THE TRANSMISSIBLE VENEREAL TUMOR OF THE DOG
STUDIES INDICATING THAT THE TUMOR CELLS ARE MATURE END CELLS OF RETICULO-ENDOTHELIAL ORIGIN *

FRANK BLOOM, D.V.M., GEORGE H. PAFF, Ph.D.,† and CHARLES R. NOBACK, Ph.D.‡
(From the Departments of Pathology and Anatomy of the State University Medical Center at New York, Brooklyn, N.Y., and the Departments of Anatomy of the Hahnemann Medical College and Hospital, Philadelphia, Pa., and of the College of Physicians and Surgeons, Columbia University, New York, N.Y.)

The transmissible venereal tumor of the dog, also known as canine condyloma, venereal granuloma, infectious sarcoma, infectious lymphosarcoma, transmissible lymphosarcoma, and contagious venereal tumor, is a neoplasm occurring naturally on the genitals of both male and female dogs. Tumors thought to be of venereal type have been described also in extragenital locations,[1-6] but transplantation experiments to establish their identity as transmissible venereal tumors have not been performed.

Although the transmissible venereal tumor has been studied by many competent pathologists, the origin and classification of the tumor cells are still subjects of controversy. Earlier workers [7,8] believed that the tumors are composed of epithelial cells and are therefore carcinomas. Others [9-11] considered them to be infectious granulomas. At present, however, there is no serious opposition to the belief that the transmissible tumors are truly neoplastic. Since transmission can be effected only by viable tumor cells, the belief that they are true tumors appears to be justified. As will be shown later, however, behavior of the tumor *in vivo* bears certain similarities to that of infectious granulomas. The consensus [1,12-15] is that the venereal tumors are round cell sarcomas or lymphosarcomas, although certain reservations concerning this concept have been made by some authors. Beebe and Ewing [1] believed the tumor to be either alveolar sarcoma or endothelioma. Feldman [13] formulated the characteristics of the neoplasms which differ from those of most lymphoid tumors and stated that the term lymphosarcoma was used only for morphologic reasons. Stubbs and Furth [15] mentioned that the designation of lymphosarcoma or endothelioma has no sound basis. Kaalund-Jörgensen and Thomsen [16] were impressed with the similarity to reticulo-sarcoma, but thought that further investigation was necessary before including venereal tumors in this group. Jackson [3,17] desig-

* Received for publication, January 16, 1950.

† Now at the Department of Anatomy, Hahnemann Medical College and Hospital, Philadelphia, Pa.

‡ Now at the Department of Anatomy, College of Physicians and Surgeons, Columbia University, New York, N.Y.

nated the transmissible tumors on the basis of morphologic and cytologic studies as neuroblastoma (sympathogonioma). Mulligan [6] stated that the venereal tumor is apparently a histiocytoma.

The aforementioned investigators have attempted to define the cell type of the tumor by its microscopic structure, and, as is apparent, without any unanimity of opinion. While the cellular character is often the only available means for classifying tumors and establishing their histogenesis, the structure of this transmissible tumor is such that histologic study alone, even by competent observers, is inconclusive in this respect. For this reason we have attempted to determine the cell type of the transmissible venereal tumor by (1) ascertaining the identity of a spontaneously occurring vaginal tumor in a dog as a true transmissible venereal tumor by experimental transplantation to other dogs; (2) vital staining with trypan blue; (3) cytologic and histochemical investigations, and (4) tissue culture studies. Several of these procedures, *e.g.*, certain histochemical and tissue culture studies, have not to our knowledge been employed previously in regard to this tumor. At the same time several hitherto undescribed observations not directly related to the problem at hand have been made. The results obtained reveal that the tumor cells are of reticulo-endothelial origin.

General Data Concerning Transmissible Venereal Tumors

Gross Appearance. Under normal circumstances the transmissible venereal tumors are found as single or multiple, small or large, firm, soft or friable, gray to gray-red, sessile or pedunculated, nodular or papillary masses on the penis and at times on the parietal layer of the prepuce. They occur on the glans, sometimes on the entire penis, at the base of the penis and adjacent prepuce, and may extend to the scrotum and perineal region. In the female the tumors are usually solitary, are found beneath the mucosa in any part of the vagina, often involve the adjoining vestibule, and may spread to the labia. Their size varies from small nodules to large masses and the latter may occlude the vulvovaginal lumen or may protrude between the labia. In both sexes regressive changes are common, so that the tumors may ulcerate and slough, bleed easily, and frequently are associated with a serous, hemorrhagic, or purulent preputial or vaginal discharge.

Microscopic Structure. The tumor cells are large, round, polyhedral or slightly oval, rarely irregular, and display a striking uniformity in size and appearance (Figs. 1 and 2). The nuclei are large, round, relatively vesicular, and contain a prominent, generally single, and eccentrically situated nucleolus. There is an abundant clear or finely granular cytoplasm that stains weakly or pale pink with eosin, or bluish when

the sections are deeply stained with hematoxylin. Specific cytoplasmic granulations (eosinophilic or basophilic granules) are absent with special stains, such as Giemsa's. Mitotic figures are numerous and from 2 to 8 occur in each high-power field. The cells are closely packed and are arranged in diffuse masses (Fig. 1). Throughout the tissue are vascular connective tissue trabeculae of varying size, which sometimes produce pseudo-alveolar arrangements of the tumor cells. The fibrous stroma usually is scanty but is increased in older growths. Connective tissue stains and silver stains reveal an absence of intercellular collagenous and reticulum fibers; the argyrophilic fibers invest groups of cells. Scattered throughout the stroma are small numbers of lymphocytes, macrophages, eosinophils, and tissue mast cells. Polymorphonuclear leukocytes commonly are present in tumors showing regressive and inflammatory changes.

Spread. Although spread may occur by direct extension to adjacent structures and by metastasis to regional lymph nodes and rarely to internal organs, in most cases the tumors are confined to the genitals. The degree of malignancy is relatively slight as the health of the animal is not seriously, if at all, impaired nor is death a sequel. Spontaneous regression is frequent and recurrence is rare even after incomplete surgical removal.

Transmission. The tumors are characterized by the ease with which they can be transmitted to other dogs by natural or artificial means. Under ordinary conditions transmission is effected by coitus, either from male to female or from female to male. Transmission is possible also by an animal licking the affected genitals of another animal and in turn licking its own genitals or those of other susceptible dogs. The neoplasms appear to have a special predilection for the genitals, unless it can be proved that those in extragenital locations are also transmissible venereal tumors.

