area (A). Hematoxylin and eosin. x 188.

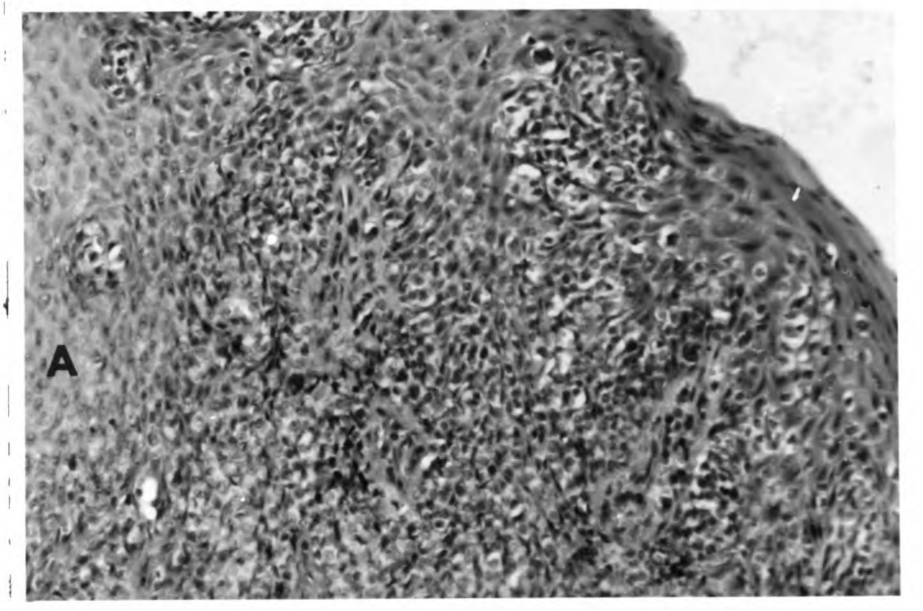

Figure 10. Adnexal carcinoma. Neoplastic cells originating from epidermis (A). Hematoxylin and eosin. x 188.

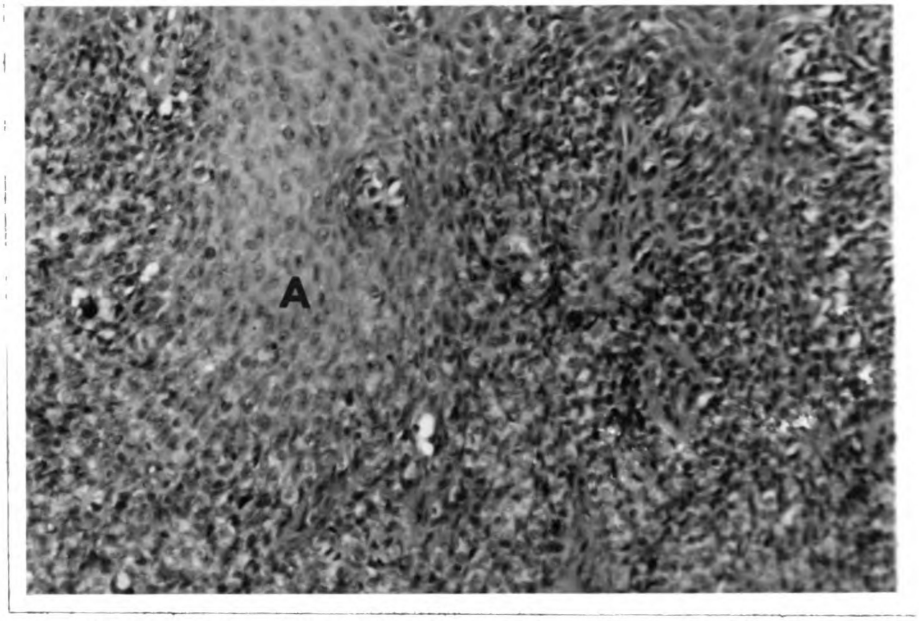

Figure 11. Adnexal carcinoma. Same as Figure 10. Note origin of the neoplastic cells from epidermis (A). Hematoxylin and eoxin. x 188.

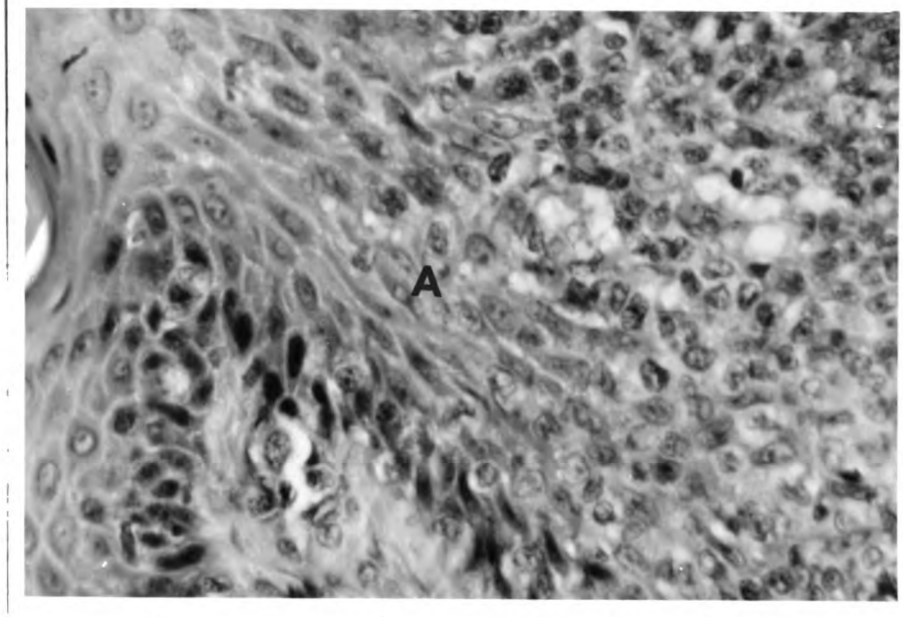

Figure 12. Adnexal carcinoma. Higher magnification of Figure 11. Note origin of neoplastic cells from epidermis (A) and the variation in their shape. Hematoxylin and eosin. x 469.

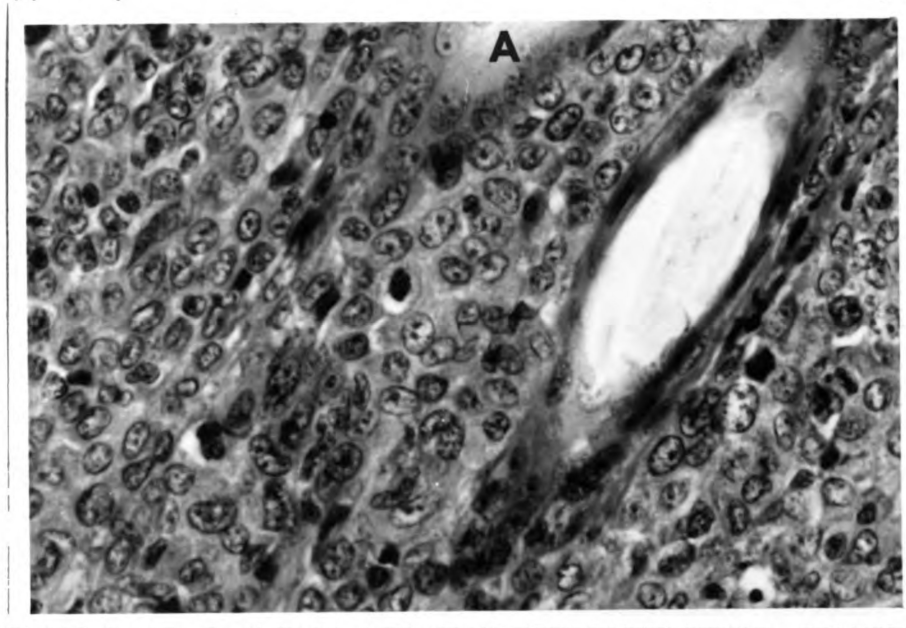

Figure 13. Adnexal carcinoma. The neoplastic cells appear to originate from the epithelium of the hair follicle (A). Also note the marked variation in the shape of the nuclei. Hematoxylin and eosin. x 469.

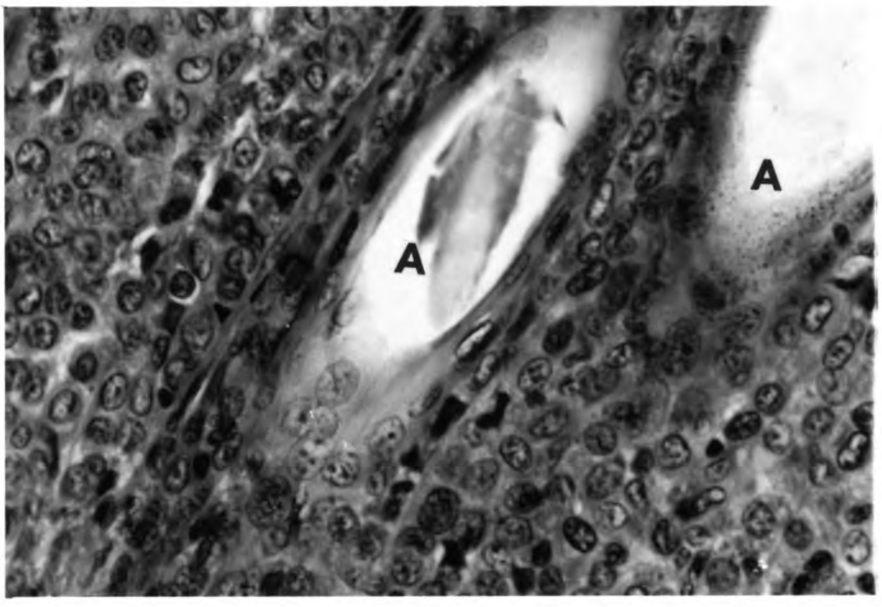

Figure 14. Adnexal carcinoma. The neoplastic cells appear to originate from the epithelial cells of two hair follicles (A). Hematoxylin and eosin. x 469.

