HISTOLOGICAL, CYTOLOGICAL, AND *IN VITRO* STUDIES OF CANINE MAST CELL TUMORS

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY

Jean L. Juday

1960

HISTOLOGICAL, CYTOLOGICAL, AND IN VITRO STUDIES OF CANINE MAST CELL TUMORS

By

Jean L. Juday

A THESIS

Submitted to the College of Veterinary Medicine
of Michigan State University in partial
fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Anatomy

1960

ACKNOWLEDGEMENTS

The author wishes to express her profound gratitude to Dr. Esther M. Smith, Associate Professor of the Department of Anatomy, for her invaluable assistance, continuous inspiration, and unfailing enthusiasm during this investigation and for her constant help and patience in the preparation of this manuscript. It has been a pleasure to work and study under one who gave so freely of her time and knowledge.

It is with sincere appreciation that the author thanks Dr. Lois M. Calhoun, Professor and Head of the Department of Anatomy, Dr. Thomas J. Bell, Jr., Associate Professor of the Department of Anatomy, and other members of the Anatomy department who gave so generously of their valuable time.

Appreciation is expressed to: Dr. Waldo F. Keller and Dr. Robert G. Schirmer of the Department of Surgery and Medicine for their assistance in the procurement of the specimens, Mr. James Tucker for his assistance in the preparation of the photomicrographs, and Mr. Karl Fisher for his help in the German translations.

Special thanks go to Dr. Madan Bharadwaj, Miss June DeHate, and the writer's parents for their help, encouragement and unlimited faith.

The author also wishes to thank the Ingham County Cancer Society for the financial support which made this research possible.

TABLE OF CONTENTS

LIST OF PLATES

LIST OF TABLES AND GRAPHS

INTRODUCTION

Since 1879 when Ehrlich first named the mast cell, this cellular component of the connective tissue has stimulated the interest and curiosity of many. Although much literature on various aspects of this cell has accumulated during the past 81 years, many basic questions still remain unanswered.

In addition to its appearance in normal tissue, tumorous proliferations of mast cells occur in the skin of various animals, especially the dog. The abundance of mast cells in these canine tumors has prompted extensive biochemical and histological studies in the hope of answering questions regarding their normal function. However, morphological and biochemical differences have been noted among the tumor cells in the same tumor and in different tumors.

The purpose of this study is to correlate the appearance of neoplastic canine mast cells in fixed sections and smear preparations with cells from the same tumors grown in vitro. It is hoped that these data will provide a basis for continuing studies, based upon variations which exist among these tumors.

LITERATURE REVIEW

The history of the mast cell stems back to the early work on connective tissue (Michels 1938). Ehrlich first named this connective tissue component the mast cell in 1879. He observed cytoplasmic granules which stained metachromatically with toluidine blue and considered this cell an overnourished connective tissue element. The literature on the normal mast cell has been reviewed extensively by Michels (1938), Asboe-Hansen (1954), Riley (1955), and Fulton et al. (1957).

It has been recognized for some time that normal tissue mast cells occasionally become neoplastic. These tumors have been reported in animals other than the dog, but they are infrequent. Meier (1957) and Head (1953, 1958) described mast cell tumors in cats. Furth et al. (1957) and Dunn and Potter (1957) worked with these tumors from mice, while Head (1958) described a similar lesion in the ox. Mast cell disturbances have also been noted in man (Drennan 1951, Lever 1954, and Beare 1958).

General Characteristics of Canine Mast Cell Tumors

Murray (1908) first reported two cases of mast cell sarcomas in the dog. It is evident from the literature that mast cell tumors occur frequently in the cutis of this animal. Larsson (1956) reported an incidence of 13% in a study of 266 cutaneous tumors. Nielsen and Cole (1958) found

mast cell tumors comprised 20% of the cutaneous neoplasms in their collection.

Various authors (Head 1953, Larsson 1956, 1957 and Nielsen and Cole 1958) reported the following: no sex predominance, an age occurrence of generally six to seven years, and a breed predisposition of Boston terriers and boxers.

Canine mast cell tumors may be solitary or multiple (Bloom 1942, Oliver et al. 1947, Nielsen 1952, Head 1953, Köhler 1954, Larsson 1957, and Head 1958). Generally the cells infiltrate the corium and sometimes extend into the subcutaneous tissue, and may be heavily grouped or loosely infiltrate the connective tissue (Bloom 1942, Oliver et al. 1947, Mulligan 1949, Nielsen 1952, Köhler 1954, Larsson 1957, and Nielsen and Cole 1958). In addition, Larsson (1957) reported some tumors to be well demarcated in the subcutis, and Nielsen (1952) stated the cells often infiltrate the epidermis.

Eosinophils, neutrophils, plasma cells, lymphocytes, and histiocytes are also present among the tumor cells (Bloom 1942, Mulligan 1949, Köhler 1954, Larsson 1957, Nielsen and Cole 1958, and Bloom et al. 1958). The number of eosinophils in the tumors varies greatly and, therefore, a relationship between the disintegration of mast cell granules and the appearance of eosinophils may exist (Nielsen and Cole 1958).

Edema and necrosis of the tumor mass may occur and the

ephithelium often becomes ulcerated due to local irritation (Bloom 1942, Mulligan 1949, Nielsen 1952, Larsson 1957, and Head 1958). Dilated lymphatics and cystic, atrophic sebaceous and sweat glands sometimes appear (Mulligan 1949). Various degrees of metastasis to regional lymph nodes and other tissue have been reported (Mulligan 1949, Nielsen 1952, Head 1953, 1958, Bloom et al. 1958, and Nielsen and Cole 1958).

Histochemistry of Mast Cells

The metachromatic staining of mast cell granules was first noted by Ehrlich (1879) and has served as an identifying characteristic of these cells since that time. It has been recognized, however, that the metachromatic reaction might vary in different cells depending on the degree of maturation. The immature normal mast cell granules may not exhibit metachromasia (Michels 1938). Taketa (1958) considered that immature normal mast cell granules stain orthochromatically and the mature, metachromatically.

Oliver et al. (1947) reported wide variation in the degree of metachromatic staining as well as granule size in the mast cells from canine tumors. They considered as immature those granulated anaplastic cells resembling early stages in the developing tissue mast cells. The cells with heavy and coarse granulation were considered to be mature. This designation of "mature" and "immature" cells has been used by other authors (Nielsen 1952, Nielsen and Cole 1958,

Stunzi 1955, and Larsson 1957).

Jorpes et al. (1948) indicated a variation in the periodic acid-Schiff reaction depending on the form of heparin present in mast cell granules. Only the heparin monosulfuric acid or some other precursor stained positively. Hyaluronic acid also stains intensely. Drennan (1951) interpreted a positive reaction to be due to a less developed variety of heparin in the granules. In his opinion, local stimulation of mast cell areas prevented full maturation of this normal secretory product. Mast cells with a positive reaction were immature by this criterion. Taketa (1958) reported immature normal mast cells were P.A.S. positive and the mature normal mast cells were P.A.S. negative.

Some authors observed P.A.S. positive granules in canine mast cell tumors (Williams et al. 1959 and Bloom et al. 1958), while Nielsen and Cole (1958) reported the granules rarely revealed a positive reaction.

Biochemical Properties

Present evidence indicates normal mast cells may be unicellular endocrines which produce heparin, hyaluronic acid, histamine, and serotonin (5-hydroxytryptamine) (Fulton et al. 1957). In 1937 Jorpes et al. demonstrated the relation between mast cells and heparin. Because of the abundance of mast cells in the neoplastic tissue these tumors have been used for biochemical studies. Oliver et al.

(1947) showed that the heparin content of canine mast cell tumors varies with the degree of anaplasia.

In 1953 Riley and West working with normal and pathological tissue found the amount of histamine in a given tissue is in direct proportion to the mast cell content. Further work has supported these studies, and in 1954 Cass et al. found six canine mast cell tumors exceedingly rich in both heparin and histamine, and those containing the most heparin had mast cells filled with strongly metachromatic granules. Experiments by Bloom et al. (1958) with canine mast cell tumors, indicated that heparin and histamine may form a complex in which histamine preserves its physiological effects while heparin loses its effect on the coagulation of blood. This correlates with the work reported previously by Bloom et al. (1955) who found that dogs with mast cell tumors showed no increase in blood coagulation. Lindel et al. (1959) gave further evidence that mast cells in canine tumors form histamine and not merely store it.

Romenelli (1953) demonstrated hyaluronic acid as well as heparin in a canine mast cell tumor. These tumors yield small amounts of serotonin, but there is no relationship with the degree of anaplasia or maturity of the mast cell and the serotonin content as there is with heparin (Meier 1959). West (1958), however, found no correlation between serotonin and the mast cells themselves.