The experimental transmissibility can be accomplished by injecting emulsions of tumor cells subcutaneously, intraperitoneally, or intravenously; by bringing fresh neoplastic tissue in contact with scarified skin or mucous membranes, or by implanting tumor fragments in the subcutaneous tissue. It is well established that transmission occurs because the transplanted tumor cells are able to grow and multiply in the tissues of the host, and not because of a separate virus or infectious agent.[1,3,12-15] Dogs that recover spontaneously from the natural or experimentally induced disease are immune to attempts at further transmission.

Incidence. The transmissible venereal tumor has a world-wide distribution. During the early part of the twentieth century numerous

cases were encountered in New York City. In the experience of one of us (F. B.) true venereal tumors are at present rare in this region and only two examples were observed in a period of 19 years. It is apparent that in recent years there has been a significant reduction in the incidence, not alone in New York but also in other states.

Material and Methods

Tumor material used for transplantation and subsequent studies was obtained from a spontaneous transmissible tumor, measuring 7 by 11 cm., located in the vagina of an adult German Shepherd. The growth was of several months' duration and was characterized by a sero-hemorrhagic vaginal discharge and marked bulging of the perineal region. No other symptoms were observed and the animal appeared well. The tumor was removed surgically under general anesthesia on October 2, 1948, and there has been no recurrence to date.

Employing the usual aseptic precautions, small fragments of fresh, intact tumor not exceeding several millimeters in size were implanted subcutaneously, with suturing of the external skin, in the dorsal neck region of 4 male and female dogs of different ages and breeds. This site was selected because of inability of the animals to lick or scratch the operative area, thus aiding in the prevention of secondary infection and trauma. At varying intervals of time after growth had occurred, similar transplantation of neoplastic tissue from the recipient animals was done with 4 additional dogs. In all instances positive takes resulted. The original donor and 7 of the 8 recipient dogs were utilized as sources of tumor for morphologic, cytologic, histochemical, and tissue culture studies.

Morphologic studies were made from tissue fixed in Bouin's solution, Zenker's formalin solution, and 10 per cent solution of neutral formaldehyde, U.S.P. The sections were stained with hematoxylin and eosin, Wilder's reticulum stain,[18] Masson's trichrome stain,[19] Giemsa's stain, and Dominici's stain.[20] Tumor imprints were prepared by gently pressing a clean glass slide upon the cut surface of the growth. These were stained with Wright's and Giemsa's stains.

The following procedures were employed for cytologic and histochemical studies. The Golgi apparatus was identified by the Ludford and Da Fano methods.[21] Mitochondria were stained with the Bensley-Cowdry method [21] and the Mallory phosphotungstic acid hematoxylin technic.[22] Acid and alkaline phosphatases were studied in acetone-fixed tissue by the Gomori technics.[22] In addition, acid phosphatase was identified in fresh tissues (without fixation) by the modification of Bartelmez and Bensley.[23] In frozen sections of formalin-fixed tissue, the

M-Nadi reagent was used for cytochrome oxidase [24] and benzidine and hydrogen peroxide for peroxidase.[25] Lipids were investigated in tumors fixed in a 10 per cent solution of neutral formaldehyde, U.S.P., and in Baker's [26] formol-calcium and formol-calcium-cadmium fixatives. Frozen sections were stained with sudan IV, sudan black, and nile blue sulfate [24,26] and with sudan III.[27] Cholesterol was tested for in sections with the histochemical Schultz technic [21] and the total content in the tumor was estimated by the Liebermann-Burchard method.[28] Baker's [29] acid hematein and pyridine extraction tests for phospholipids were applied to tissues preserved in formol-calcium. Plasmals were investigated by the mercuric chloride-Schiff method [24] and by the dinitrophenylhydrazine method of Albert and Leblond.[30] Unstained frozen sections were observed under polarized light for birefringence. Glycogen was tested for by the periodic acid-Hotchkiss method [31] in picric acid-alcohol-formol and 10 per cent formalin-fixed tissues; glycoproteins, by the periodic acid-Hotchkiss method [31] in formalin-fixed and Zenker's formalin-fixed tissues, and metachromasia with toluidine blue in tissues fixed in 4 per cent lead subacetate.[32]

The procedures employed in tissue culture studies were as follows. Tumor fragments were grown in hanging drop preparations in a medium consisting of two parts chicken blood plasma, two parts dog serum, and one part chick embryo extract. Over 300 fragments were cultured. Some were stained supravitally with either methylene blue, Janus green, or toluidine blue; others were fixed in a 10 per cent solution of neutral formaldehye, U.S.P., and stained with iron hematoxylin, and still others were examined for acid and alkaline phosphatases by the technic of Bartelmez and Bensley.[23] To note the effect of "immune" serum on living neoplastic tissue *in vitro*, a separate experiment was conducted in which 50 tumor fragments were planted in a medium containing "immune" serum. The "immune" serum is so designated because it was obtained from a dog in which a transplanted tumor spontaneously regressed and finally completely disappeared. Many time-lapse photomicrographs were taken.

Vital staining was performed with trypan blue in the eighth animal when the transplanted tumor reached a size of 1.5 cm. A sterile 1 per cent solution of the dye at the dose rate of 1 cc. per lb. of body weight was injected intravenously and repeated in 48 hours. Twenty-four hours after the second injection a small piece of the tumor was removed surgically, from which imprints and sections were made. The tissue was fixed in a 10 per cent solution of neutral formaldehyde, U.S.P., and paraffin sections were counterstained with carmine. Imprints were examined unstained. The same dog then received five additional con-

secutive injections of the dye at 48-hour intervals. Twenty-four hours after the last injection, a small tumor fragment was again removed and treated as before. Another course of five injections of the dye was administered. Forty-eight hours after the last injection, several cubic centimeters of the dye was injected subcutaneously around the tissues of, but not into, the tumor itself. Twenty-four hours later a section of the growth was removed and treated as previously described.

Observations

Transmission Experiments

Following subcutaneous implantation of the tumor, healing occurred by first intention and the sutures were removed in 7 days. In 2 to 3 weeks after transmission there appeared a palpable and sometimes visible spherical nodule from 3 to 6 mm. in diameter. The nodule gradually enlarged so that by the fifth to seventh week it varied from 2 to 4 cm. in size. At this stage the growth projected above the surrounding skin and the covering epithelium appeared reddened and thinned. Shortly afterwards, the covering skin became ulcerated and the tumor broke through, resulting in a discharging necrotic mass. This process was hastened in dogs from which tumor sections were removed for study, despite the exercise of complete aseptic operative technic. In from 3 to 8 weeks after the appearance of regressive changes, there was gradual healing and ultimately a fibrous scar resulted. The latter persisted for several months and could barely be palpated or observed on incision at the end of this time.