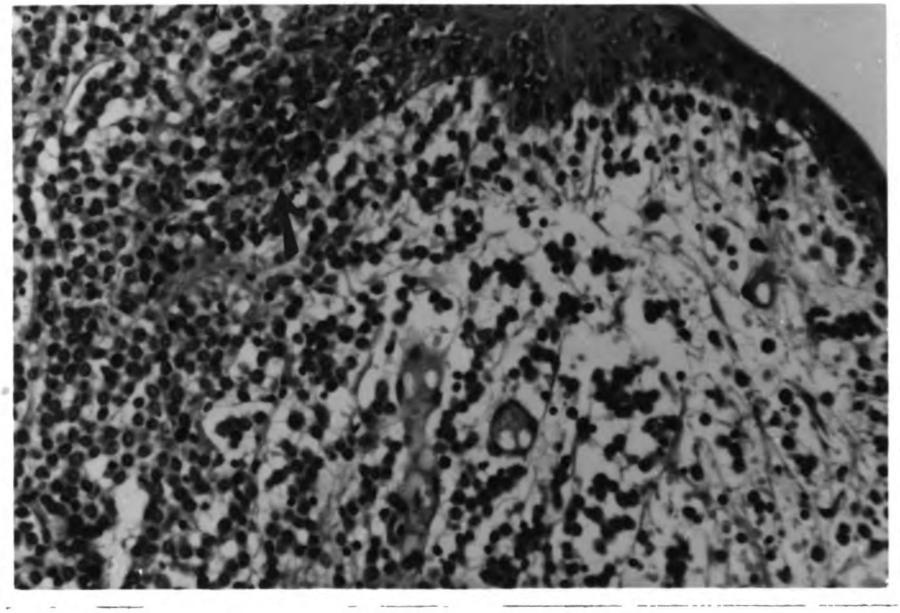

Figure 15. Adnexal carcinoma. Individualization of neoplastic cells due to subepidermal edema. Also some cells appear to originate from the epidermis (arrow). Hematoxylin and eosin. x 188.

Shape and size of nuclei. The nuclei of the neoplastic cells were spherical, oval or pleomorphic (Figures 16, 17 and 18), and they were indented in some cases (Figure 19). A few bilobed nuclei were also noted. The staining characteristics of the nuclei were variable. In some cases the nuclei of cells in the deeper areas of the dermis were more pleomorphic than those of the superficial areas. For all 10 animals, the average nuclear diameter was 7.7 microns.

Nucleoli. Nucleoli were mostly solitary and placed either in the center of the nuclei or eccentrically.

Mitoses. The number of mitoses in adnexal carcinomas varied from 0 to 15 per hpf, with an average of 4 per hpf.

Histochemical characteristics. Seventeen neoplasms from this group of 25 were studied with special stains. The characteristics are recorded in TABLE 3.

Reticulum. The amount of reticulum varied from small to large and was distributed between neoplastic cells or around small groups of cells (Figures 20, 21, 22, and 23). This distribution was irregular in most cases. The fibers appeared thin and wavy, and some were branched. There was no evidence that these fibers were formed by the neoplastic cells.

PAS reaction. Occasionally very small amounts of pink-staining granules were seen in the cytoplasm of some neoplastic cells.

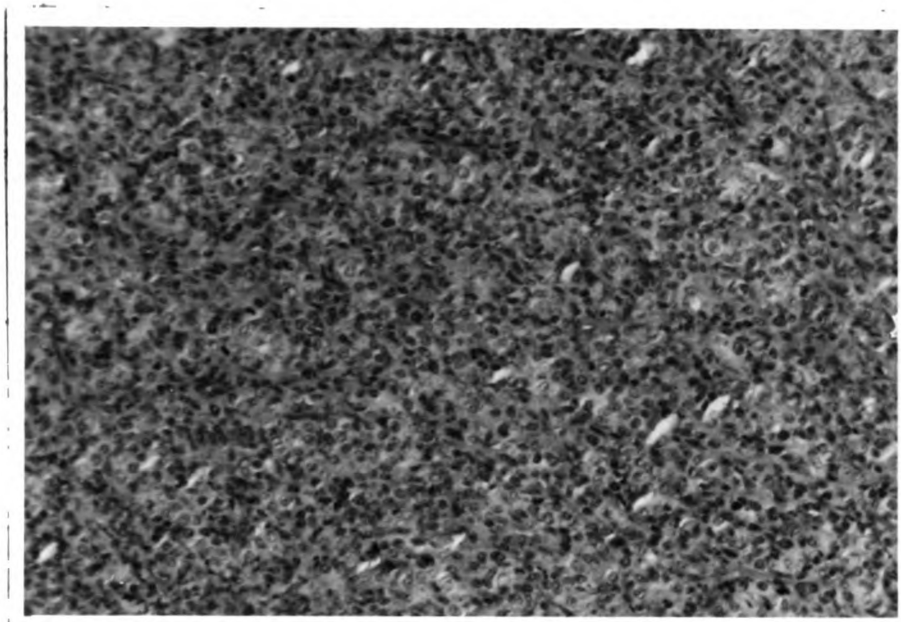

Figure 16. Adnexal carcinoma. Highly cellular neoplasm with very little visible supporting stroma. Hematoxylin and eosin. x 188.

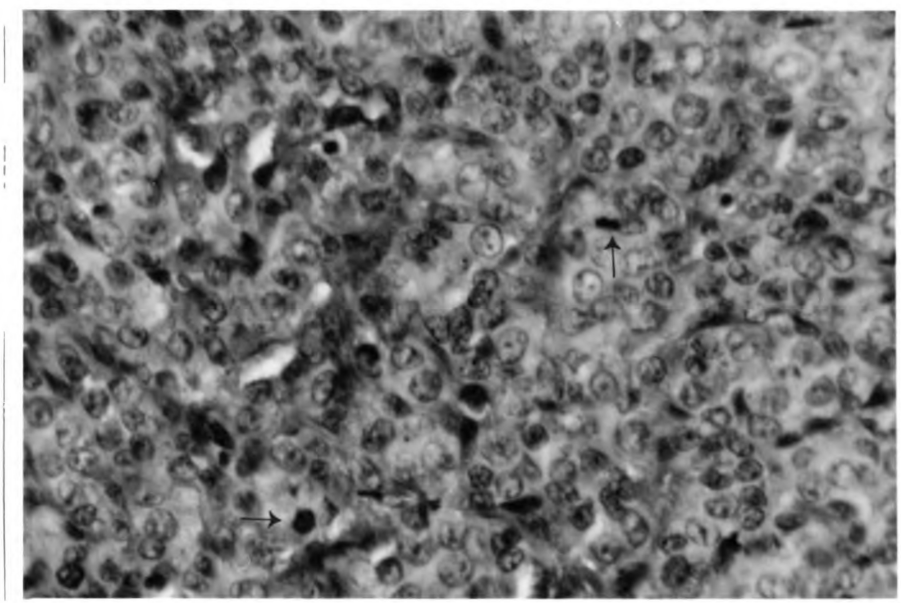

Figure 17. Adnexal carcinoma. Note indistinct cytoplasmic outlines with some variation in the shape of the nuclei. A few mitoses (arrows) are also seen. Hematoxylin and eosin. x 469.

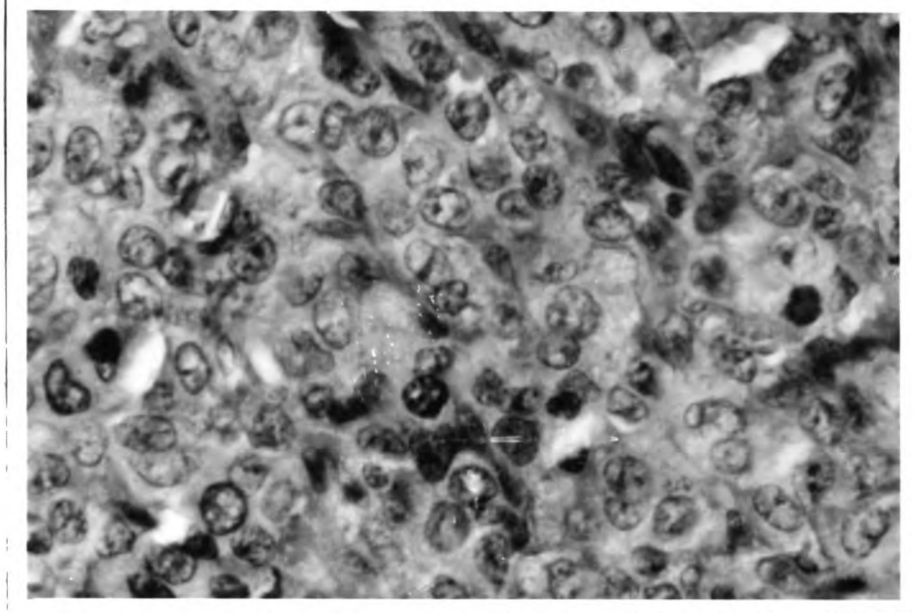

Figure 18. Adnexal carcinoma. Higher magnification of Figure 17. Note variation in the shape of the nuclei. Hematoxylin and eosin. x 750.

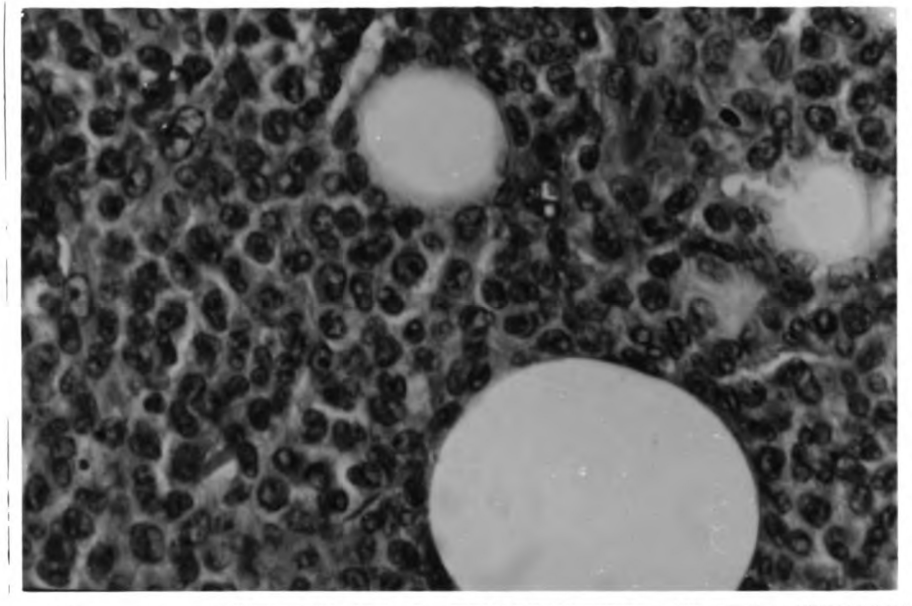

Figure 19. Adnexal carcinoma. Higher magnification of Figure 8. Note variation in the shape of the nuclei. Hematoxylin and eosin. x 469.