Histopathology

Bloom (1942) was the first to propose the name mastocytoma for these neoplasms. He defined them as primary, subepithelial neoplasms that occur spontaneously and are characterized by many atypical tissue mast cells. Bloom gave an extensive pathological description of five canine mastocytomas, and noted that differences existed between the "solitary benign" and the "multiple malignant" tumors. He reported the cytological characteristics, tinctorial reaction, and general distribution and arrangement of the neoplastic mast cells in the different tumors varied only slightly. However, the multiple tumor cells decolorized more easily, showed mitosis, were more often binucleated, and contained more intracytoplasmic bodies than the solitary tumor cells.

In 1947 Oliver et al. in a study of 15 mast cell tumors, observed that the "immature" tumor cells were less distinct than the "mature" cells which resembled normal tissue mast cells except that they were somewhat larger. They noted that all gradations between the two types of cells were present.

Mulligan (1949) used the name mast cell sarcoma and described the differences between mature and more anaplastic tumors. He stated that the cells of a mature tumor had a polyhedral shape, a discrete border, a pale acidophilic cytoplasm, and had small nuclei in comparison to the volume of cytoplasm. The nuclei were round or slightly oval, and

often were obscured by coarse dark granules. The more anaplastic tumor cells had a fuzzy border, pale and moderately basophilic cytoplasm, large nuclei in relation to the volume of cytoplasm, and fine granules.

Nielsen (1952) in a clinicopathological correlation of 60 mast cell tumors, also observed that the cells had a spherical but indistinct outline with large round nuclei and a small amount of chromatin material. He further observed that cells in rapidly growing malignant tumors were packed closely together while the mature, slow growing tumor cells had more space between them. The number of stainable granules increased with progressive degrees of maturity.

Köhler (1954), reporting on five cases of mast cell tumors, noted hyalinized collagen in small arteries. He believed these tumors were mesenchymal neoplasms of limited malignancy.

Eighteen cases of mast cell tumors were studied by Stunzi (1955). The only relationship he found between the maturity of the cells and the architecture of the tumor was that when tight masses of tumor cells occurred, the mast cells almost always appeared "immature" and had little chromatin in their nuclei. He preferred to call these tumors mastocytomas.

Electron and light microscopy studies by Bloom et al. (1955) of cells from canine tumors, indicated the shapes varied from round to oval and the nuclei from round to

kidney-shaped, and the granules measured about 0.7 μ. A general feature was a great number of small filamentous cytoplasmic protrusions on the surface of the cell. Further electron microscopy by Bloom et al. (1956) showed that vacuoles occurring on the periphery of the cell were probably functionally related to the cytoplasmic protrusions. Both of these structures might be connected with the exchange of fluid from the cell to the surrounding tissue.

Larsson (1957) classified 60 cases of mast cell tumors in dogs according to their clinical appearance. He used four divisions based on morphology, and a fifth, based on recurrent tumors. He found the degree of maturity of the mast cells varied in the different types of tumors, and he described the occurrence of a tumor that showed considerable edema and an abundance of eosinophils.

Bloom et al. (1958) in a study of 60 mast cell sarcomas by light and electron microscopy, noted the size of the cells varied from 7 to 13 μ . The cells were usually rounded in shape but some were thin and elongated. The nuclei varied from oval to kidney-shaped and were about five μ in diameter, and contained one to three nucleoli. The nucleoplasm stained with basic dyes and was generally homogeneous with a fine chromatin network. The cells usually contained one nucleus but sometimes binucleated cells were seen. The cytoplasm had granules which measure 0.6 to 0.7 μ , and frequently contained vacuoles.

Head (1958) compared mast cell tumors of the dog, cat, and ox. His classification of them was similar to that used by Larsson (1957). In Head's study the mast cell tumors revealed indistinct borders and strands of tumor cells penetrating the surrounding connective tissue. The more malignant tumors tended to become multiple and had large numbers of "immature" tumor cells. The main difference between the three species was that cat tumors were smaller and ox tumors larger than canine tumors. Head believed the benign form could be called mastocytoma, and the malignant form a mast cell sarcoma.

Nielsen and Cole (1958) conducted an extensive study on 100 canine mast cell tumors. They observed that the tumor cells were larger than normal mast cells, spherical to rectangular in shape, and contained granules which vary greatly in number and size. The tumor cells measured 10 to 12 μ in diameter and had well outlined cytoplasmic borders. The round or ovoid centrally located nuclei measured 5 to 8 μ in diameter. Few mitotic figures were seen even in the rapidly growing tumors, and a few giant and multinucleated mast cells were present. Generally the rapidly growing mast cell tumors consisted of "immature" cells with only fine dustlike granules. They believed that these tumors were true neoplasms.

Orkin and Schwartzman (1959) described 13 mast cell tumors. Eight of these were considered "typical" and 5 "atypical." The "typical" tumors consisted of cells which

measured 8 to 18 μ in diameter. Their outlines varied but were usually polygonal. The large vesicular, "basophilic" nuclei measured 5 to 10 μ in diameter, were centrally located and had a variable nuclear outline. Binucleated cells and mitotic figures were occasionally seen. The "atypical" tumors consisted of cells with a reduced cytoplasmic nuclear ratio. These cells had a foamy appearance, showed more mitotic figures, and were more often multinucleated.

Tissue Culture

In order to gain more information about both normal and neoplastic mast cells, in vitro cultivation of these cells has been undertaken. Paff et al. (1947) cultivated tissue fragments from two canine tumors using the clot technique. Studies of the living and of fixed, stained preparations revealed that only mast cells grew from the original tumor fragments, although other cells were present. In some of the cultures the cells grew in a sheet resembling epithelium, while in others they were separate and irregularly spindle or star-shaped, with long protoplasmic processes. The granules varied in size, number, and tinctorial properties. In these cultures most of the granules stained metachromatically, but in a few none could be stained. There seemed to be a difference in the number of stainable granules in the two tumors. Only one of the tumors showed mitotic divisions.

Paff and Bloom (1949) grew tissue fragments from a

canine tumor and allowed them to age in culture. They noted that the cells underwent changes which resulted in the liberation of a metachromatic substance which was presumed to be heparin or some heparin derivative. Vacuolation in the cells varied and it was observed that in some of the cells large vacuoles occurred which eventually ruptured, releasing granules into the surrounding medium. In other cells the granules underwent dissolution and a metachromatic material was discharged. There were also other cells in which the granules supplied metachromatic material to the formation of vacuoles which, in turn, discharged this material. They concluded that possibly the secretory cycle of heparin production and liberation involves degenerative changes and death of the mast cell.

Mast cells from a case of urticaria pigmentosa were successfully grown in vitro for 13 months (Zitcer et al. 1953). These cells stained metachromatically with toluidine blue, and the cytoplasmic granules varied in size, number, and staining ability. They also reported the possible cyclic production and loss of granulation.

In vitro cultivation of a murine mast cell tumor (Schindler et al. 1959) indicated that this tumor required a high folic acid concentration (or a small amount of citrovorum factor) in the growth medium plus an unknown growth factor supplied by undialyzed horse serum. The generation time of these cells was 24 hours, and a high intracellular

level of serotonin and histamine as well as infectivity was maintained.

Williams et al. (1959) successfully grew neoplastic canine mast cells in monolayer cultures for a period of two years. These cells maintained the same morphology during this time forming a fibroblastic growth pattern. The cytoplasmic granules stained metachromatically with toluidine blue and were periodic acid-Schiff positive. These granules were the most plentiful in culture three to six days after transfer. The nuclei were vesicular, contained 1 to 20 nucleoli, and often exhibited bizarre outlines. Multinucleated cells and mitotic figures were seen often. An extracellular fibrous material occurred in all of the cultures and appeared to originate from the cell surface.

MATERIALS AND METHODS

The canine mast cell tumors used in this study consisted of 11 surgical specimens received from the Department of Surgery and Medicine, Michigan State University, during the period from August 1957 through May 1960.

The tumors were obtained from the Veterinary Hospital immediately after excision. Under aseptic conditions they were divided into two portions; one part was saved for tissue culture and the other was used for impression smears and paraffin sections. To make the impression smears the freshly cut tumor surface was pressed gently on the slide, fixed by drying in air, and stained with Wright's stain. If the smears indicated a mast cell tumor, the remaining portion was fixed in 10% formalin-alcohol. The breed, age, and sex of each animal and the duration, location, and appearance of each tumor were noted.

Fixed Specimens

The fixed specimens were dehydrated and infiltrated according to the butyl alcohol-paraffin-mush method (Johnson et al. 1943), embedded in Tissuemat* and sectioned at seven μ. They were stained routinely with hematoxylin and eosin according to the method of Malewitz and Smith (1955). To study special characteristics of the tumor cells, the

---

*Fisher Scientific Company, Pittsburgh, Pennsylvania.

following stains were used: May-Grunwald-Giemsa, Mallory's phosphotungstic acid hematoxylin, periodic acid-Schiff (Gridley 1957), pinacyanol erythrosinate (Bensley 1952), methylene blue extinction (Pearse 1953), and toluidine blue.