Microscopic examination of the early developmental stages of the transmitted tumors revealed structural characteristics identical with those of the original vaginal tumor (Figs. 1 and 2). Tumors undergoing regression exhibited edema, hemorrhage, necrosis, fibroblastic proliferation, and variable numbers of polymorphonuclear leukocytes, lymphocytes, and plasma cells. The covering epithelium was ulcerated and showed pseudo-epitheliomatous hyperplasia at its junction with normal skin. In the end stages there was conspicuous fibrosis with scattered nests of tumor cells. Terminally, no vestiges of tumor cells were seen.

Cellular Characteristics in Imprints

The tumor cells were from two to four times larger than red cells and generally showed a uniform appearance (Fig. 3). They were round or oval and occasionally irregular or polyhedral. The nuclei were spherical or oval, usually eccentrically located in the cytoplasm, and contained a single nucleolus. The nuclear structure was more or less leptochromatic and was made up of fine chromatin granules without a sharp

nuclear membrane. The nuclei comprised about one-half to two-thirds of the entire cell volume. The cytoplasm stained a pale to deeper shade of blue, and the peripheral rim was deep blue in some cells. Specific cytoplasmic granules were absent and small vacuoles occurred in occasional cells. Different stages of karyokinesis were observed frequently. The cells exhibited no evidence of phagocytosis. Also seen were occasional eosinophils, polymorphonuclear leukocytes, tissue mast cells, macrophages, lymphocytes, and plasma cells. Several of these elements, notably the polymorphonuclear leukocytes, macrophages, lymphocytes, and plasma cells, were greatly increased in tumors undergoing regressive changes. Simultaneously, the tumor cells were decreased numerically and often showed marked vacuolization.

The Tumor Cells in the Vitally Stained Animal

Neither in imprints nor in sections was there any visual evidence of granules of dye in the cytoplasm of tumor cells from the vitally stained animal. Diffuse staining of the nucleus and cytoplasm likewise was absent except in dead or dying cells. The cytoplasm of all of the stromal macrophages, however, was very heavily loaded with trypan blue granules.

Cytologic and Histochemical Observations

Golgi Apparatus. The Golgi apparatus appeared as a network adjacent to the eccentric nucleus of the tumor cells (Fig. 4).

Mitochondria. The mitochondria occurred as occasional fine granules scattered throughout the cytoplasm. In the Da Fano preparations for Golgi bodies, minute silver granules, presumably mitochondria, were present in the cytoplasm.

Phosphatases. Fresh unfixed frozen sections showed localization of acid phosphatase in moderate amounts in the nuclei and in traces in the cytoplasm of tumor cells (Fig. 5). No histochemical evidence for either acid phosphatase or alkaline phosphatase was noted in tumor cells which were acetone-fixed.

Oxidases. No cytochrome oxidase or peroxidase activity was seen in the tumor cells. The scattered granulocytes in the sections gave positive reactions for both of these enzymes.

Lipid Substances. Lipid substances were examined in 4 tumors and in all 4 the tumor cells stained faintly with sudan black. In 3 of the tumors no other lipids could be detected with the technics used. The fourth tumor contained, in addition, cytoplasmic lipids occurring as droplets of variable size (Fig. 6). These droplets stained positively with sudan III, sudan IV, and sudan black. The last dye gave the most intense staining reaction. Histochemical tests to characterize the lipids

in more detail were negative. Although the tumor contained 3 mg. of cholesterol per gm., this substance could not be identified histochemically. Phospholipids and plasmals (tissue aldehydes) were not visualized. No pink coloration was observed after staining with nile blue sulfate. Except for the anisotropicity of the stromal collagenous fibers, no anisotropic substances were present.

Miscellaneous. Metachromatic substances, glycogen, glycoproteins, and periodic acid-Schiff positive substances were not identified in the tumor cells.

The Tumor Grown in Vitro

Fragments of tumor grown *in vitro* exhibited a fairly constant behavior pattern. Within 24 hours a less dense zone could be distinguished toward the periphery (Fig. 7). This zone was of considerable width and was caused by a general separation and loosening of the closely packed cells. From the edge of the loose zone, round cells which were undoubtedly tumor cells migrated into the surrounding medium in large numbers (Fig. 8). Also at 24 hours a few spindle-shaped cells could be seen here and there along the periphery. At 48 hours the pattern was one of migration of round cells accompanied by growth and migration of spindle cells (Fig. 8).

With continued cultivation and transplantation of the cultures a pronounced shift in cell population occurred. In the area of new growth the round cells, originally in the majority, could no longer be found. This transformation occurred in from 1 to 3 weeks and upon completion the preparations resembled pure cultures of fibroblasts (Figs. 9 and 10).

The Round (Tumor) Cells. The living round cell as observed with the ordinary microscope and with phase contrast microscopy was quite active. It showed ameboid movements, extended restless tentacles and veils of cytoplasm into the surrounding medium, and sometimes even assumed the characteristics of fibroblasts. Retraction and re-extension of cytoplasmic "feelers" often occurred so actively as to make good photomicrographs difficult to obtain at body temperature (Figs. 11, 12, and 13). The cells exhibited thigmotropism to a relatively high degree. A small piece of hair shaft inadvertently included in a culture was almost completely covered with cells. Spikes of fibroblasts growing out from the parent fragment were commonly surrounded by many round cells. The nucleus was characteristically eccentric in position and was either spherical or ovoid. Occasionally 2 (Fig. 14) and rarely 3 nuclei were found in a single cell. At least one nucleolus was observed in each nucleus, a feature best seen in cells stained with iron hematoxylin. The nucleus showed a strong acid phosphatase reaction (Fig. 14), but little or no alkaline phosphatase activity. The cytoplasm contained so many

fine granules that nuclear identification was often difficult. Supravital staining with Janus green revealed the presence of a few, small, intensely staining, dot-like mitochondria (Fig. 15). The cytoplasm, like the nucleus, gave a strong acid phosphatase reaction (Fig. 14). Attempts to demonstrate the presence of alkaline phosphatase in the cytoplasm were unsuccessful. When cultures were stained with toluidine blue, either a very few or a great many round cells reacted metachromatically, depending upon the age of the culture after the last transplantation. More cells stained as the time interval increased. In different cultures, at the end of 1 day only 10 to 15 cells were stained; at the end of 2 days 100 to 200 were stained; at the end of 3 days it was estimated that about one-fourth of all the round cells stained. The metachromatic material appeared either as blobs or irregularly shaped masses. This reaction was observed only in round cells and in all of these the nuclei stained blue, indicating that the cells were dead.

The Spindle Cells (Fibroblasts). The spindle cells possessed no distinctive characteristics. Occasionally cells with 2 nuclei were observed (Fig. 10). No evidence was obtained that round cells were transformed into spindle cells.