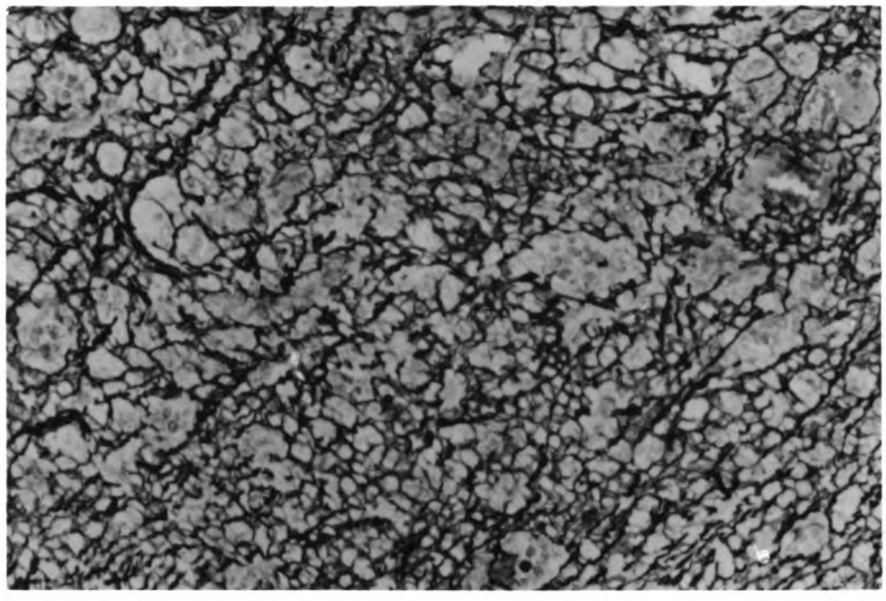

Figure 20. Adnexal carcinoma. Large amounts of reticulum seen around small groups of cells. Lithium Silver. x 188.

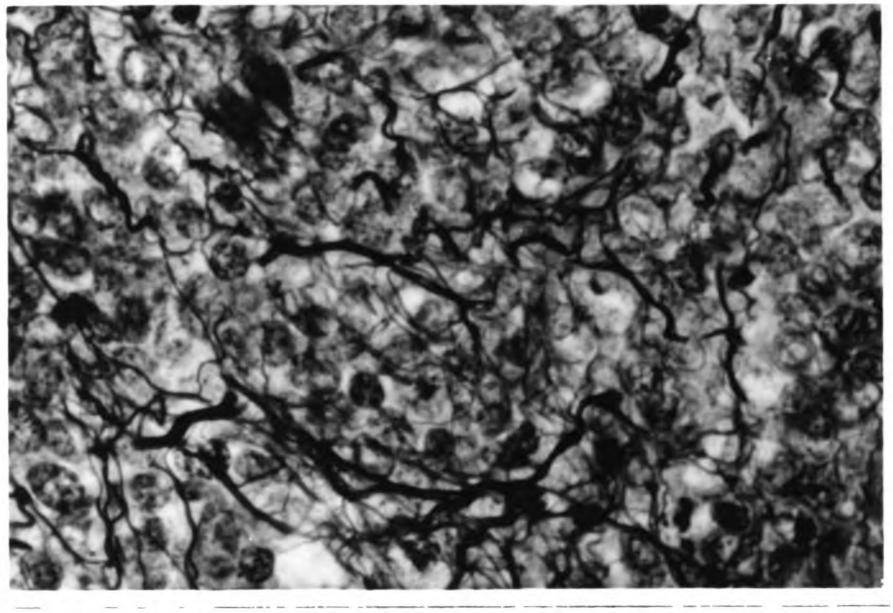

Figure 21. Adnexal carcinoma. Moderate amount of reticulum distributed irregularly around groups of cells. Lithium Silver. x 750.

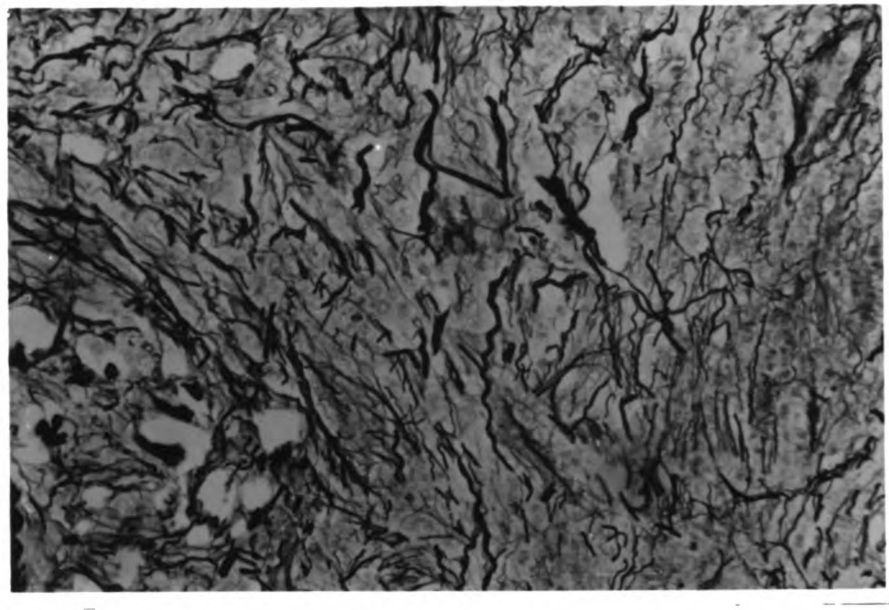

Figure 22. Adnexal carcinoma. Moderate amount of reticulum irregularly distributed around and between neoplastic cells. Lithium Silver. x 188.

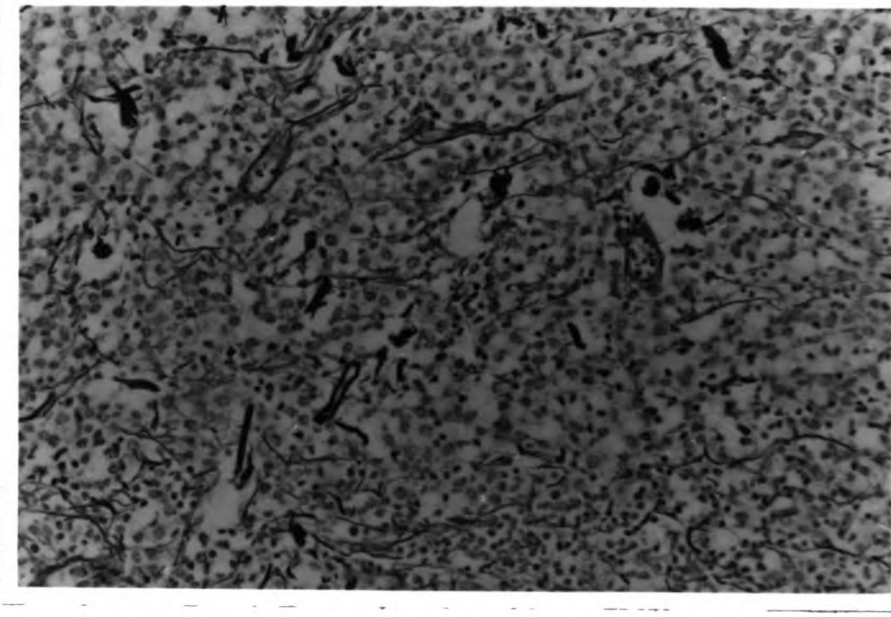

Figure 23. Adnexal carcinoma. Very small amount of reticulum present. Lithium Silver. x 188.

Giemsa stain. With Giemsa stain, some mast cells were seen in the cellular areas and elsewhere in the sections examined.

Basal Cell Carcinoma

Breed incidence. Of 114 basal cell carcinomas recorded, the highest incidence was noted in Cocker Spaniels (15%), Collies (13%), Poodles (9%), Boxers (7%) and Kerry Blue Terriers (7%), respectively (TABLE 1). Information regarding breed affected was not available for 8 cases.

Sex distribution. Fifty-three neoplasms were recorded in male dogs, compared to 46 in females, the ratio being approximately 1.2:1.

Age distribution. Even though the highest incidence was noted in older age groups, 1 instance was recorded in which a 6-month-old dog was affected. The mean age was 8 years and dogs as old as 16 years were reported as having the neoplasms.

Primary location. Basal cell carcinomas were commonly located around the head and neck area.

Metastases. One neoplasm with a history of metastasis to submaxillary lymph node was submitted for histopathologic examination. Although some lymphocytic tissue was found at the periphery of the neoplastic cells, definite evidence of metastasis to a lymph node could not be established.

Microscopic characteristics. Tumors from 25 dogs were examined histologically, 19 with the aid of special stains. The characteristics are recorded in TABLE 2.

The neoplasms in this group consisted of cells which were fairly uniform in shape. In only 2 instances was the origin of the neoplastic cells from the basal cell layer of the epidermis suggested (Figure 24). Evidence of origin of the cells from the epithelium of the hair follicle was seen in 1 case. Moderate to large amounts of fibrous tissue surrounded the cells and appeared hyalinized and edematous in most instances. Ulceration of the overlying epidermis, necrosis (Figure 25) and inflammatory cells were seen occasionally.

Shape of the cells. The cells were fairly uniform in morphology. Cytoplasm was scanty and cell outlines were indistinguishable. The cytoplasm stained faintly with eosin. Two chief patterns of cellular arrangement were noted. In one, the cells were in solid nests (Figures 26 and 27), while in the other they were in serpentine cords (Figure 28). The cells in the cords were arranged perpendicular to the long axes of the cords (Figure 29). Occasionally both forms were present in the same neoplasm. A third type of cellular arrangement, described as the Medusa head type in the literature, was much less common. In some, nests of cells were arranged in tubular form (Figure 30). Peripheral cell nuclei in some of these tubules were arranged with long axes perpendicular to the basement membrane (Figure 31). A few instances of formation of glandular and cyst-like structures were noted (Figures 32 and 33).

Shape of the nuclei. The nuclei were round, oval, cylindrical, or slightly pleomorphic. The nuclear chromatin was often increased in amount. The size of the nuclei in their longest diameter varied from 8.7 to 10.3 microns, with an average of 9.2 microns.

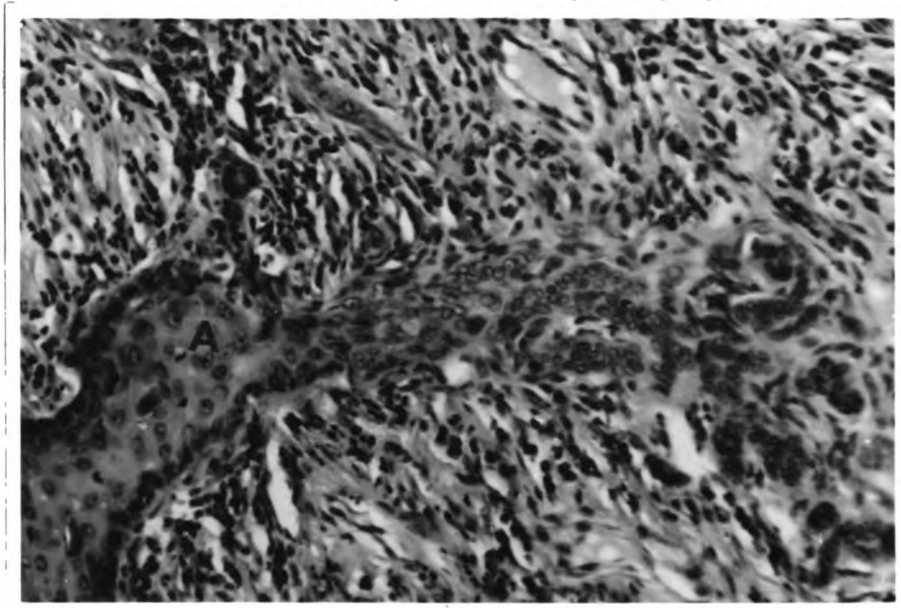

Figure 24. Basal cell carcinoma. Note apparent origin of the neoplastic cells from the epidermal basal cells (A). Hematoxylin and eosin. x 188.