Tissue Culture

From the remaining sterile portion of the tumor saved for tissue culture, the epithelium and connective tissue were trimmed away, and the tissue was minced in Hanks' (1949) Balanced Salt Solution (BSS) and treated with 0.25% trypsin at 37.5°C until the greater part of the tumor cells were in suspension. The suspension was filtered through sterile gauze to remove any large tissue fragments, centrifuged at 760 rpm for 5 minutes, the BSS and trypsin removed, and the cells resuspended in Hanks' BSS. The centrifugation and washing were repeated three times. The cells were then suspended in Eagle's medium (1955) containing 10% horse serum and dispensed into four ounce prescription bottles and Leighton tubes containing cover slips.

The cells formed a monolayer on all the glass surfaces after 2 to 3 days. The preparations on the prescription bottles were used for low power observations, and the cover slip preparations for high power microscopic studies. Following varying time intervals, the coverslips were removed from the Leighton tubes, fixed in methyl alcohol and stained with Wright's stain, dehydrated, and mounted on slides with

Permount.* The cells used in this study were from primary and secondary cultures.

Histological Studies

Histological studies were made on the tissue culture, fixed and smear preparations to compare the characteristics of the mast cells from the various tumors. The tumors in this study were numbered from 1 through 11; one being the most anaplastic and eleven, the most differentiated.

The largest and smallest cells and their nuclei were measured in each tumor to obtain the size range of each in the different specimens. To determine variations in the number of tumor cells and eosinophils present in the different tumors, cell counts were made using a 5 mm square ocular reticule. All of the cells were counted in three 25 sq mm areas totaling 39,675 sq. μ.

*Fisher Scientific Company, Pittsburgh, Pennsylvania.

RESULTS AND DISCUSSION

General Characteristics

Many of the specimens used in this experiment were from animals which had been referred to the Veterinary Clinic. Consequently, their case histories were often scanty. Available information indicated that 5 of the 11 dogs had neoplasms removed previously, but only 3 of these were known to have been mast cell tumors. The 5 recurring tumors included numbers 1 and 2 which contained cells with only a few, fine granules, and 4, 6, and 9 exhibiting cells which usually had evenly distributed granulation (Table I). According to Mulligan (1949) and Larsson (1957), recurring mast cell tumors contained more anaplastic or "immature" cells. In this study it appears that this is generally true since the two most anaplastic tumors were in this group. However, some recurrent tumors showed the same type of granulation and degree of anaplasia present in original tumors, and some original tumors have been observed by other workers to contain extremely anaplastic cells.

The number of animals used in this study was too small for valid statistical studies with respect to age, breed, and location of tumors. However, it is interesting to note the average age of the animals was 7.8 years, and 4 of the 11 tumors came from boxers. These findings are in fair agreement with those of Larsson (1956, 1957) which demonstrated

a mean age of nearly seven years and a breed predisposition for boxers. Nielsen and Cole (1958) found that the majority of their 100 specimens were from dogs at least six years of age. The greater percentage of these cases occurred in Boston terriers.

Although Nielsen (1952), Larsson (1957), Nielsen and Cole (1958), and Head (1958) reported the tumors were located most frequently on the hindquarters, it can be seen in Table I that the location of the tumors used in this study varied considerably.

Gross Characteristics

The consistency of the tumors in the fresh state varied greatly. The slow growing tumors were generally firm and greyish-white, probably because of the abundance of coarse connective tissue. This agrees with Bloom (1942), Mulligan (1947) and Larsson (1957). The more rapidly growing tumors appeared reddish yellow with an almost gelatinous consistency. Gradations between these two extremes were noted.

Nielsen (1952) stated that mast cell tumors never involve the deeper fascia and muscle. However, in this study, tumors 1, 2, and 4, which were located in the axillary region, showed infiltration into the endomysium separating the skeletal muscle fibers. Larsson (1957) also noted this type of involvement.

Nomenclature

The use of the terms "mature" and "immature" has led to some confusion in describing the mast cells in these neoplasms. When these terms were first reported, Oliver et al. (1947) compared the appearance of the tumor cells to that of the normal developing mast cells. Recent evidence indicates that the tumor mast cells vary considerably in their histological and biochemical behavior from the normal connective tissue mast cells, and for these reasons the terms "mature" and "immature" should be avoided.

The term anaplasia better describes the morphological characteristics of the tumor mast cells, since they are really neoplastic and consequently include more dramatic biochemical variation than present in the normal cells.

Microscopic Appearance of Fixed Sections

The cells from all of the tumors ranged in size from 5 to 25 μ, and though they were approximately the same size in each of the specimens, tumors 2, 4, and 9 contained a greater number of the extremely large multinucleated cells (Plate III, Figure 1; Plate VII, Figure 1). The shape of the cells varied from round to elongate. In the more anaplastic tumor cells, the cellular outlines were generally indistinct, and the cytoplasm had a vacuolated appearance (Plate I, Figure 1). The round to oval nuclei ranged in size from 3 to 11 μ. They were generally centrally located with the concentration

of chromatin at the periphery. The nuclei of the very anaplastic tumor cells were larger, more vesicular, and stained lighter than the less anaplastic cells.

The microscopic arrangement of the tumor cells was similar to that described by previous authors. The mast cells appeared as clumps or chains in the tumor areas, separated by strands of loose connective tissue. Usually these tumors were not encapsulated, thus allowing the cells to infiltrate the surrounding tissue. The tumor cells rarely invaded the epithelium but were present in varying amounts in the corium and often in the subcutaneous tissue. They surrounded the appendages of the skin, sometimes pushing them aside. The ducts of the apocrine glands often appeared dilated and active; this was probably due to the pressure of the tumor growth.

Mitotic figures were seen in 9 of the tumor sections (Plate I, Figure 2). This is in agreement with the observations of Oliver et al. (1947), Mulligan (1949) and Stunzi (1955) who described the frequent appearance of mitosis. However, it is in contrast to reports by Nielsen and Cole (1958) and Bloom et al. (1958) who rarely encountered mitotic figures.

Metastasis to regional lymph nodes was observed in six of the animals. Animal 1 also showed involvement of the liver, and animal 6 of the lung and spleen. Sections of the lymph nodes, lung, spleen, and liver contained foci of mast cells which were morphologically identical to those

seen in the primary tumors. Plate IV, figure 1 illustrates the large multinucleated neoplastic mast cells in a regional lymph node. They are identical to the cells seen in the primary tumor (Plate III, Figure 1). These findings support the studies of previous authors who have observed similar metastasis (Bloom 1942, Mulligan 1949, Nielsen 1952, Larsson 1957, and Head 1958).

The presence of large numbers of eosinophils in mast cell tumors has been a subject of much concern and discussion. According to West (1958) the eosinophils are associated with detoxification and disposal of histamine, and edematous fluid is formed with the local release of amines. Drennan (1951) stated that the presence of the edema seems to be an immediate stimulus for the mast cells to secrete, resulting in the loss of granulation. Thus, in the tumors with the most edema, one would expect to find eosinophils, and partially degranulated mast cells.

Eosinophil and mast cell counts made on given areas of each of the tumors used in this study indicated a relationship between eosinophil population, edema, and mast cell granulation. Generally, the most edematous tumors had the highest eosinophil/mast cell ratio and the least amount of granules in the tumor cells (Graph I). Tumors 1 and 2 had an almost complete absence of granulation of mast cells and a large number of eosinophils in the 39,675 square μ area. Good evidence of tissue edema is shown by the low total cell

count when compared to the cell counts from tumors containing heavily granulated cells.

The data in Graph I give considerable support to a possible relationship between eosinophils, edema, and mast cell granulation. The author is aware of the necessity of determining normal tissue eosinophil counts for the various breeds of dogs in order to give this theory firm support. However, on the basis of these data, it could be postulated that the very anaplastic tumors are more active, secreting considerable histamine or other substances which could cause an increase in eosinophils with subsequent edema and degranulation.

Histochemical Studies of Fixed Sections

Toluidine blue and pinacyanol erythrosinate were used as the metachromatic stains to note the amount and character of the granules in the mast cells. Some of the cells exhibited fine, light staining and evenly distributed granules while others demonstrated coarse, intensely stained granules. The variations in size and staining reaction were correlated with the degree of anaplasia. Generally, the most anaplastic cells demonstrated few or no metachromatic granules, but rather a hazy metachromatic cytoplasm. In fact, granules were rarely seen in cells from tumors 1 and 2. Conversely, cells which were more differentiated contained heavy, definitely metachromatic granules. Table I shows the intensity (graded

from 0 to 5) of the metachromatic reaction in the different tumors. It must be emphasized that the granules in the tumor cells never stained as intensely, or were as abundant, as those of normal mast cells in the same section (Plate VIII, Figure 2). If the intensity of the metachromatic reaction is due to the concentration of heparin in the granules (Cass et al. 1954), then the more differentiated cells have the greatest amount of heparin.