Fate of the Round Cells. The shift in cell population from a mixed mass of many round cells and a few spindle cells to a pure culture of spindle cells was the result of the fact that the round cells degenerate and die more rapidly than they divide. The nuclei showed lysis and the cytoplasm lost its organization. Granular débris of these degenerate and dead cells could be found in the areas of new growth as well as within the parent fragment itself. Concomitant with this round cell disintegration, spindle-shaped fibroblasts grew luxuriantly and ultimately they were the only cells present in the areas of new growth. This transformation occurred within a week or 10 days.

Behavior in "Immune" Serum. Tumor fragments placed in "immune" serum showed no differences in lag phase, rate of round cell migration, rate of growth of fibroblasts, type of growth, type of cells, or general behavior from control fragments placed in normal serum.

Discussion

That the investigations herein described were performed on a true transmissible venereal tumor is proved not only by the morphologic structure, but by the fact of greater importance that the tumor was readily transmitted to other dogs. With the exception of oral papillomatosis, of viral origin, the venereal tumor is the only known transmissible tumor of the canine species. Whether the transmissible venereal tumor occurs in extragenital locations is not within the province

of this discussion, although transmission experiments from these tumors should be done to prove or disprove this contention. Considering the ease with which the venereal tumor can be artificially transmitted, it appears probable that it can occur also in extragenital sites, especially in the cutaneous tissues.

Dry imprints were employed because it is known that there is loss of cellular details in sectioned material and it was hoped that this procedure would be of aid in the recognition of the tumor cell type. Unfortunately, this was not possible as the tumor cell could not be identified positively in the imprints. In any event the appearance of the cells in imprints bore no resemblance to the usual cells seen in smears of blood and in imprints of bone marrow, normal or abnormal. The cells also showed no similarity, in the experience of one of us (F. B.), to neoplastic cells of a large number of canine epithelial and mesenchymal tumors studied by means of dry imprints stained by the Giemsa method. In both imprints and sections the tumor cells maintain their structural type, regardless of whether the tumors occur naturally or are transplanted, and show no evidence of differentiation or dedifferentiation.

Although the cytologic and histochemical procedures were of little aid in ascertaining the origin and classification of the tumor cell, these studies showed that the cells possess more or less specific cytologic and histochemical characteristics.

The well developed Golgi apparatus has been noted also by Jackson.[17] Mitochondria are said to be absent [14] or to stain more weakly than in other cells.[17] In our material, mitochondria were demonstrated in sections with the usual mitochondrial stains and *in vitro* with Janus green, a specific mitochondrial stain. The small silver granules seen in Da Fano preparations are interpreted by many as mitochondria.[21] The evidence therefore indicates that the cytoplasm of the tumor cells contains fine granular mitochondria in sparse numbers and not that they are absent or stain weakly.

Demonstrable lipid droplets are stated to occur in considerable quantities in the tumor cells.[14,17] This was found to be true in only one transmitted tumor in which sudanophilic lipid globules were localized in the cytoplasm. In three other tumors lipid droplets were absent, but because of the faint staining with sudan black it is believed that lipids are present and they are interpreted as "bound lipids." As the tumor with lipid droplets microscopically showed degenerative changes of its cells, it is possible that the visible lipids simply indicated fatty metamorphosis. Further attempts to characterize the lipids histochemically were unsuccessful, although it is known that they are not doubly refrac-

tive and the absence of a pink coloration with nile blue sulfate signifies that neutral fats (nonsaturated glycerides) are not present. The inability to identify cholesterol histochemically, despite its occurrence in the tumor, is probably due to the fact that this substance is not concentrated in sufficient amounts in restricted foci.

A positive acid phosphatase reaction was demonstrated in the nuclei and cytoplasm of tumor cells in both tissue cultures and in sections, but, however, in fresh unfixed tissue only and not in acetone-fixed tissue. This is explained by the fact that from 70 to 95 per cent of the enzyme is lost during fixation and embedding.[33]

The presence of metachromatic substances in the tumor cells *in vitro* is difficult to evaluate and explain. The cultivated tumor cells showing metachromasia were entirely different in cultural characteristics from either normal or tumor mast cells.[34] In cultures only tumor cells and fibroblasts grew, but other cellular elements of the tumor, including tissue mast cells, did not. The complete absence of metachromatic granules in tumor cells of sections and imprints, whether of young, old, or degenerating natural or transplanted tumors, proves that the cells are not mast cells. It is true [35] that in immature mast cell neoplasms of the dog, anaplastic tumor cells have a greatly reduced mast granule content, although the granules can always be demonstrated in suitably fixed and stained sections as well as in imprints and *in vitro*.[34] Since the degree of metachromasia was associated with ageing of the cultures, it appears that this change is one either of degeneration or senility of the tumor cells.

Crile and Beebe [36] found that when whole blood from dogs that had recovered spontaneously from the transmissible venereal tumor is transfused into animals with active tumors, either a cure or regression of the growth was effected in the recipient. One might therefore expect that growth *in vitro* would be interfered with or non-existent when serum from an immune animal is used in the culture medium. However, no cultural differences existed in tumor grown in "immune" and in normal serum. Apparently the conditions *in vivo* and *in vitro* are different in respect to the reaction of the tumor cells to "immune" serum.

With the exception of the time element, the behavior of the tumor *in vivo* and *in vitro* showed certain similarities. In the tissues of the host there is, at first, growth of the tumor cells which is then followed by regressive changes of the cells and a proliferation of fibroblasts. This process from the initial transplantation occupies a period of from 2 to 4 months and may be accelerated by surgical procedures and secondary infection. Similar changes may occur in spontaneous tumors

located on the genitals, although in these instances fibrous replacement may be only partial or requires greatly extended periods of time. A probable explanation for the delayed involution of spontaneous tumors is that the genital tissues are the natural sites for the development of the tumor and that conditions here are most suitable for the maintenance of growth. In transplanted extragenital subcutaneous locations, on the other hand, even though initial growth occurs it appears that optimal conditions to maintain this growth are not present, so that a relatively early spontaneous regression takes place.

In tissue culture the round tumor cells fail to multiply to a degree sufficient to maintain themselves and they disappear in from 1 to 3 weeks. The shift in cell population from many round cells and a few fibroblasts to practically a pure culture of fibroblasts suggests a plausible explanation for the regression of the tumors *in vivo*. The unusual feature in both circumstances is the eventual replacement of the tumor cells by fibroblasts, very rapidly *in vitro*, more slowly in transplanted extragenital subcutaneous sites, and partially or greatly delayed in spontaneous genital locations. The inability of the tumor cells to maintain themselves *in vitro* may be due to the fact that they are mature end cells and thus unable to undergo further growth, and that the environment in tissue culture is lacking in specific substances present in the living tissue essential for their optimal growth.