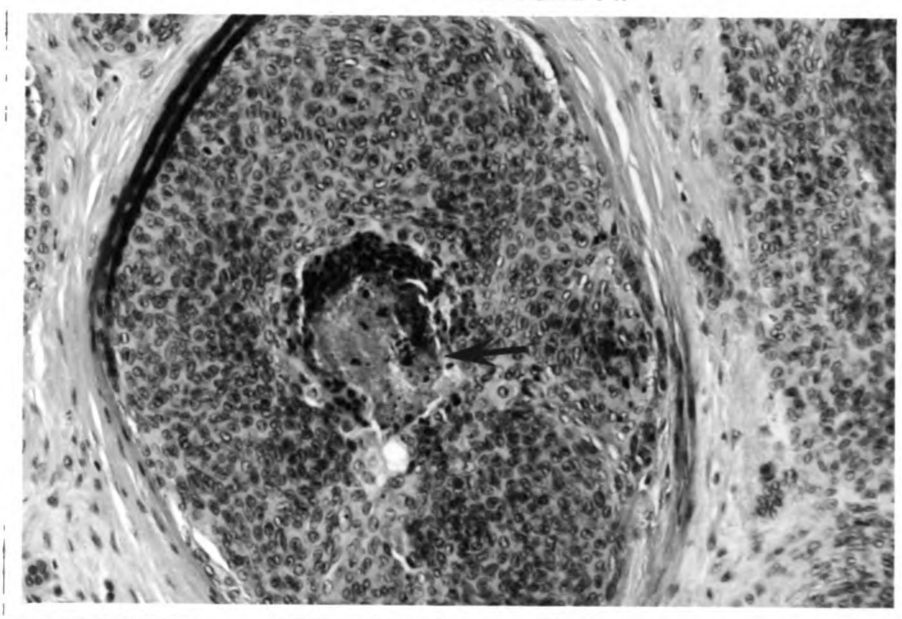

Figure 25. Basal cell carcinoma. Solid nests of neoplastic cells with necrosis of the cells in the center (arrow). Hematoxylin and eosin. x 188.

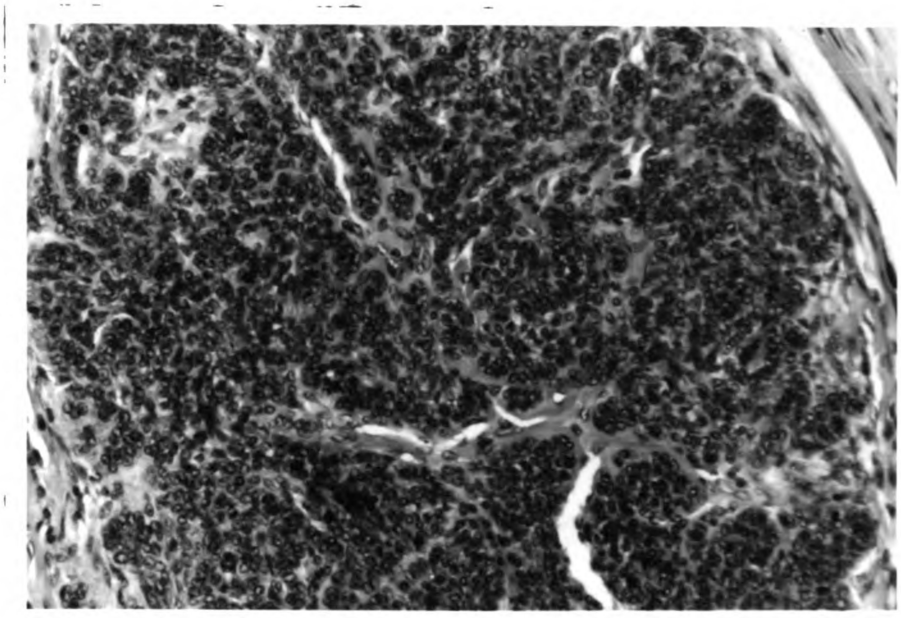

Figure 26. Basal cell carcinoma. Neoplastic cells arranged in solid nests surrounded by connective tissue stroma. Hematoxylin and eosin. x 188.

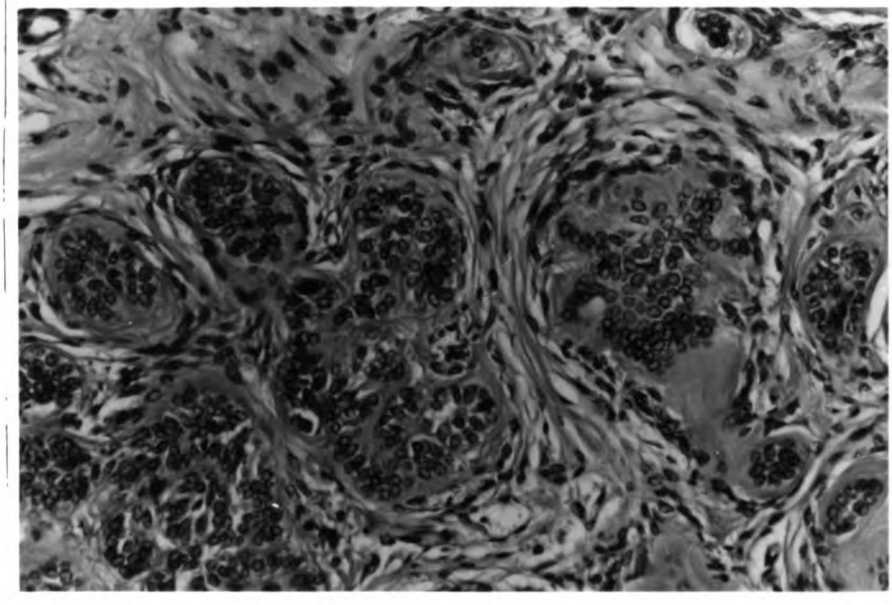

Figure 27. Basal cell carcinoma. Neoplastic cells arranged in small, solid nests surrounded by large amounts of cellular stroma. Hematoxylin and eosin. x 188.

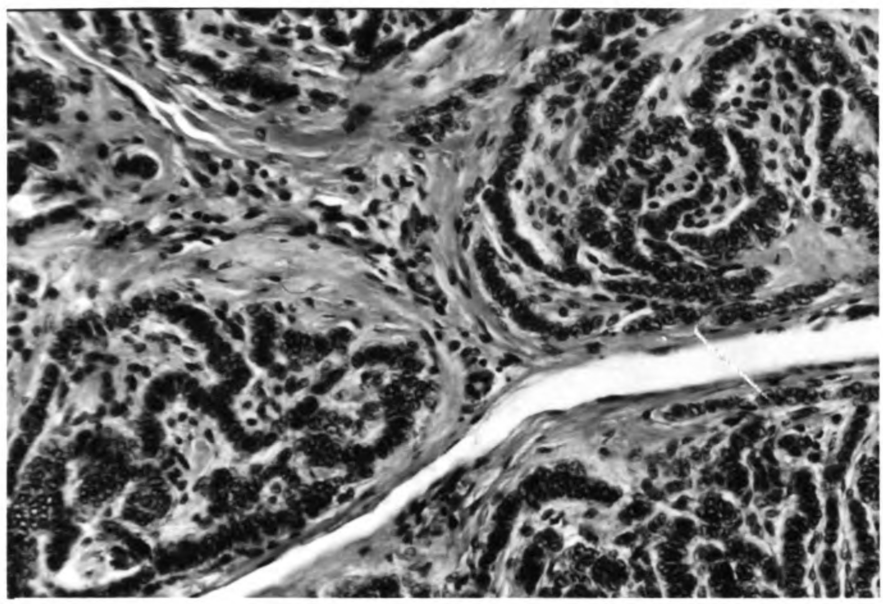

Figure 28. Basal cell carcinoma. Neoplastic cells arranged in serpentine cords and surrounded by large amounts of connective tissue stroma. Hematoxylin and eosin. x 188.

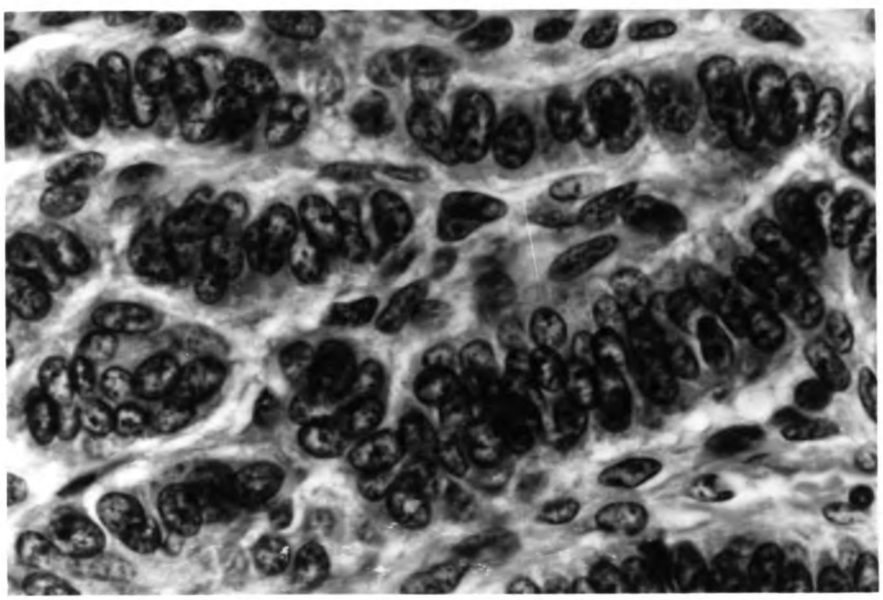

Figure 29. Basal cell carcinoma. Neoplastic cells arranged in cords with their long axes perpendicular to the basement membrane. Also note large amounts of nuclear chromatin. Hematoxylin and eosin. x 750.