The methylene blue extinction test was used to determine the pH at which the mast cells stained intensely basophilic. Only cells containing coarse granules stained this way at a pH as low as 2.0 (Table I). Most of the tumor cells began to stain in a pH range of 3.75 to 4.13, and at higher pH ranges all the tissue elements stained the same. However, cells from tumors 1 and 2 failed to stain differently from those of other tissues. These two tumors that failed to stain at low pH's probably did not contain acid mucopolysaccharides in the same concentration, or form of that present in the other tumors (Pearse 1953).

Variations in the response of the tissue mast cell to the periodic acid-Schiff (P.A.S.) reagent have been reported by several authors, and similar variations were encountered in this study. Not only were there differences within the same tumor but extreme variations were seen in cells from the different tumors. Cells from tumors 1 and 2 showed a negative P.A.S. reaction (Plate II, Figure 1), while those

from tumors 10 and 11 (Plate XII, Figure 1) exhibited large, intensely positive P.A.S. granules. The P.A.S. response increased with the degree of metachromasia (Table I). Therefore, the most anaplastic cells exhibited a negative P.A.S. response, and generally did not contain metachromatic granules. Since the monosulfuric acid form of heparin stains P.A.S. positive, it is tempting to postulate that the heparin in the anaplastic tumors, if present at all, may exist in some other form, or is combined or inactivated. Conversely, in those tumors whose cells showed a definite P.A.S. response, the heparin is probably in the monosulfuric acid form within the granules. The fact that the most heavily granulated cells in tumors 10 and 11 are positive, indicates that even the most differentiated tumors cells probably never have the same histochemistry as that observed in mature normal tissue mast cells.

Mallory's phosphotungstic acid-hematoxylin was used to demonstrate the intracytoplasmic inclusions previously noted by Bloom (1942) as crystalloid, acidophilic, and basophilic bodies. A few cells from tumors 3 and 5 contained the crystalloid bodies. In addition, cells from tumor 3 demonstrated acidophilic and basophilic bodies. Plate VI, Figure 1 illustrates these inclusions seen in the cells from the regional lymph node of animal 3. In all instances the number of these bodies was very small, and there was no apparent pattern of distribution. The reason for their occurrence is not known.

Impression Smears

Impression smears provided the most valuable information in regard to mast cell granulation. Even the cells from the most anaplastic tumors (1 and 2) demonstrated definite granules which had been difficult to discern in paraffin sections. The degree of anaplasia of the tumor cells could be determined by this method, and the presence of eosinophils and multinucleated cells could also be noted. These are some reasons why this procedure is so important as an aid in the correct and rapid diagnosis of the tumor.

Tissue Culture

Unfortunately, there are not many reports of successful culture of mast cells from canine tumors. In those which have been reported (Paff et al. 1947, Paff and Bloom 1949, and Williams et al. 1959), too often one or two tumors were used as a source of cells and since we know that these tumor cells can vary morphologically and histochemically, it is impossible to make definite conclusions under these limited circumstances.

Of the cell preparations made from 11 of the tumors only 9 were successfully cultivated in vitro. The cells remained viable from 1 to 11 months, but they never attained a typical growth phase, and as subsequent subcultures were made the number of cells decreased until the cell lines ultimately died out. The unsuccessful cultivation of these cells was probably due to nutritional deficiencies of the medium.

The primary cultures from all of the tumors used in this study developed a fibroblastic-like pattern. In general the cells had an indistinct cellular outline with long protoplasmic extensions. They usually had one nucleus, although some multinucleated cells were seen (Plate XI, Figure 1). Mitotic figures were never present in any of the cell preparations.

The most outstanding feature among the cells from the various tumors grown in tissue culture, was the amount of granulation which persisted during the culture period. The cells from tumors 1 and 2 which showed few to no granules in sections revealed the same picture in cell culture (Plate II, Figure 2 and Plate IV, Figure 2). Tumor cells from the intermediate group rarely showed metachromatic granules, but they generally exhibited hazier appearing cytoplasm (Plate VI, Figure 2). Preparations from tumors 10 and 11 showed definite heavily granulated cells in culture (Plate X, Figures 1 and 2 and Plate XII, Figure 2). When granules were present in the cells they appeared to be concentrated near the nuclei; sometimes there was a perinuclear halo.

Thus the picture of the mast cells in smear preparations, sections, and tissue culture indicated that there were definite histological and histochemical differences among the cells from various tumors. For these reasons, it is difficult to make definite conclusions about the behavior pattern of mast cells from these tumors. Regardless of the reason for these

differences, it is clear from the evidence here that the mast cells from these canine tumors cannot be equated to the normal mast cells.

SUMMARY AND CONCLUSIONS

Eleven canine mast cell tumors from the Michigan State University Veterinary Clinic were used to study the morphological and histochemical variations of the mast cells from these canine tumors. Paraffin sections, impression smears, and tissue culture preparations from each tumor were compared.

1. Under the conditions of this experiment the mast cells from all canine tumors studied differed from each other not only in smear preparations and fixed sections, but also in their appearance in tissue culture.

2. There was a marked difference in the degree of anaplasia among the cells from the various tumors. Some tumors were composed of very anaplastic cells, and some contained more differentiated cells.

3. The cells from the different tumors varied in the intensity of their reaction to metachromatic stains. This was directly related to the amount and type of granules present in the cells and the degree of anaplasia. The intensity increased as the cells became more differentiated, but the reaction was never the same as that observed in normal tissue mast cells.

4. The periodic acid-Schiff reaction differed; the most differentiated cells stained intensely positive while the most anaplastic cells were P.A.S. negative. These differences

are probably due to the amount and form of heparin present in the granules.

5. There was a relationship between the number of eosinophils and the amount of granulation in the tumor mast cells. As the number of eosinophils increased the amount of mast cell granulation decreased. The anaplastic tumor cells are probably the most secretory, causing mast cell degranulation accompanied by a marked accumulation of eosinophils.

6. Mast cells from 9 of the 11 tumors were cultivated in vitro for various periods of time. The most differentiated cells retained their characteristic granules during the cultivation period.

7. Impression smears provided the most valuable information with regard to mast cell granulation.

8. These differences that exist among the cells from the different tumors make it apparent that generalizations about canine mast cell tumors must be closely evaluated.

LITERATURE CITED

Asboe-Hansen, G. 1954. The mast cell. Int. Rev. Cytol. 33:399-431.

Beare, M. 1958. Urticaria pigmentosa and allied disorders. Brit. J. Derm. 70:418-425.

Bensley, S. H. 1952. Pinacyanol erythrosinate as a stain for mast cells. Stain Tech. 27:269-273.

Bloom, F. 1942. Spontaneous solitary and multiple mast cell tumors (mastocytoma) in dogs. Arch. Path. 33:661-676.

Bloom, G., U. Friberg, B. Larsson, and B. Aberg. 1955. Morphology of tissue mast cells in dog mastocytoma and clinical chemistry of these tumors. Acta Path. et Microbiol. Scand. 37:163-166.

Bloom, G., U. Friberg, and B. Larsson. 1956. Some observations on the fine structure of mast cell tumors (mastocytoma). Nordisk Veterinaermed. 8:43-55.

Bloom, G., B. Larsson, and B. Aberg. 1958. Canine mastocytoma. Zbl. Veterinärmedizin. 5:443-458.

Cass, R., J. F. Riley, G. B. West, K. W. Head, and S. W. Stroud. 1954. Heparin and histamine in mast-cell tumours from dogs. Nature (Lond). 174:318-319.

Drennan, J. M. 1951. The mast cells in urticaria pigmentosa. Jour. Path. Bact. 63:513-520.

Dunn, T. B., and M. Potter. 1957. A transplantable mast-cell neoplasm in the mouse. Jour. Natl. Cancer Inst. 18:587-596.

Eagles, H. 1955. The minimum vitamin requirements of the L and HeLa cells in tissue culture, the production of specific vitamin deficiencies, and their cure. Jour. Exptl. Med. 102:595-600.

Ehrlich, P. 1879. Beitrage zur Kenntnis der granulierten Bindegewebszellen und der eosinophilen Leukocyten. Arch. Anat. Physiol 3:166-169.

Fulton, G. P., L. F. Maynard, J. F. Riley, and G. B. West. 1957. Humoral aspects of tissue mast cells. Physiol. Rev. 37:221-232.

Furth, J., P. Hagen, and E. Hirsch. 1957. Transplantable mastocytoma in the mouse containing histamine, heparin, and 5-hydroxytryptamine. Proc. Soc. Exptl. Biol. and Med. 95:824-828.