With respect to the behavior of the tumor cells in tissue culture, they resemble macrophages of the type seen in normal cultures, *i.e.*, the wandering cell which is isolated, rounded, and exhibits active pinocytosis. In structure and behavior they appear and act like the epithelioid cells which were cultured and photographed by Warren H. and Margaret R. Lewis in their film of "Normal and Abnormal White Blood Cells in Tissue Culture." However, certain criteria considered characteristic of macrophages, such as phagocytosis and the ability to store acid vital dyes, were not exhibited by the tumor cells. In sections and imprints a careful search indicated no evidence of phagocytic activity by the tumor cells, even in areas of hemorrhage and necrosis. Intensive and prolonged vital staining of the host with trypan blue, together with injection of the dye into the tissues immediately surrounding the tumor, revealed no segregation of the dye in the cytoplasm of the tumor cells. In human histiocytomas the tumor cells are phagocytic as shown by their ability to be vitally stained with colloidal iron.[37] Therefore, despite the fact that the tumor cells *in vitro* resemble macrophages in structure and behavior, they do not possess certain properties considered characteristic of the latter elements *in vivo*. When one recalls, in addition,

that the tumor cells show no evidence of differentiation or dedifferentiation, that they are unable to maintain themselves *in vitro* for more than a week or so, and that spontaneous regression occurs *in vivo,* the conclusion is probable that the tumor cells are mature definitive cells. As such, they are unable to show unlimited growth, despite their active appearance as judged by frequent mitotic figures. This concept is supported by the relatively limited growth potentialities, once growth has begun, that are observed both *in vivo* and *in vitro.* Since the cultured cells show structural and behavioristic patterns similar to those of cultured macrophages, even though they do not morphologically resemble macrophages in sections and in imprints, an origin from the reticulo-endothelium is suggested. This assumption seems appropriate because the only visible, conveniently available means for observing the reactions of the living cell is *in vitro,* albeit in an acknowledged artificial environment. In culture the mature definitive cells manifested some physical attributes of their assumed ancestral prototype, namely, the macrophage, of reticulo-endothelial genesis. This concept appears all the more justified when it is considered that we are dealing here with a tumor unique in many respects. Following its original transformation from the reticulo-endothelium, the mature definitive cell has apparently lost its original morphologic and physiologic characteristics as they can be observed in sections and imprints, so that its origin is completely obscured.

On the assumption that the tumor cells are mature end cells of reticulo-endothelial origin, how should the transmissible venereal tumors be correctly designated? Carcinoma is obviously incorrect since the cells are not epithelial. Lymphosarcoma is likewise inappropriate as the cells do not belong to the lymphoid series. Sarcoma in its broadest sense is less objectionable because the cells are of mesenchymal type, although no exact information is conveyed concerning the cell type. Reticulum cell sarcoma has perhaps some histogenetic justification, but not on a morphologic basis. Neuroblastoma is manifestly incorrect and requires no further comment. Histiocytoma is inappropriate because the cells do not exhibit the morphologic and physiologic attributes of histiocytes, and, furthermore, the transmissible tumor is entirely different structurally from human histiocytoma. Since the naming of the cell type of the tumor offers an unusual problem in oncology, obviously some new term not previously used would have to be formulated. This is perhaps not really necessary as changes in medical nomenclature generally meet with considerable resistance and may complicate an already confused situation. It is therefore suggested that the designa-

tion of "transmissible venereal tumor" be retained, with the knowledge that the tumor cells have the morphologic and functional characteristics of mature end cells of reticulo-endothelial origin.

Summary and Conclusions

Previous attempts to classify the cell type of the transmissible venereal tumor of the dog have been based only on histologic examination. As a result the tumors have been designated by a variety of names with little unanimity of opinion. Our investigations, utilizing transmission, morphologic, cytologic, histochemical, and tissue culture studies, reveal that the tumor cell is a mature end cell of reticulo-endothelial origin. Instead of formulating a new name for the tumor, it is suggested that the designation "transmissible venereal tumor" be retained.

References

1. Beebe, S. P., and Ewing, J. A study of the so-called infectious lymphosarcoma of dogs. *J. M. Research,* 1906, **15,** 209–227.
2. Feldman, W. H. So-called infectious sarcoma of the dog in an unusual anatomic situation. *Am. J. Path.,* 1929, **5,** 183–195.
3. Jackson, C. The incidence and pathology of tumours of domesticated animals in South Africa. *Onderstepoort J. Vet. Sc.,* 1932, **6,** 387–413.
4. Lesbouyries, G. Neóplasies vénériennes du chien et de la chienne. *Rec. de méd. vét.,* 1946, **122,** 5–19.
5. Lacroix, J. V., and Riser, W. H. Transmissible lymphosarcoma of the dog. *North Am. Vet.,* 1947, **28,** 451–453.
6. Mulligan, R. M. Neoplasms of the Dog. Williams & Wilkins Co., Baltimore, 1949, p. 93.
7. Wehr. Demonstration der durch Impfung von Hund auf Hund erzeugten Carcinomknötchen. *Verhandl. d. deutsch. Gesellsch. f. Chir.,* 1888, **17,** 52–53.
8. Geissler. Gelungene Carcinom-Uebertragung beim Hunde. *Verhandl. d. deutsch. Gesellsch. f. Chir.,* 1895, **24,** 87–92.
9. Duplay, S., and Cazin, M. Tumeurs expérimentales chez les animaux. *Atti d. xi Cong. med. internaz., Roma,* 1894, **2,** 103–104. (Cited by Woglom, W. H. The Study of Experimental Cancer. In: Studies in Cancer and Allied Subjects. Conducted under the George Crocker Special Research Fund, Columbia University. Columbia University Press, New York, 1913, **1,** 227–236.)
10. Bashford, E. F., Murray, J. A., and Cramer, W. Comparison Between the Transmission of an Infective Granuloma of the Dog and Carcinoma of the Mouse. Scientific Reports on the Investigations of the Imperial Cancer Research Fund. Taylor & Francis, London, 1905, No. 2, pp. 33–37.
11. Wade, H. An experimental investigation of infective sarcoma of the dog, with a consideration of its relationship to cancer. *J. Path. & Bact.,* 1907–08, **12,** 384–425.
12. Novak, E., and Craig, R. G. Infectious sarcoma ("venereal granuloma") of the vagina in dogs. *Arch. Path.,* 1927, **3,** 193–202.
13. Feldman, W. H. Neoplasms of Domesticated Animals. W. B. Saunders Co., Philadelphia, 1932, pp. 343–356.
14. De Monbreun, W. A., and Goodpasture, E. W. An experimental investigation concerning the nature of contagious lymphosarcoma of dogs. *Am. J. Cancer,* 1934, **21,** 295–321.