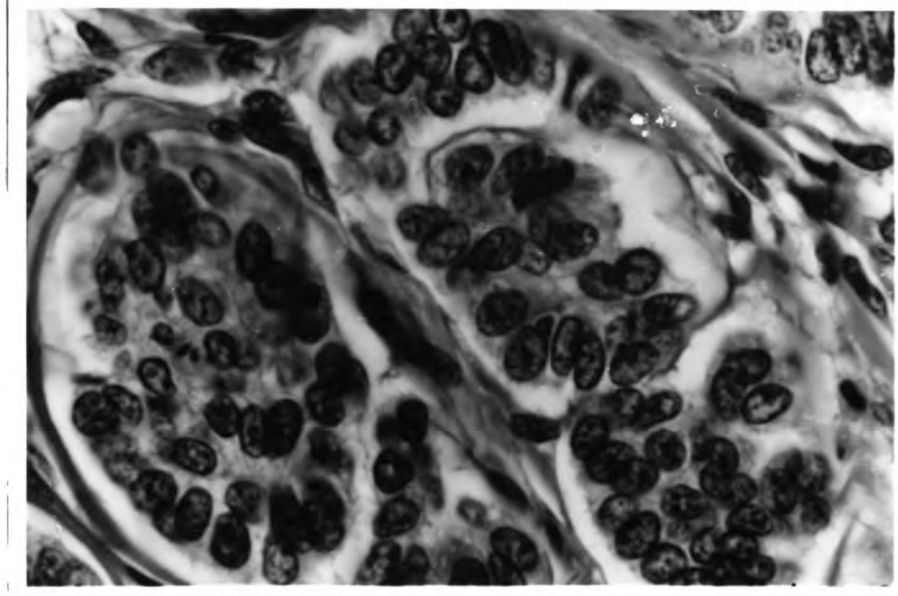

Figure 30. Basal cell carcinoma. Small nests of cells forming tubule-like structures. Hematoxylin and eosin. x 750.

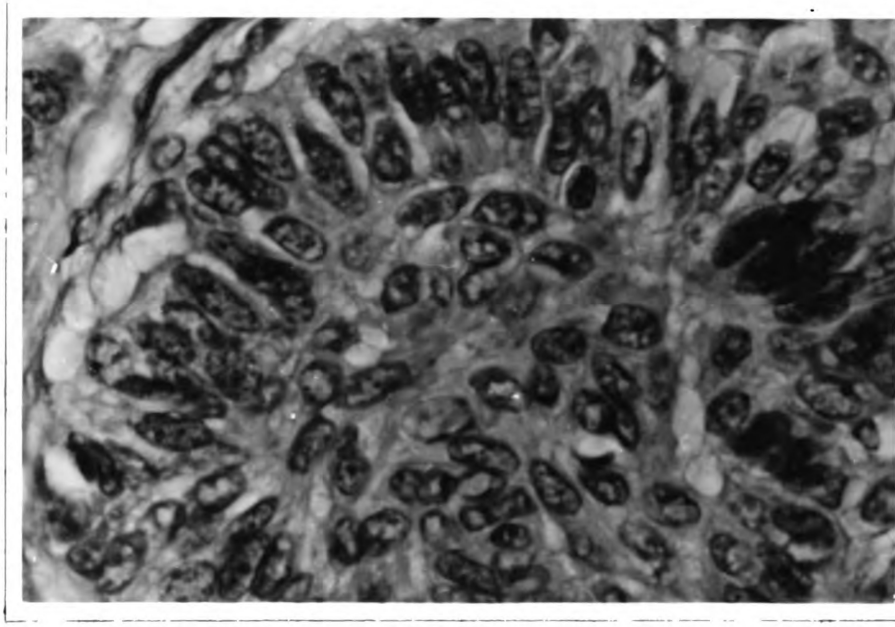

Figure 31. Basal cell carcinoma. Small nests of neoplastic cells with the peripheral cell nuclei arranged with long axes perpendicular to the basement membrane. Hematoxylin and eosin. x 750.

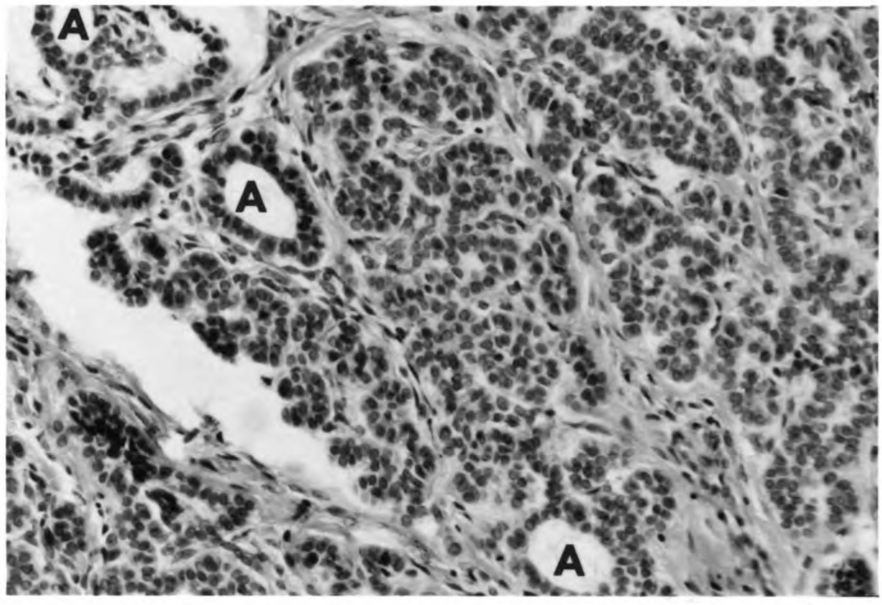

Figure 32. Basal cell carcinoma. Small nests of cells separated by small amount of stroma. Note formation of gland-like structures (A). Hematoxylin and eosin. x 188.

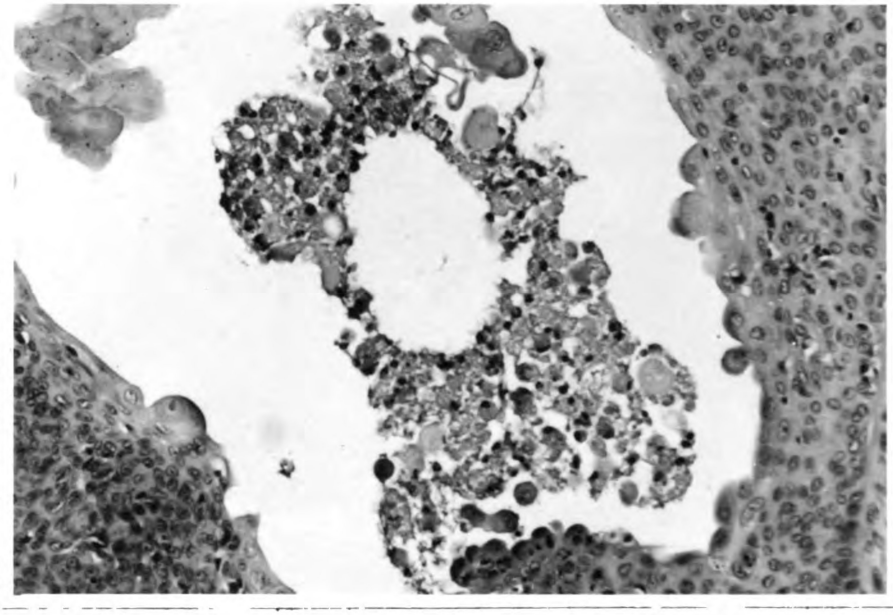

Figure 33. Basal cell carcinoma. Note formation of a cyst-like structure with desquamated neoplastic cells in its lumen. Hematoxylin and eosin. x 188.

Nucleoli. The nucleoli were mostly solitary and basophilic. They were not very prominent.

Histochemical characteristics. Histochemical characteristics of 19 neoplasms studied under this group are recorded in TABLE 3.

Reticulum. Reticulum was small to large in amount and distributed mostly around cell nests (Figures 34 and 35). Some of the fibers extended from the periphery of the nests toward the center (Figure 36). Fibers were also distributed between small groups of cells and were predominantly unbranched. There was no evidence that they were formed by the neoplastic cells.

PAS reaction. Pink-staining PAS-positive materials were seen in the cytoplasm of most of the neoplastic cells and varied from small to large in amount (Figure 37). There was no characteristic pattern of distribution except that, in rare instances, an increased amount of PAS-positive material was seen toward the center of the cell nests. This feature, however, was not constant. The distribution was not uniform even in neoplasms containing large amounts of pink-staining material. Three neoplasms from the entire group did not have any detectable reaction.

After digestion with diastase, most of the pink-staining, PAS-positive materials were dissolved, leaving only traces (Figure 38).

Giemsa stain. Variable numbers of mast cells were constantly noted in the stroma surrounding the neoplastic cells (Figure 39).

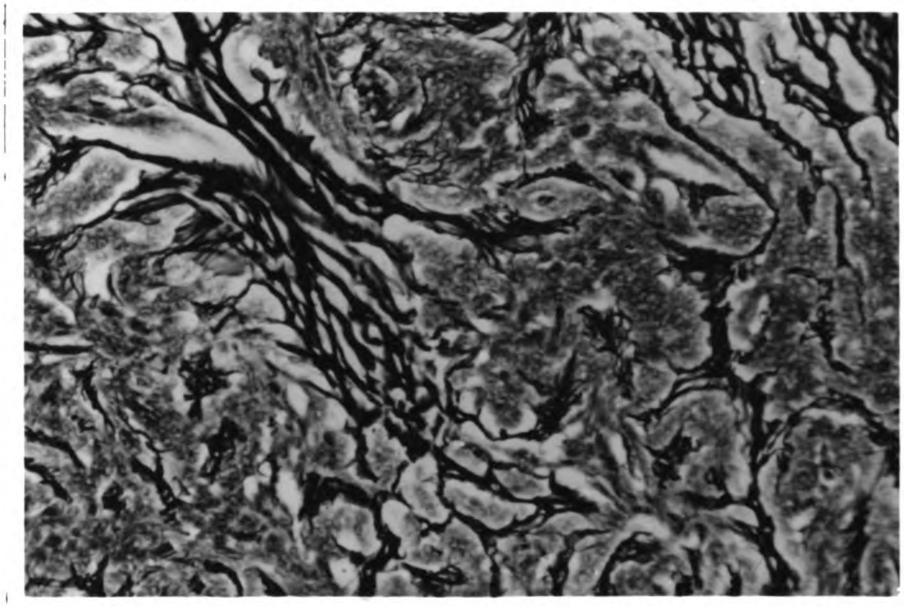

Figure 34. Basal cell carcinoma. Moderate amount of reticulum arranged irregularly around groups of neoplastic cells. Lithium Silver. x 188.