Gridley, M. F. 1957. Manual of Histology and Special Staining Technics. Armed Forces Institute of Pathology. Washington, D. C.

Hanks, J. H., R. E. Wallace. 1949. Relation of Oxygen and temperature in the preservation of tissues by refrigeration. Proc. Soc. Exptl. Biol. and Med. 71:196-200.

Head, K. W. 1953. Tumours in animals. Vet. Rec. 65:926-929.

Head, K. W. 1958. Cutaneous mast cell tumors in dog, cat, and ox. Brit. J. Derm. 70:389-408.

Johnson, Eunice, F. N. Andrews, and C. L. Shrewsburg. 1943. The preparation of muscular tissue for histological study. Jour. Animal Sci. 2:244-250.

Jorpes, J. E., H. Holmgren, and O. Wilander. 1937. Über das Vorkommen von Heparin in den Gefasswanden und in den Augen. Ztschr. Mikr.-anat. Forsch. 42:279-300.

Jorpes, J. E., F. B. Werner and B. Aberg. 1948. The fuchsin-sulfurous acid test after periodate oxidation of heparin and allied polysaccharides. Jour. Biol. Chem. 176:277-282.

Köhler, H. 1954. Über das Mastozytom beim Hund. Deutsch. Tier. Wschr. 61:460-466.

Larsson, B. 1956. Statistical analysis of cutaneous tumours in dogs with special reference to mastocytoma. Nordisk Veterinaermed. 8:130-139.

Larsson, B. 1957. Some aspects of canine mastocytoma. Nordisk Veterinaermed. 9:241-256.

Lever, W. F. 1954. Histopathology of the Skin. J. B. Lippincott Co. Philadelphia

Lindell, S. E., H. Rorsman, and H. Westling. 1959. Formation of histamine in a canine mastocytoma. Experientia 15:31.

Malewitz, T. D., and E. M. Smith. 1955. A nuclear stain employing a dilute Harris' hematoxylin. Stain Tech. 30:311.

Meier, H. 1957. Feline mastocytoma - two cases. Cornell Vet. 47:220-226.

Meier, H. 1959. Mastocytoma, serotonin content in dogs. Proc. Soc. Exptl. Biol. and Med. 100:815-817.

Michels, N. A. 1938. The mast cell. Downey Handbook of Hematology. Vol. I. Paul B. Hoeber, Inc., New York. 4:230-373.

Mulligan, R. M. 1949. Neoplasms of the Dog. Williams and Wilkin Co. Baltimore.

Murray, J. A. 1908. The zoological distribution of cancer. Third Scient. Rep. Invest. Imp. Cancer Res. Fund. 41-60.

Nielsen, S. W. 1952. Clinical aspects of mastocytoma in dogs: frequency, regional involvement, metastasis, recurrence, and differential clinical and pathological diagnosis. Proceedings Amer. Vet. Med. Assoc. 89th annual meeting.

Nielsen, S. W. and C. R. Cole. 1958. Canine mastocytoma; a report of one hundred cases. Amer. Jour. Vet. Res. 19:417-432.

Oliver, J., F. Bloom, and C. Mangieri. 1947. On the origin of heparin. Jour. Exptl. Med. 86:107-116.

Orkin, M. and R. M. Schwartzman. 1959. Mastocytoma, comparison in man and dog. Jour. Invest. Derm. 32:451-466.

Paff, G. H., F. Bloom, and C. Reilly. 1947. The morphology and behavior of neoplastic mast cells cultivated in vitro. Jour. Exptl. Med. 86:117-124.

Paff, G. H. and F. Bloom. 1949. Vacuolization and the release of heparin in mast cells cultivated in vitro. Anat. Record. 104:45-60.

Pearse, A. G. E. 1953. Histochemistry Theoretical and Applied. Little, Brown, and Co. Boston.

Riley, J. F. and G. B. West. 1953. Mast cell and histamine in normal and pathological tissue. Jour. Physiol. 119:44.

Riley, J. F. 1955. Pharmacology and function of the mast cell. Pharmacol. Rev. 7:267-277.

Romanelli, V. 1953. Contributo allo studio delle funzioni die mastociti nel cane. Atti Della Soc. Ital. Delle Sci. Vet. 7:580-584.

Schindler, R., M. Day, and G. A. Fischer. 1959. Culture of neoplastic mast cells and the synthesis of 5-hydroxytryptamine and histamine in vitro. Cancer Res. 19:47-51.

Stunzi, H. 1955. Zur Pathologie des sogenannten Mastozytoms des Hundes. Schweiz. Ztschr. f. Allg. Path. u. Bakt. 18:1271-1277.

Taketa, Y. 1958. On the origin of the tissue mast cell. Okajimus fol. Anat. 31:143-159.

West, G. B. 1958. Pharmacology of the tissue mast cell. Brit. J. Derm. 70:409-417.

Williams, W. J., E. Larson, and T. L. Phillips. 1959. A neoplastic connective tissue mast cell capable of continuous growth in tissue culture. Jour. Biophys. and Biochem. Cytol. 6:361-367.

Zitcer, E. M., W. H. Elsasser, and P. L. Kirk. 1953. Cultivation and morphology of human tissue mast cell in vitro. Growth 17:111-122.

TABLE I. TUMOR CHARACTERISTICS

Animal, Tumor Number*	Location	Previous Tumors Removed	Metastasis	Mitosis	MBE** pH	PAS***	Toluidine Blue****	Tissue Culture Granulation
1	axillary region	+	+	+	-	-	0-1	none
2	axillary region	+	+	+	-	-	0-1	none
3	superior gingiva	-	+	+	3.75	±	2.5-3	none
4	axillary region	+	+	+	3.75	±	2.5	no growth
5	prepuce	-	+	-	4.13	+	3	no growth
6	scrotum	+	-	+	3.75	+	3	a few cells with granules
7	supra scapular	-	-	+	4.13	+	2-3	none
8	lateral pelvic limb	-	+	+	3.75	+	3	possible granulation
9	lateral pectoral limb	-	+	+	2.00	+	3-4	questionable
10	medial ventral thorax	-	-	+	2.00	++	4	definite granules
11	lateral pectoral limb	-	-	-	2.00	+++	5	many granules

*Tumor number and animal number are the same, **MBE pH = pH where tumor cell first stained with methylene blue extinction test, ***PAS = periodic acid-Schiff reaction, ****Toluidine blue reaction graded from 0-5.