15. Stubbs, E. L., and Furth, J. Experimental studies on venereal sarcoma of the dog. *Am. J. Path.*, 1934, 10, 275–286.
16. Kaalund-Jörgensen, O., and Thomsen, A. S. Det overførbare veneriske sarkom hos hunde. *Maanedsskr. Dyrlaeg.*, 1937, 48, 561. (Cited by Engelbreth-Holm, J. Spontaneous and Experimental Leukaemia in Animals. Oliver & Boyd, Edinburgh & London, 1942, p. 32.)
17. Jackson, C. The cytology of the contagious (venereal) tumour of the dog. *Onderstepoort J. Vet. Sc.*, 1944, 20, 97–117.
18. Wilder, H. C. An improved technique for silver impregnation of reticulum fibers. *Am. J. Path.*, 1935, 11, 817–819.
19. Masson, P. Carcinoids (argentaffin-cell tumors) and nerve hyperplasia of the appendicular mucosa. *Am. J. Path.*, 1928, 4, 181–211.
20. McClung, C. E. Handbook of Microscopical Technique. Paul B. Hoeber, Inc., New York, 1937, p. 340.
21. Lee, A. B. The Microtomist's Vade-Mecum. (Edited by: Gatenby, J. B., and Painter, T. S.) P. Blakiston's Son & Co., Philadelphia, 1937, ed. 10, pp. 284–329.
22. Lillie, R. D. Histopathologic Technic. The Blakiston Co., Philadelphia, 1948, pp. 94–111.
23. Bartelmez, G. W., and Bensley, S. H. "Acid phosphatase" reactions in peripheral nerves. *Science*, 1947, 106, 639–641.
24. Lison, L. Histochemie Animale. Gauthier-Villars, Paris, 1936, pp. 189–273.
25. Mallory, F. B. Pathological Technique. W. B. Saunders Co., Philadelphia, 1938, p. 187.
26. Baker, J. R. The structure and chemical composition of the Golgi element. *Quart. J. Micr. Sc.*, 1944–45, 85, 1–71.
27. Quensel, U. Untersuchungen über die Morphologie des organisierten Harnsediments bei Krankheiten der Nieren und der Harnwege und über die Enstehung der Harnzylinder. *Nord. med. Ark.*, 1918, 50, Avd. II, 319–662.
28. Hawk, P. B., Oser, B. L., and Summerson, W. H. Practical Physiological Chemistry. The Blakiston Co., Philadelphia, 1947, ed. 12, p. 261.
29. Baker, J. R. The histochemical recognition of lipine. *Quart. J. Micr. Sc.*, 1946, 87, 441–470.
30. Albert, S., and Leblond, C. P. The distribution of the Feulgen and 2,4-dinitrophenylhydrazine reactions in normal, castrated, adrenalectomized and hormonally treated rats. *Endocrinology*, 1946, 39, 386–400.
31. Hotchkiss, R. D. A microchemical reaction resulting in the staining of polysaccharide structures in fixed tissue preparations. *Arch. Biochem.*, 1948, 16, 131–141.
32. Holmgren, H. Studien über Verbreitung und Bedeutung der chromotropen Substanz. *Ztschr. f. mikr.-anat. Forsch.*, 1940, 47, 489–521.
33. Stafford, R. O., and Atkinson, W. B. Effect of acetone and alcohol fixation and paraffin embedding on activity of acid and alkaline phosphatases in rat tissues. *Science*, 1948, 107, 279–281.
34. Paff, G. H., Bloom, F., and Reilly, C. The morphology and behavior of neoplastic mast cells cultivated *in vitro*. *J. Exper. Med.*, 1947, 86, 117–124.
35. Oliver, J., Bloom, F., and Mangieri, C. On the origin of heparin. An examination of the heparin content and the specific cytoplasmic particles of neoplastic mast cells. *J. Exper. Med.*, 1947, 86, 107–116.
36. Crile, G. W., and Beebe, S. P. Transfusion of blood in the transplantable lymphosarcoma of dogs. *J. M. Research*, 1908, 18, 385–405.
37. Senear, F. E., and Caro, M. R. Histiocytoma cutis. *Arch. Dermat. & Syph.*, 1936, 33, 209–226.

DESCRIPTION OF PLATES

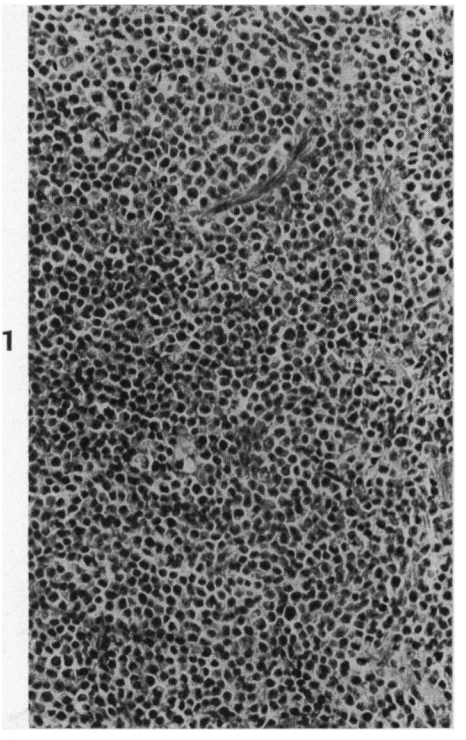
PLATE 24

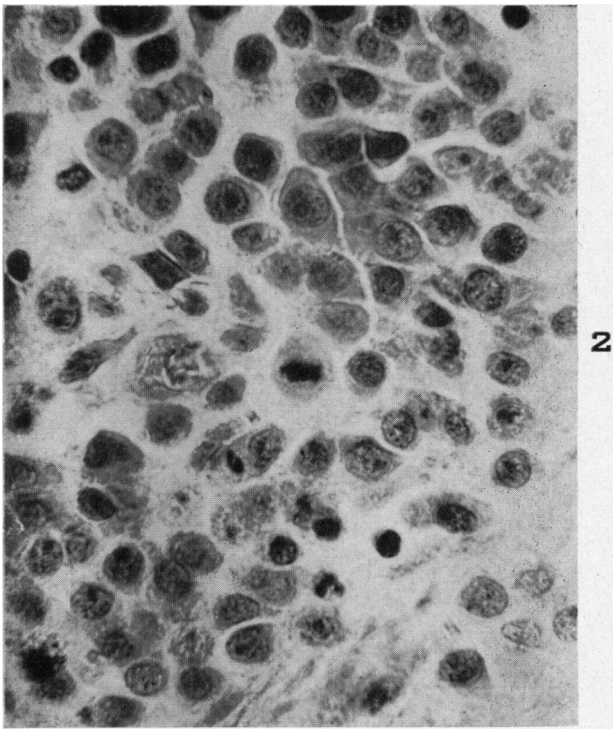
FIG. 1. Low-power view showing general structure of the canine transmissible venereal tumor. Hematoxylin and eosin stain. × 200.