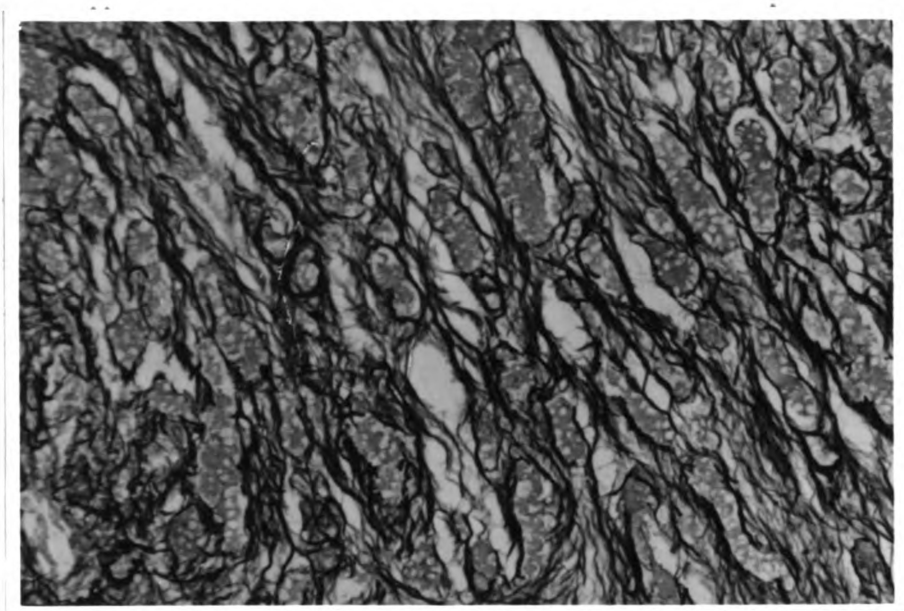

Figure 35. Basal cell carcinoma. Note presence of large amounts of reticulum distributed around small groups of neoplastic cells. Lithium Silver. x 188.

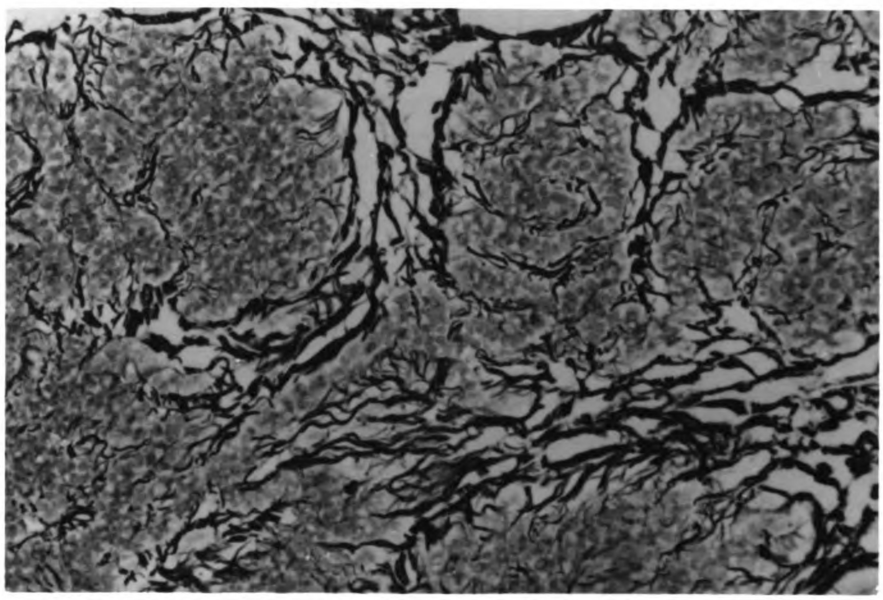

Figure 36. Basal cell carcinoma. Note presence of moderate amount of reticulum mostly distributed around nests of cells and occasionally coursing from the periphery toward the center. Lithium Silver. x 188.

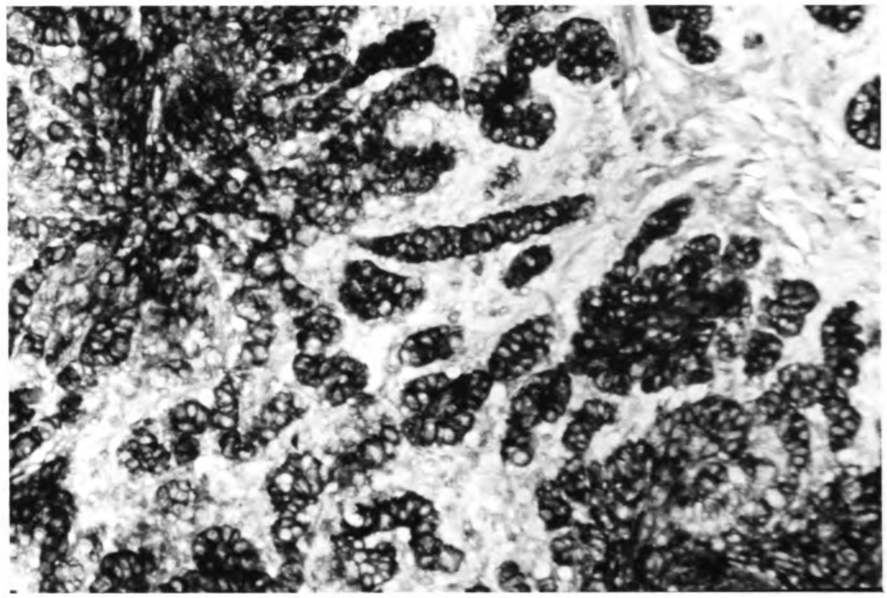

Figure 37. Basal cell carcinoma. Note presence of deeply staining, periodic acid-Schiff-positive material in the cytoplasm of the neoplastic cells. Periodic acid-Schiff stain. x 188.

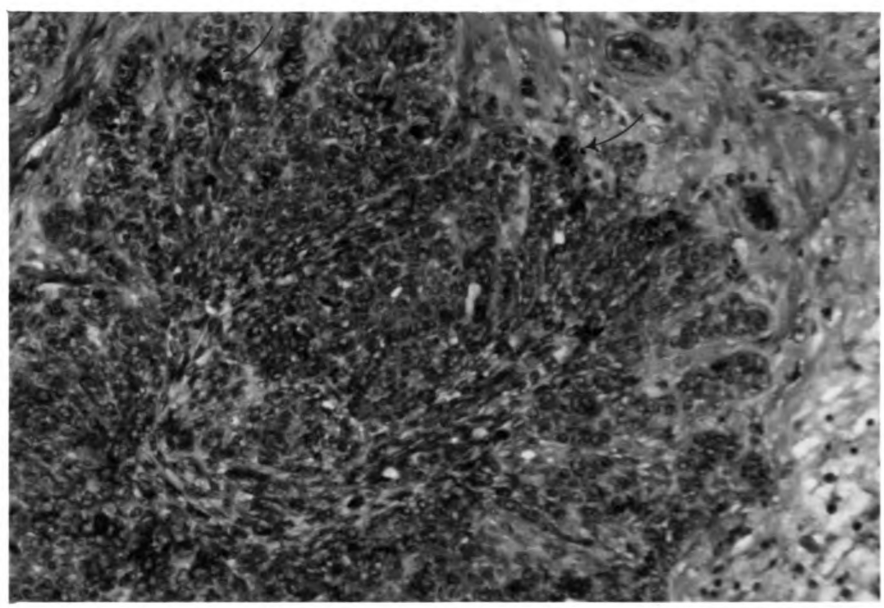

Figure 38. Basal cell carcinoma. Note presence of only very small amount of PAS-positive material (arrows) after diastase digestion. Periodic acid-Schiff stain. x 188.

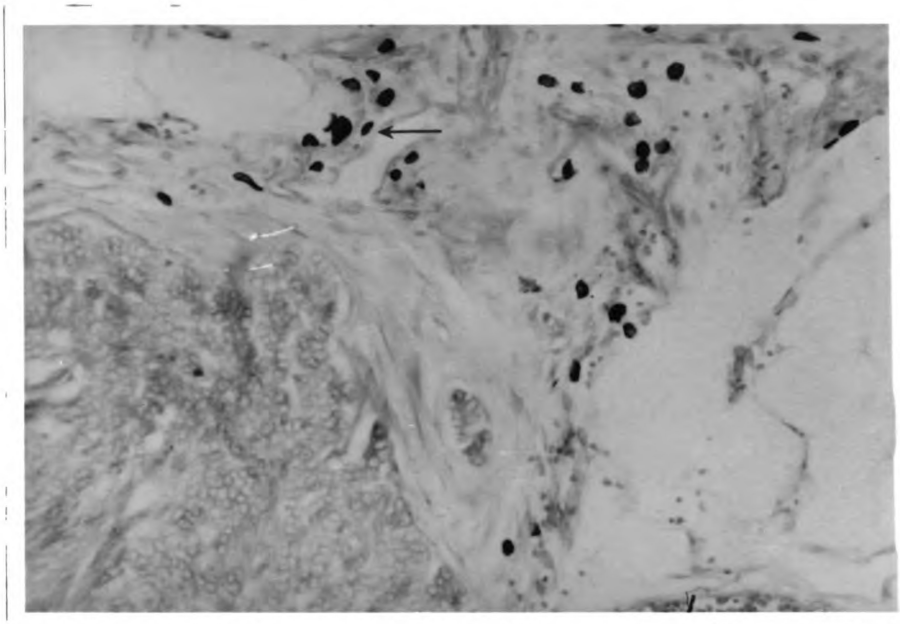

Figure 39. Basal cell carcinoma. Note presence of moderate numbers of mast cells (arrow) in the stroma surrounding the neoplastic cells. May-Grunwald-Giemsa. x 188.

DISCUSSION

Transmissible Venereal Tumor

The transmissible venereal tumor is a relatively rare neoplasm in the canine population. In the present study of the 25 neoplasms originally placed in this category, only 3 were characteristic of transmissible venereal tumor histologically. The remaining 22 were reclassified and will be discussed later.

Breed incidence. Although the Boxer was the predominant breed in the original 40 cases, breed incidence could not be considered significant when these neoplasms were re-evaluated, since only 3 of the 25 cases studied histologically were retained as transmissible venereal tumors. This was because of the confusion that has long existed regarding genital and extragenital locations of the transmissible venereal tumors. No particular breed distribution was found in the literature, and this neoplasm apparently occurs in all breeds of dogs.

Sex distribution. The ratio between male and female dogs was 2:1. This ratio was probably not of significance due to the small number of animals involved. Moulton (1961) pointed out a greater incidence of these neoplasms in female than male dogs. In this study, however, of the 3 neoplasms reclassified as transmissible venereal tumors, 2 occurred in male dogs and 1 in a female.

Age distribution. The age at which these neoplasms occurred in dogs varied from 4 months to 13 years. The average age for the 38 cases for which information was available was 5 years. Three neoplasms, re-evaluated as transmissible venereal tumors, occurred at 9 months, 9 years and 10 years of age, respectively. The data presented earlier might be debatable as it would be quite unusual for a dog to have venereal tumor at the age of 4 months because it would not be sexually mature and infection by coitus would be improbable.