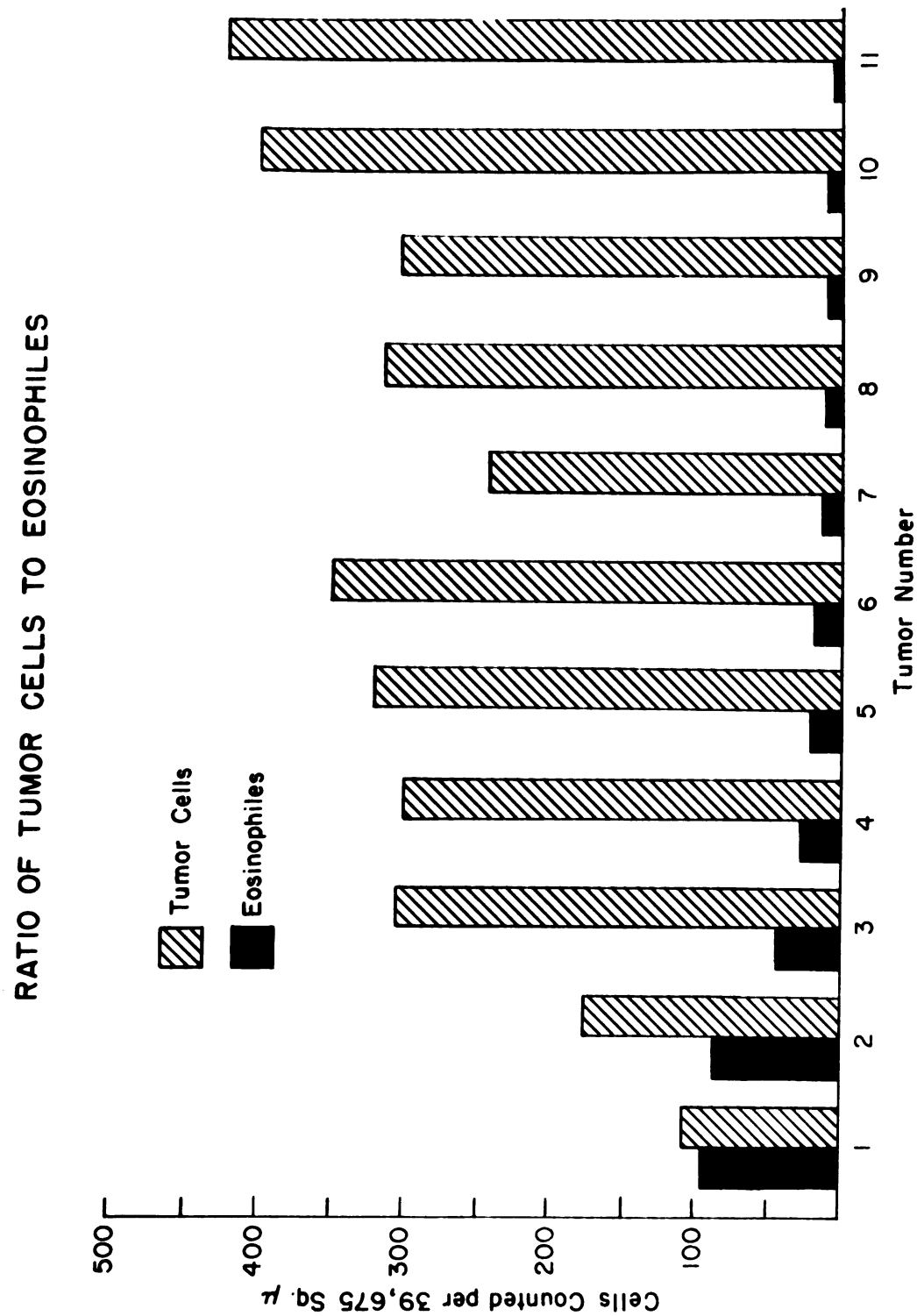
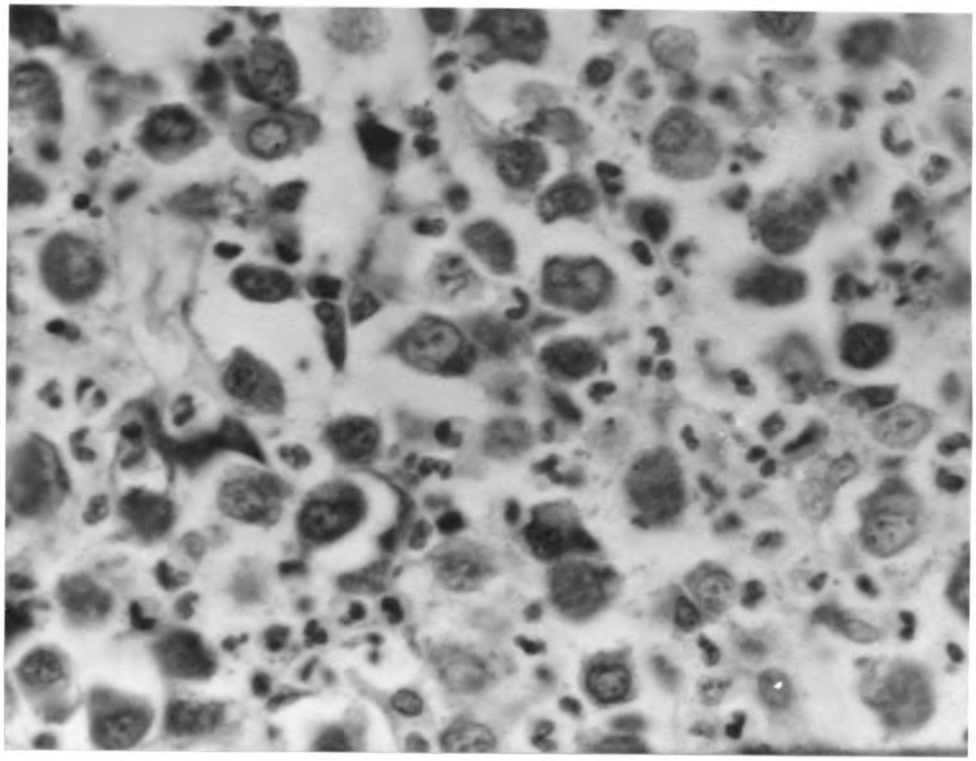

RATIO OF TUMOR CELLS TO EOSINOPHILES
Tumor Cells
Eosinophiles
Cells Counted per 39,675 Sq. μ
500
400
300
200
100
1
2
3
4
5
6
7
8
9
10
11
Tumor Number

PLATE I

Tumor number 1

Figure 1. Section from deep in the axillary region. Little granulation is present in the tumor cells and many eosinophils can be noted. Toluidine Blue; x 722.

Figure 2. Section from the same region showing mitotic figures and eosinophils. H. and E.; x 720.



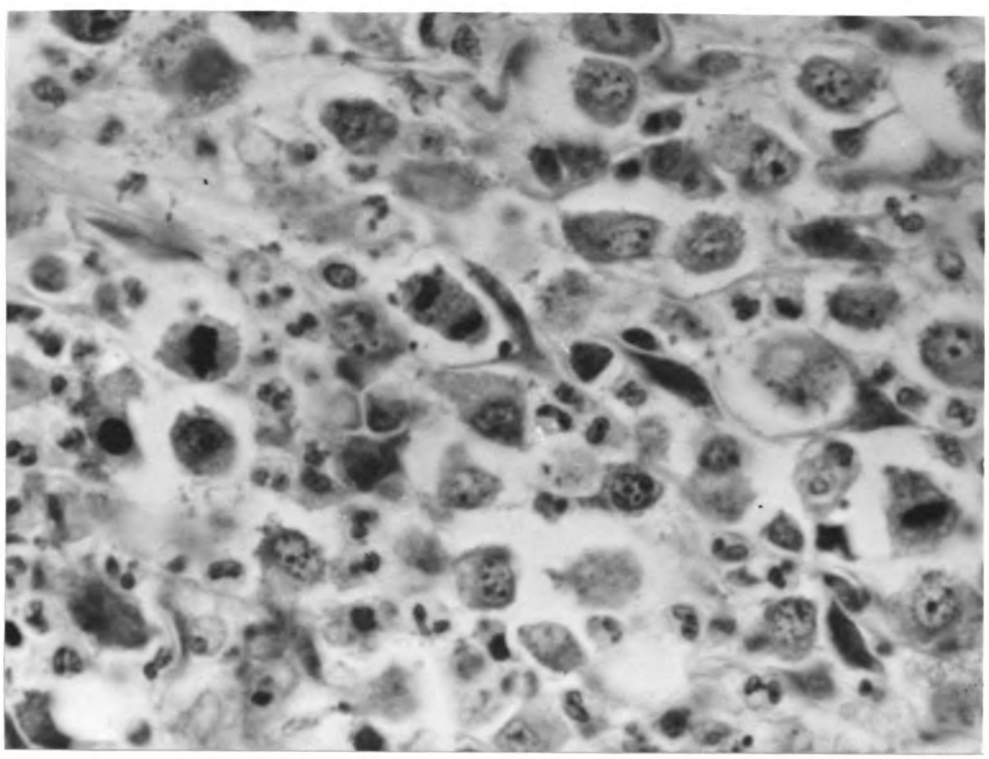


PLATE II

Tumor number 1

Figure 1. Section showing absence of P.A.S. positive granules. Periodic Acid-Schiff; x 731.

Figure 2. Tissue culture preparation showing a cell with foamy vacuolated cytoplasm, absence of granules, and an indistinct cellular outline. Wright's; x 710.