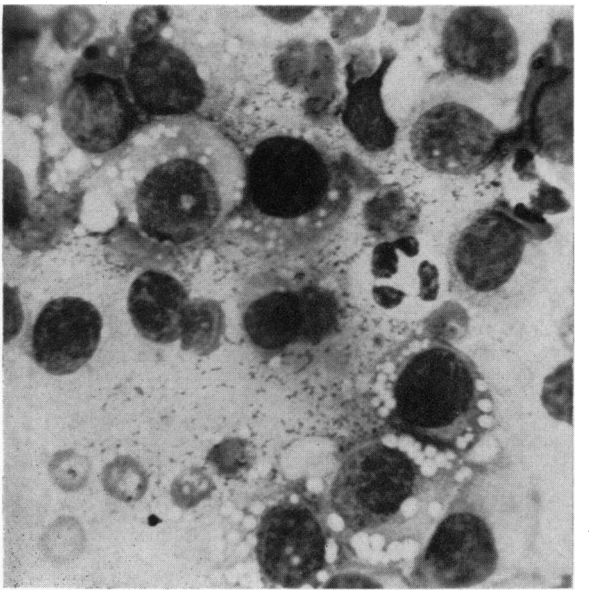
FIG. 2. High-power view of the cellular structure. Of note are the mitotic figures. Hematoxylin and eosin stain. × 900.

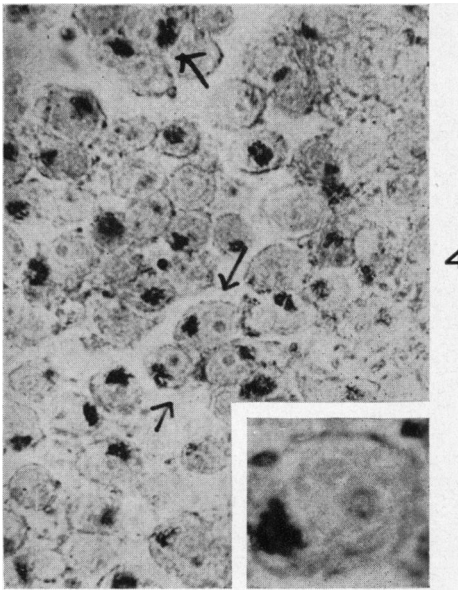
FIG. 3. Imprint of a mature, regressing, transplanted venereal tumor. Cytoplasmic vacuolization and two polymorphonuclear leukocytes may be observed. Wright's stain. × 900.

FIG. 4. Photomicrograph of the Golgi apparatus adjacent to the slightly eccentric nucleus. Ludford technic,[21] no counterstain. × 900. The inset shows the Golgi apparatus at a higher magnification. × 2700.









Bloom, Paff, and Noback

Venereal Tumor of the Dog

PLATE 25

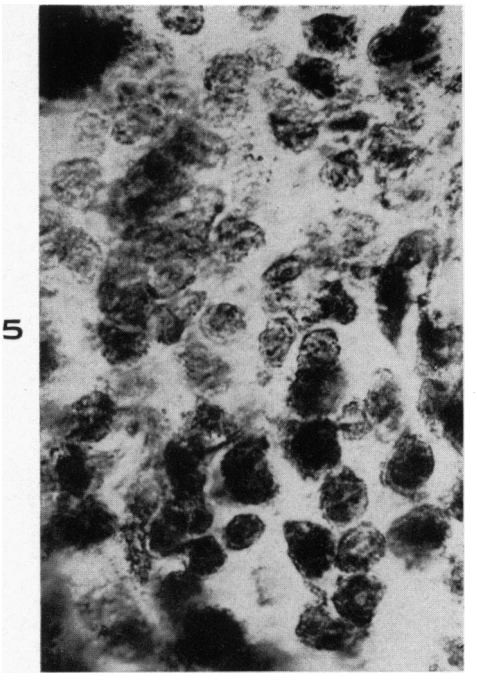
FIG. 5. Acid phosphatase in tumor cells. The reaction is more intense in the nucleus than in the cytoplasm. The variability between the reactions in different cells is due in part to the thickness of the section. Enzyme was detected in unfixed frozen sections (25 μ thick) according to the method of Bartelmez and Bensley.[23] No counterstain. × 900.

FIG. 6. Lipids in the form of droplets in tumor cells of a regressing tumor. A thick frozen section was fixed in formol-calcium and stained with sudan black. No counterstain. × 900.

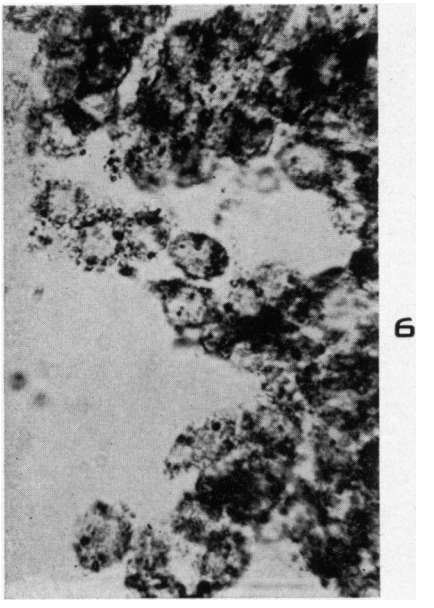
FIG. 7. Peripheral zone of a living unstained tumor fragment cultured for 24 hours. This shows the beginning of separation and migration of the round tumor cells. × 200.

FIG. 8. Same tumor fragment as seen in Figure 7 at the end of 48 hours of cultivation. The clearer zone is due to further separation and migration of round cells. The appearance of fibroblasts may be noted. × 100.

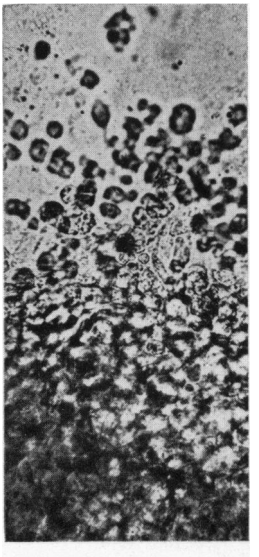
FIG. 9. Same culture as shown in Figures 7 and 8 but 2 weeks later. The round cells have completely disappeared and only fibroblasts are present. The shift in cell population is complete. Acid phosphatase stain. × 200.