Primary location. Two of the 3 neoplasms in the study with microscopic characteristics of transmissible venereal tumor were located in the genital organs of male dogs and 1 in the perianal region of a female dog. This observation was in agreement with that of Moulton (1961), who mentioned the occasional location of these neoplasms in the scrotum or perianal area in addition to the genital region.

Metastases. Metastatic lesions in the eye and in visceral organs were reported by Karlson and Mann (1952) and Barron et al. (1963). The 3 neoplasms reclassified as transmissible venereal tumors in this group, however, had no history of metastasis.

Microscopic characteristics. It appeared from the literature that there was some disagreement regarding the histologic characteristics of transmissible venereal tumors. In the present study, the neoplastic cells were spherical or oval and more or less uniform in shape and size. Cell nuclei were round, oval or rarely indented and were fairly uniform in size. This finding was in agreement with that of Bloom et al. (1951), Karlson and Mann (1952), Stewart et al. (1959), and Moulton (1961).

Smith and Jones (1966) stated that,

> "In spite of these general statements, a characteristic of some value in diagnosis is a rather pronounced variation in size of individual cells and nuclei."

This statement was contradictory to the present findings and that of others. The eccentric location of the nucleoli in the nuclei was reported by Bloom et al. (1951) and Moulton (1961), but it was noted from this study that the nucleoli, although predominantly present eccentrically, also were seen in the center of some nuclei. There was some disagreement between Bloom and co-workers (1951) and Moulton (1961) regarding the amount of chromatin in the nuclei of transmissible venereal tumor cells. This might have been due to visual error or improper fixation. However, hyperchromatism was observed in the neoplastic cells studied in this group. This supported the findings of Moulton (1961). Also an increase in the number of mitoses, reported by most of the investigators, was in agreement with the present work.

Histochemical characteristics. Thin fibrils of reticulum surrounding groups of cells in transmissible venereal tumor were reported by Bloom and co-workers (1951), Stewart et al. (1959) and Moulton (1961). This type of arrangement was also found in this study. In addition, absence of reticulum from certain areas of the section, except around the capillaries, was also noted. This emphasized the irregularity in the distribution of these fibers in this neoplasm. However, this point might not have a strong basis as only 1 neoplasm was studied with special stains.

Occasional PAS-positive granules were seen in the cytoplasm of some cells. The nature of these granules was not ascertained, although they would appear insignificant because of their small numbers. Some

of these granules might represent granules of mast cells and other leukocytic granulocytes. However, the absence of PAS-positive material from the neoplastic cells was observed by Bloom et al. (1951).

Four neoplasms of the remaining 22 were classified as neoplasms of an undifferentiated type, and their origin could not be ascertained. It was quite clear that they were not histologically similar to typical transmissible venereal tumors. These neoplasms would probably fit the classification of "reticulosarcomata" of Ottosen (1949).

The remaining 17 neoplasms were located on various areas of the body; they resembled adnexal carcinomas histologically and are discussed with this group.

Adnexal Carcinoma

This neoplasm probably would be designated as histiocytoma or extragenital venereal tumor by some investigators. Even though it was encountered quite frequently, unfortunately only a few reports were found in the literature. Moulton (1961) stated that its neoplastic nature has been questioned by some, who thought it to be a special type of inflammatory process rather than neoplastic. From the present study it was evident that these lesions resembled neoplasms histologically. In the following discussion it is assumed that this neoplasm is comparable to the histiocytoma described by others.

Breed incidence. Almost all breeds were affected. Highest incidence was noted in Boxer dogs. Howard and Nielsen (1965) reported the high incidence of histiocytoma in Boxer dogs, which was quite in agreement with this study. Mulligan (1948) reported a high incidence of histiocytoma in Cocker Spaniel dogs. This observation of Mulligan was explained

by Howard and Nielsen (1965) to be due to the increased population of Cocker Spaniels during that time and also to the locality. Incidences of adnexal carcinomas in cats have been noted by Moulton (1961) and Schmidt (1966). This suggests that histiocytoma, as previously described, is a separate entity from transmissible venereal tumor, the latter being uncommon in cats.

Sex incidence. Mulligan (1948) noted that histiocytomas occurred 2.5 times as frequently in males as in females. This also corresponded to the finding of Head (1953), who found the neoplasm more commonly in male dogs. The male:female ratio was only 1.1:1 in the present investigation. The results of this study seem to support Moulton (1961), who found no sex predisposition.

Age distribution. Mostly young dogs were affected, the average age being 4 years. This observation is in agreement with those of Mulligan (1948) and Moulton (1961). A significant number of these neoplasms was seen in older dogs in the present group. The observation reported by Ottosen (1949) was quite interesting and contradictory to that of others. He noted "histiocytic sarcomata" in older dogs, and not a single tumor was present in the younger age group. As was pointed out before, it cannot be determined with certainty if he was describing the same neoplasm as the one presently studied.

Metastases. Of 56 cases followed by Mulligan (1961) after surgical excision there was no recurrence. Also, there was no history of metastasis. This report was contradictory to that of Head (1953), who found recurrence in 7 of 127 cases. Moulton (1961) reported that these

neoplasms did not metastasize, although local malignancy was evidenced by rapid growth and increased numbers of mitoses. Only 3 cases were noted in this study in which metastatic foci were reported in the skeletal muscles and visceral organs. Histologic examination revealed metastatic foci in visceral organs in only 1 case. Tissues from metastatic foci were not received in another case for histopathologic examination. The incidence of metastasis and recurrence following surgical removal may have been greater than reported in this study due to the difficulty in obtaining accurate follow-up information. The occurrence of several miliary tumors in the skin of 1 dog affected with adnexal carcinoma was also reported.

Primary location. The results of this study are in agreement with those of Mulligan (1948), in which the author noted the predominant occurrence of histiocytomas around the head and anterior extremities of the body.

Microscopic characteristics. Variation in the size and shape of the neoplastic cells and their nuclei identified in this study with adnexal carcinoma was also reported by Mulligan (1948) and Moulton (1961) in histiocytoma. Binucleate forms of the nuclei were also reported in the histiocytoma by Mulligan (1948). Although the numbers of mitoses were variable, they were nevertheless significant in some neoplasms in this group. Moulton (1961) stated, "A chracteristic of this tumor is the presence of mitotic figures". Ottosen (1949) reported the presence of giant cells in the "histiocytic sarcomata" he studied. This was not observed by others, to the knowledge of the author. Ottosen also noted the presence of numerous fat globules in the neoplastic cells and

speculated that these are due to phagocytosis by the cells or represent some degenerative changes in these cells. His finding was contradictory to that of Mulligan (1948), who did not find any fat in the cells of histiocytoma. This suggests that Ottosen dealt with a group of neoplasms separate from the histiocytoma originally described by Mulligan. On the other hand, Ottosen's (1949) description of "reticulosarcomata" was in agreement in many respects with the appearance of adnexal carcinomas presently studied. However, the occasional presence of giant cells in the neoplasms studied by Ottosen was not seen in the present study. The nomenclature applied by Ottosen needs further clarification.

The presence of neoplastic cells in clusters around hair follicles and other adnexal structures in the histocytoma was reported by some investigators (Moulton, 1961; Mulligan, 1961). Although this feature was commonly noted in the present study, the origin of the cells from the epithelium of some hair follicles was also apparent histologically. Epidermal atrophy and hyperplasia were also mentioned by Mulligan (1961) and were commonly seen in this study. Several instances were noted in which the neoplastic cells appeared to originate from the epidermis or from stratified squamous epithelium. Of interest was the common occurrence of ulceration of the epidermis over the neoplastic mass and in other areas. This was probably due to trauma but may have been a result of rapid multiplication of the cells without sufficient blood supply. The inflammatory cells in these neoplasms were probably secondary to ulceration and necrosis.

Runnells et al. (1965) did not clearly differentiate between "histiocytic sarcoma" and transmissible venereal tumors of dogs, and their descriptions were not very helpful in establishing criteria for diagnosing

these neoplasms. An alveolar arrangement of the neoplastic cells surrounded by connective tissue stroma was noted by these authors. However, this type of arrangement was not commonly seen in the neoplasms studied presently. The authors also pointed out the neoplastic involvement of turbinate bones due to inhalation of tumor cells. This would probably be more applicable to the transmissible venereal tumor than to histiocytoma. Runnells and co-workers (1965) mentioned the possibility of confusing "histiocytic sarcoma" with "mastosarcoma" occurring in the skin of the dog, especially when the latter is devoid of metachromatic granules. This is a very important consideration and deserves the attention of pathologists. So far as the present study is concerned, much attention has been paid to differentiation between mastocytoma and adnexal carcinoma. The presence of metachromatic granules, as demonstrated by Giemsa's stain, and other microscopic characteristics were used in this study for differentiation between these 2 entities.

Histochemical characteristics. Mulligan (1948) observed reticulum fibrils around groups of tumor cells and pointed out that these were part of the stroma and not produced by the neoplastic cells. This was similar to the observation made in the present study. It is emphasized, however, that the demonstration of these fibrils is not of any significance in diagnosis due to considerable variation in their numbers and distribution even in the same neoplasm. Ottosen (1949) also observed differences in the amount and distribution of the argyrophilic fibers in the "reticulosarcomata" (which were similar to adnexal carcinomas in many respects) he studied. Absence of demonstrable argyrophilic fibers from certain areas of the section was noted by him, and he thought that those areas were probably "growth centers".

The presence of occasional PAS-positive granules in the cytoplasm of some cells was noted in this study. Some of the granules were in cells other than the neoplastic cells. Nevertheless, some of the granules were present in the neoplastic cells. This could have been due to the presence of several materials, since a number of substances have an affinity for this stain. However, no attempt was made in this study to determine the nature of these granules, as they were insignificant in number.

With the Giemsa stain, the cytoplasm of the neoplastic cells was slightly basophilic. No metachromatic granules were seen in the cells. The presence of mast cells in variable numbers was noted in these sections.

The term "atypical epithelioma" is suggested for the neoplasm previously diagnosed as adnexal carcinoma due to the following reasons:

1. None of these neoplasms formed adnexal structures. The neoplastic cells did not originate from all the adnexal structures, so the term adnexal carcinoma is believed to be unsuitable.

2. The neoplastic cells appear to originate from the stratified squamous epithelium of the epidermis and other areas and epithelium of the hair follicles, hence are considered epithelial in nature.

3. The cells are atypical due to the fact that they do not resemble the parent cells from which they originate and are pleomorphic.