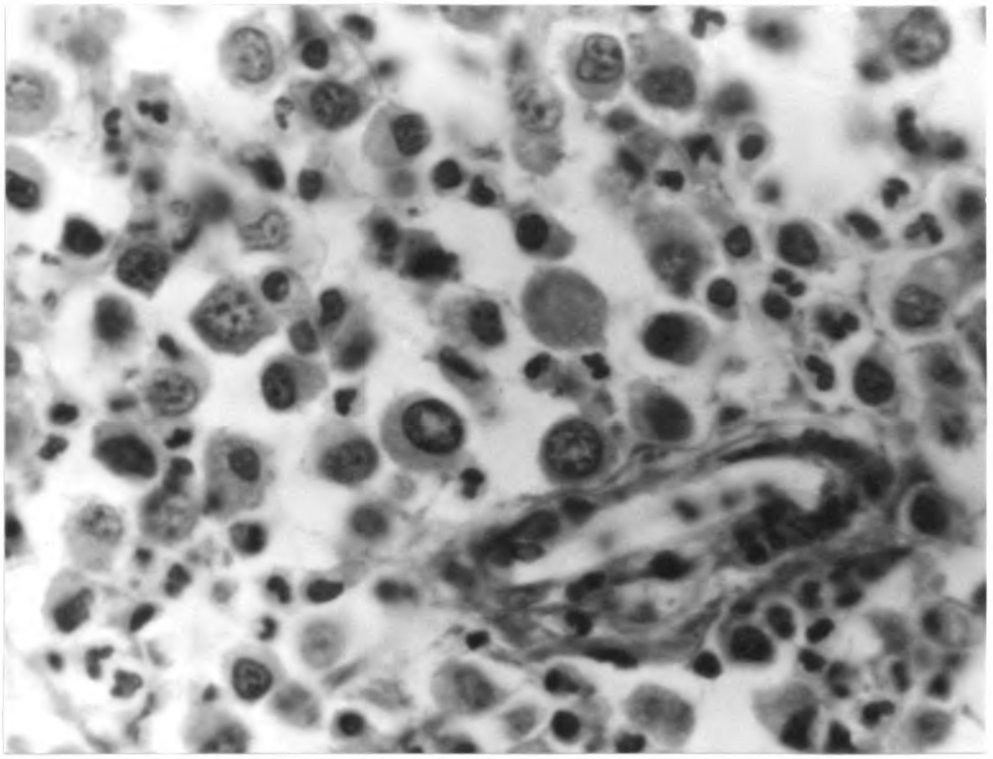
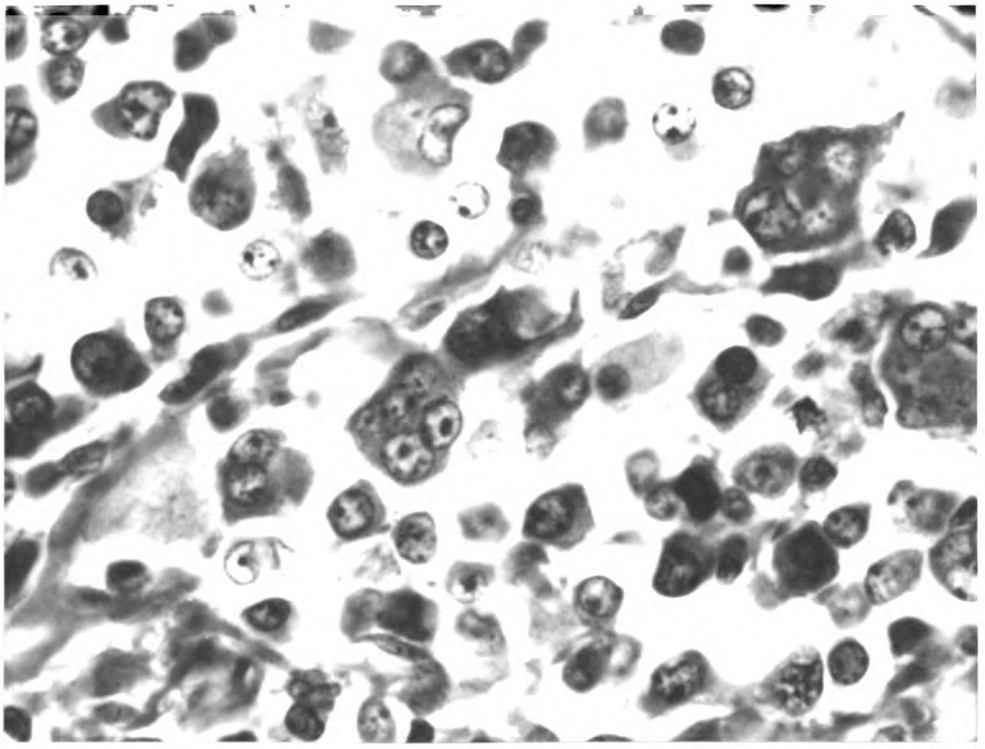


PLATE III

Tumor number 2

Figure 1. Section showing multinucleated giant cells and little metachromatic granulation. Pinacyanol Erythrosinate; x 735.

Figure 2. Impression smear demonstrating variations in granulation and multinucleated giant cells. Wright's stain; x 717.



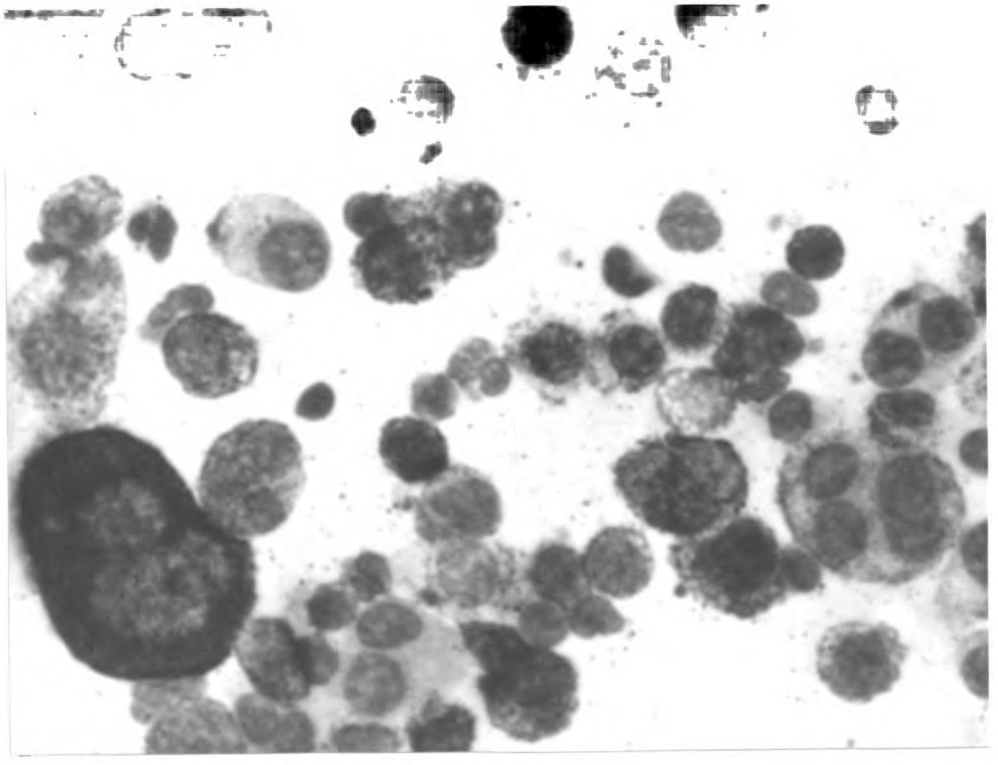
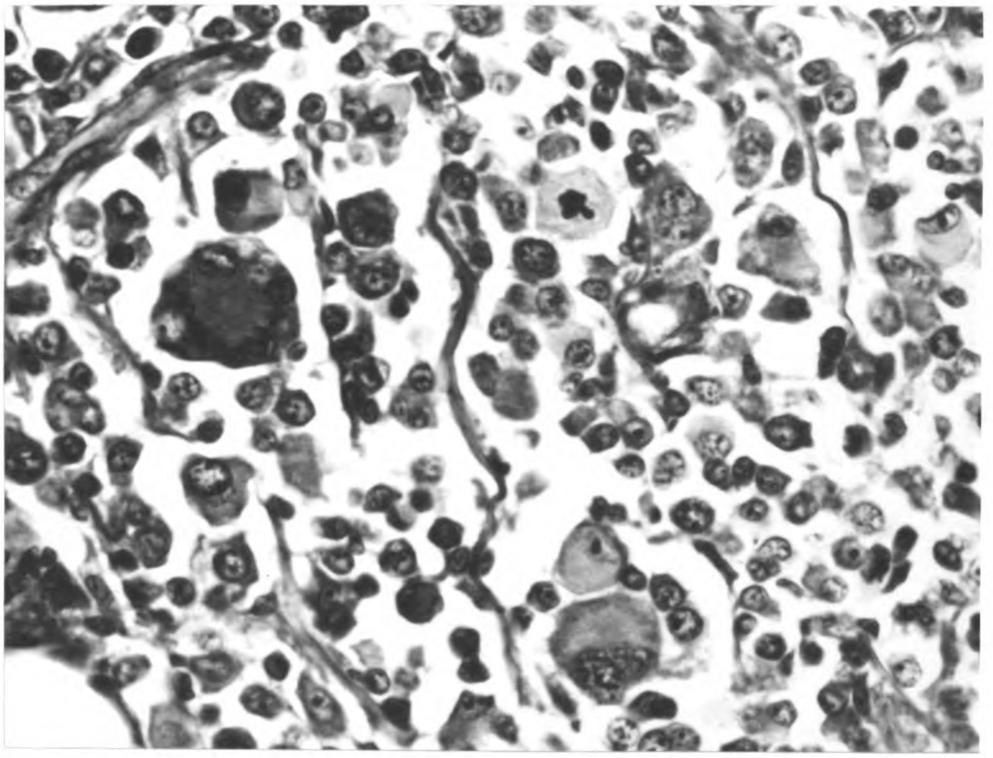


PLATE IV

Tumor number 2

Figure 1. Section of regional lymph node from animal number 2. Giant tumor cells can be noted. Pinacyanol Erythrosinate; x 454.