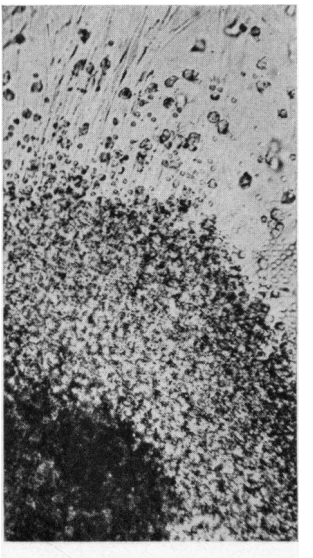
5



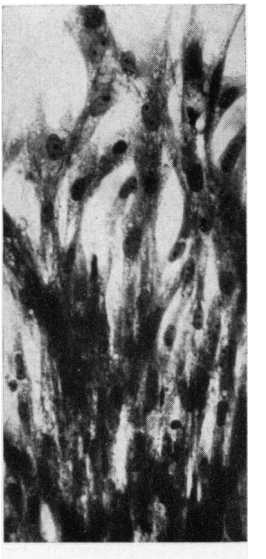
6



7



8



9

Bloom, Paff, and Noback Venereal Tumor of the Dog

PLATE 26

FIG. 10. Living, unstained, binucleated fibroblast from a tumor fragment cultivated for 3 weeks. Phase contrast. × 1455.

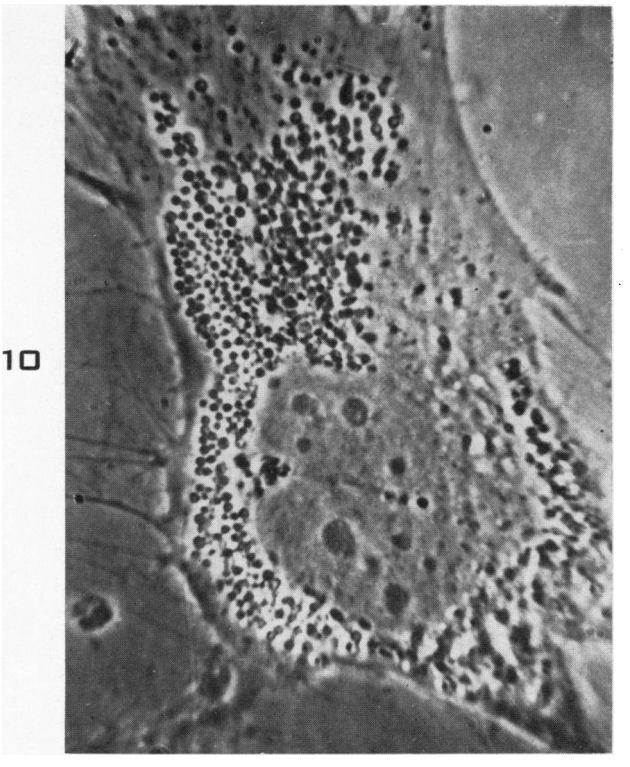
FIG. 11. Living, unstained round cell extending a large process into the surrounding medium. Three-day-old culture. Phase contrast. × 970.

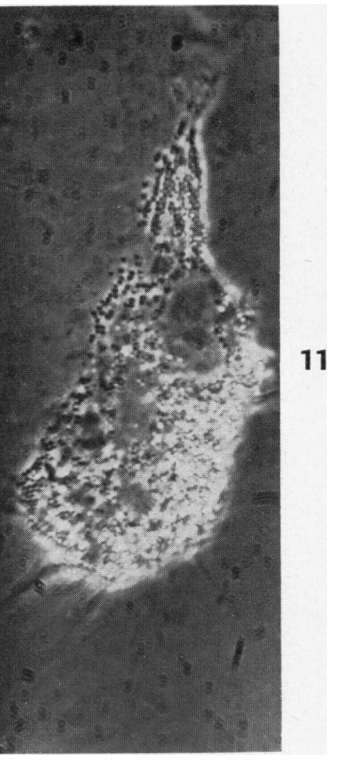
FIG. 12. Living, unstained round cells exhibiting ameboid movement. The cell to the right is the same as the one in Figure 11. Three-day-old culture. Phase contrast. × 970.

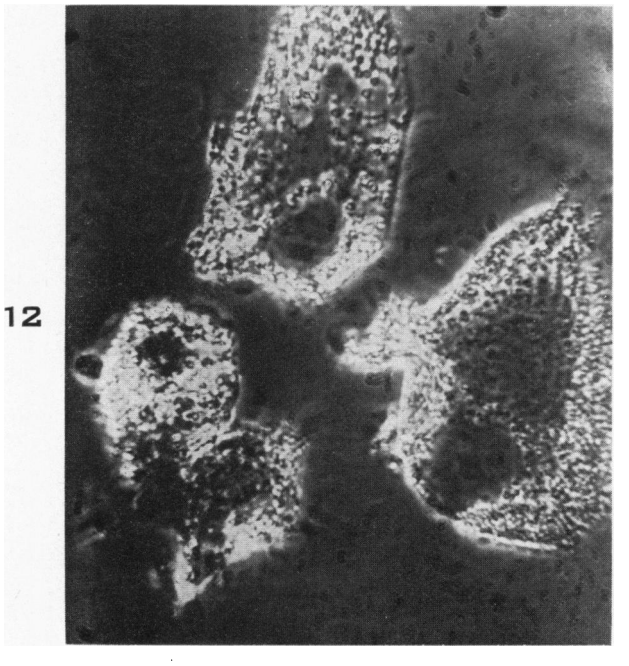
FIG. 13. Living, unstained round cell showing pinocytosis. Three-day-old culture. Phase contrast. × 970.

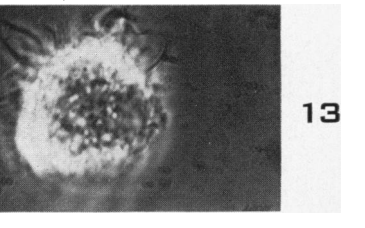
FIG. 14. Binucleated round cell stained for acid phosphatase. Two-day-old culture. × 970.

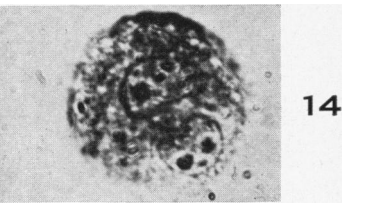
FIG. 15. Round cell with dot-like granular mitochondria, a few of which stain intensely, in the cytoplasm. Supravital staining with Janus green of a 3-day-old culture. × 970.

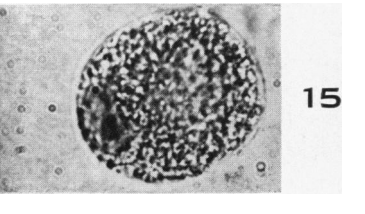












Bloom, Paff, and Noback Venereal Tumor of the Dog