Basal Cell Carcinoma

Several names have been applied to this neoplasm in the past. Moulton (1961) called it "basal cell tumor" because it never metastasized, and hence the term "carcinoma" was not acceptable to him. However,

metastatic lesions in the regional lymph nodes and in the lung were reported by Head (1953) in 2 cases. One neoplasm with a history of metastasis or direct extension to a lymph node was also recorded in the present study. It is evident, therefore, that the term basal cell carcinoma is probably the name of choice because metastasis has been encountered in some cases, even though it is infrequent. In this study one neoplasm originated from the epidermal basal cells and another from the epithelium of the hair follicle. This demonstrates that this neoplasm may arise from the basal cell layer of epidermis or from dermal adnexa.

Breed incidence. Moulton (1961) noted this neoplasm occurring commonly in the Cocker Spaniel. His finding was in agreement with the present study, in which a relatively high incidence was noted in this breed. However, Nielsen and Cole (1960), in their studies with 37 neoplasms of this group, did not observe any breed predisposition.

Sex incidence. Several authors (Nielsen and Cole, 1960; Runnells et al., 1965) did not note any sex preference. Head (1953) found these neoplasms predominantly in male dogs. Males were more commonly involved than females in the present study, although the differences were small.

Age distribution. Basal cell carcinoma was found to occur commonly in older age groups by the author, a finding which coincides with that of Nielsen and Cole (1960) and Moulton (1961). This was contradictory to the report of Runnells et al. (1965), who stated that the information regarding age predisposition was not established.

Primary location. The neoplasms were commonly located around the head and neck. Jackson (1936), Nielsen and Cole (1960), and Moulton (1961) also reported similar locations of basal cell carcinomas.

Metastases. Reports on the malignant nature of basal cell carcinoma were meager. Based on their clinically benign and locally invasive character, these neoplasms were termed basal cell epithelioma (Jackson, 1936) and basal cell tumor (Nielsen and Cole, 1960; Moulton, 1961). Head (1953), however, reported 3 neoplasms in his study with evidence of metastasis -- 2 to regional lymph nodes and 1 to the lung. This finding was of significance, but unfortunately there was no description of the microscopic characteristics of the neoplasms in their metastatic sites. It was difficult to evaluate the degree of malignancy in the present study, since not all of the neoplasms studied were followed closely for data on recurrence or metastasis.

Microscopic characteristics. Jackson (1936) stated,

> "It would give a wrong impression to say that the cells are uniform, close study showing that there are minor variations in size and shape which are associated with a tendency of these cells to undergo a certain amount of differentiation; but the term 'uniformity' is useful in indicating the prime characteristic of the histological picture, which is the absolute lack of anaplastic changes."

Based on this statement the term "uniform" was also applied in this study, although there was moderate variation in the shape of the cells. Continuity of the neoplastic cells with the epidermis was noted in 1 instance. Chiefly, 2 types of cellular arrangement were recorded in this study -- the solid type and the serpentine type -- with the former

being more common. This finding is not in agreement with Nielsen and Cole (1960), who stated that the ribbon or serpentine type is the most frequent form in the dog. Head (1953), from his study of 49 neoplasms in this group, classified 28 as solid type and 21 as Medusa head type. From his description it was evident that the Medusa head type was similar to those classified as serpentine in the present study. Head's observation regarding the greater incidence of solid types than serpentine types was confirmed by this study. The invasiveness of the neoplasms associated with the solid type of arrangement was stated by Head (1953) and Nielsen and Cole (1960). This was also noticed in some of the neoplasms presently studied. Nielsen and Cole (1960) described the Medusa head arrangement as consisting of cords of epithelial cells projecting from the solid islands of cells like the "tentacles of an octopus". This type of cellular arrangement was relatively rare in this study.

The presence of hyalinized, coarse, collagenous stroma with edema in some cases, as described by Jackson (1936) and Moulton (1961), was also observed in this study. Jackson (1936) reported the presence of 1 or 2 nucleoli in each of the neoplastic cells. THis was not in agreement with the observations of Nielsen and Cole (1960), who did not find any visible nucleoli. The results of this study, although they supported the findings of the former author, still might be debatable as the structures resembling nucleoli could have been confused with large chromatin granules occurring in the hyperchromatic nuclei. However, a statement made by Ham and Leeson (1961) in this connection is worth mentioning. They stated,

> "Nucleoli may be acidophilic or basophilic. The reasons for their behaving two ways are not entirely clear but are probably related to their staining reactions being determined by both the nucleic acid and the protein that they contain."

They also mentioned that the chromatin granules in fixed tissues tend to adhere to the nucleolus, thereby forming a basophilic envelope around it, and the nucleolus finally appears basophilic.

In this study the nucleoli appeared basophilic, possibly because of chromatin envelopes around them.

Haythorn (1931) and Mulligan (1949) noted the presence of structures resembling hair follicles in basal cell tumors. In this study a few hair follicles in deeper areas and surrounded by neoplastic cells were noted in some sections. No continuity between these structures and the neoplastic cells was demonstrated.

The data obtained by measuring the neoplastic cells of transmissible venereal tumor, adnexal carcinoma and basal cell carcinoma in their longest diameters were analyzed statistically, using an analysis of variance and the "F" test (Snedecor, 1948). These data were then checked for significant differences between group averages, using the multiple range test as described by Duncan (1955). It was found that the average diameter of the nucleus of the basal cell carcinoma was statistically greater (at the 1% level) than the average diameter of the nucleus of the other 2 neoplasms. There was no statistical difference between the average size of the nuclei of the canine transmissible venereal tumor and the adnexal carcinoma. This was to be expected, in view of the fact that many of the neoplasms originally diagnosed as canine transmissible venereal tumors were, in the present study, diagnosed as adnexal carcinomas.

Histochemical characteristics. Sams and co-workers (1963), using Wilder's stain, demonstrated the presence of argyrophilic fibers between and around the neoplastic cells in human basal cell epithelioma. These authors were able to dissolve these fibers by treating the tissue with dilute acetic acid. These fibers were also seen in variable numbers in this study and were distributed irregularly between and around the cell nests. Sometimes they appeared to course into the cell nests from the periphery and were predominantly unbranched. There was no indication that the fibers were being formed by the neoplastic cells, and they were assumed to be part of the stroma. Treatment with dilute acid was not possible as no fresh material was available for study.

With the PAS stain, pink-staining, PAS-positive granules were seen in the cells of most of the basal cell carcinomas studied, although the amount varied considerably from tumor to tumor. No positive correlation could be established between the cellular arrangement and the numbers of granules present. These granules were identified as glycogen by the fact that they were almost completely digested by diastase. The presence of glycogen in basal cell carcinomas probably has limited diagnostic value because of variations in the amount and distribution in the neoplasms. The author is not familiar with any other such report in the veterinary literature. Goltz and co-workers (1959) and Sams and co-workers (1963) pointed out the presence of PAS-positive material in basal cell carcinomas. While no effort was made by the latter authors to identify the nature of the PAS-positive materials, Goltz and co-workers (1959) tried to classify them by using special stains. They demonstrated 2 different kinds of materials, diastase-resistant and diastase-extractable, the latter being glycogen. Glycogen was present occasionally

in the tumors they studied compared to its common occurrence in the tumors studied in this series. It was interesting to note the presence of glycogen in these neoplasms, since the parent basal cells of the epidermis do not contain glycogen in the normal skin. Lovell (1955) found glycogen in the epithelium of the vulva, lip, conjunctiva and prepuce of normal dogs. Also glycogen was noted by him in the middle third of the outer root sheath of hair follicles, but not in the basal cells of the epidermis. The mechanism of the formation of glycogen in some of the basal cell carcinomas, however, still remains unexplained. It was difficult to ascertain whether the diastase-resistant material was intracytoplasmic or extracellular. This material could probably represent any one of or a combination of substances, such as melanin, connective tissue stroma entrapped by the tumor cells, or breakdown products of neoplastic cells. The presence of diastase-resistant substances, which were mostly extracellular, was also reported in the neoplasms from human cases by Goltz and co-workers (1959). These authors were unable to determine the exact nature of these substances and suggested that they might be any one or a combination of the 3 substances described above.

The reason 3 of the neoplasms studied did not have any detectable PAS reaction is not known. It is believed that the time elapsing between surgical removal and fixation probably played some part in these cases, and further work is needed to prove this point. It was also found that glycogen occurred more commonly in basal cell carcinomas from dogs than is reported from the same neoplasm in man.

Variable numbers of mast cells were noted in the stroma surrounding neoplastic cells in this study. This was also reported by Sams and co-workers (1963) in basal cell carcinomas of man.

SUMMARY

A study was made of the canine skin neoplasms diagnosed as transmissible venereal tumor, adnexal carcinoma or basal cell carcinoma in the Department of Pathology over a 10-year period. Of the 459 neoplasms in these groups, 43 were diagnosed as transmissible venereal tumor, 302 as adnexal carcinoma, and 114 as basal cell carcinoma. These neoplasms were compared with regard to breed, sex and age incidences and primary location in the body. Microscopic study was made of 25 transmissible venereal tumors, 25 adnexal carcinomas and 25 basal cell carcinomas. Histochemical studies were done on 22, 17 and 19 neoplasms, respectively, from the 3 groups.

Of the 25 transmissible venereal tumors studied microscopically, only 3 had the typical histologic characteristics described for this neoplasm. Most of the remaining neoplasms in this group histologically resembled the adnexal carcinoma. This discrepancy probably represents a change in the diagnostic criteria used in recent years in evaluating skin neoplasms of dogs. The 3 neoplasms typical of transmissible venereal tumors occurred on the genital organs and in the neighboring area.

The adnexal carcinoma appears to represent a distinct group of neoplasms seen in dogs and is probably sometimes diagnosed as histiocytoma or extragenital venereal tumor. It occurs frequently in the skin of young dogs. Boxer dogs are commonly affected. The term atypical epithelioma is suggested by the author for this group of neoplasms, for the following reasons:

1. The neoplastic cells appear to originate from the stratum germinativum of the stratified squamous epithelium of the epidermis and other areas such as lips and oral cavity, and the epithelium of hair follicles.

2. The cells are atypical due to the fact that they are pleomorphic and do not resemble the parent cells from which they originate.

This neoplasm may easily be confused with the transmissible venereal tumor when the latter is present in extragenital sites. No specific criteria could be established in this study to differentiate atypical epithelioma from transmissible venereal tumor. The use of special stains within the limitations of fixed tissues is not very helpful for their differentiation. Microscopic characteristics, history and the area of location of the neoplasm are of some value to the pathologist. More work with histochemistry on fresh tissues, chromosome count, tissue culture, and electron microscopy is needed to define the nature of the neoplastic cells.

The basal cell carcinoma is microscopically distinct from the transmissible venereal tumor and adnexal carcinoma. This tumor occurred frequently in Cocker Spaniel dogs. Mostly older dogs were affected. These neoplasms were commonly located in the head and neck areas. The occurrence of glycogen in the cytoplasm of the neoplastic cells in some of them is of interest. The average greatest nuclear diameter is significantly greater in basal cell carcinoma than in transmissible venereal tumor or adnexal carcinoma.

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