Figure 2. Tissue culture preparation demonstrating cells without granules, with a foamy cytoplasm, and indistinct cellular outlines. Wright's stain; x 895.



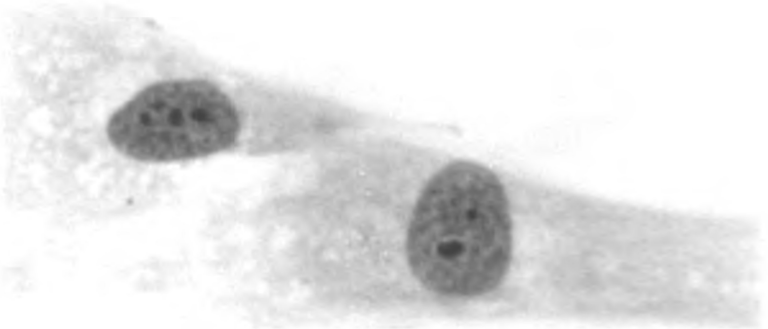
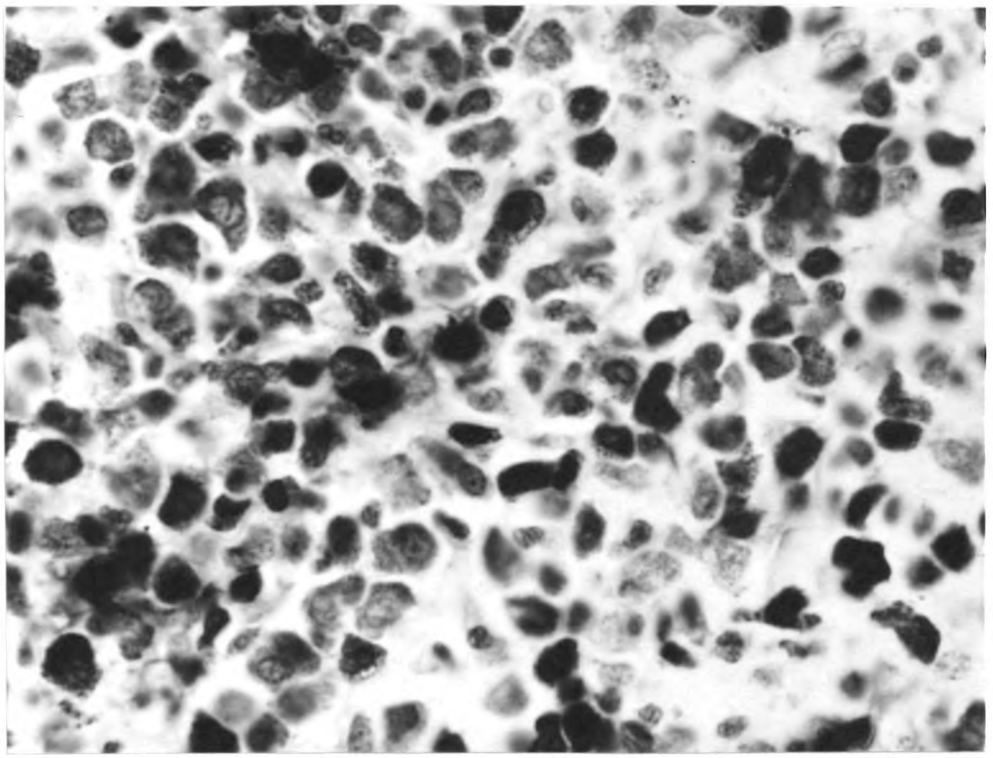


PLATE V

Tumor number 3

Figure 1. Section showing distinct granules in the tumor cells. Toluidine Blue; x 705.

Figure 2. Impression smear exhibiting light to heavy granulation in the tumor cells. Wright's stain; x 719.



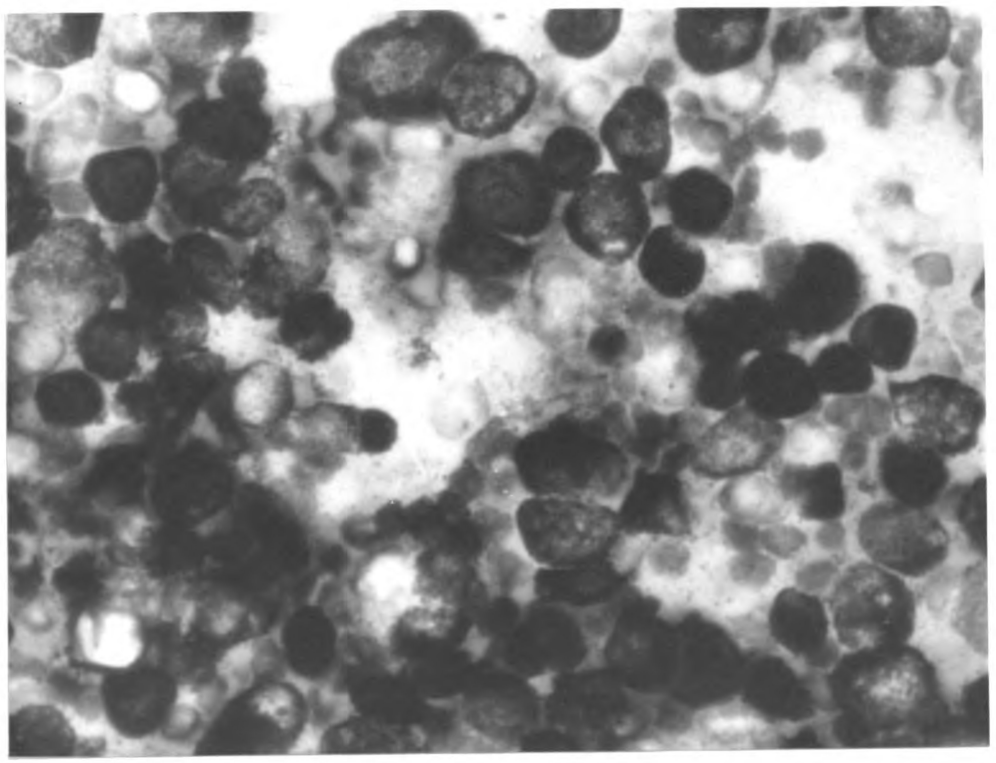
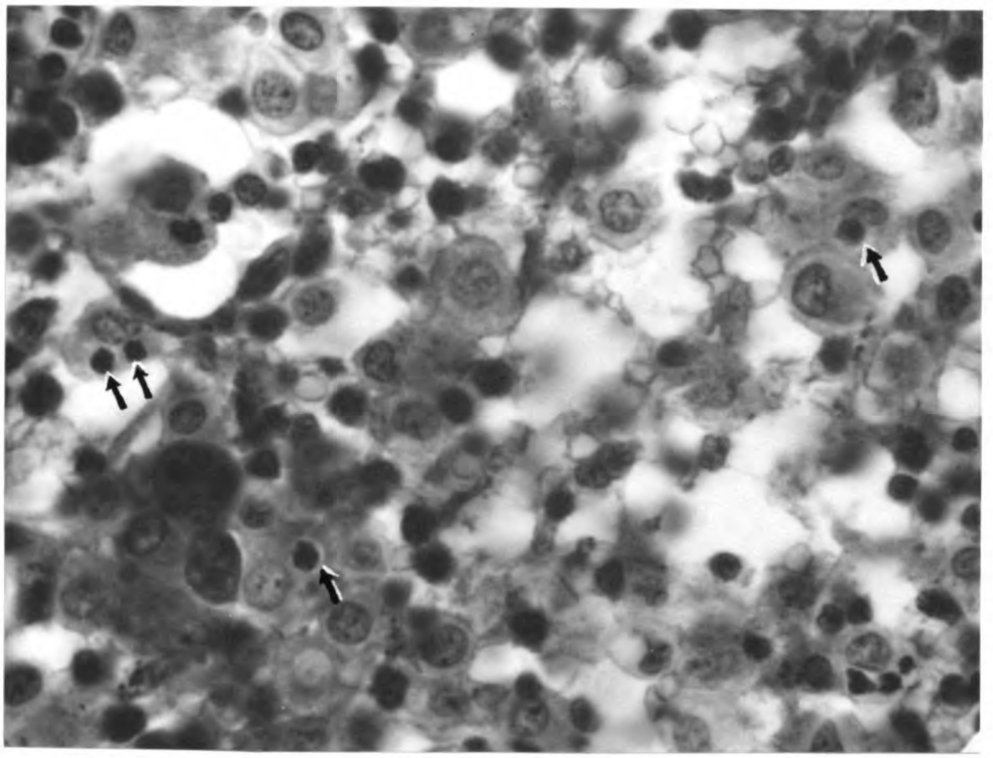


PLATE VI

Tumor number 3

Figure 1. Section of the regional lymph node of animal number 3. Arrows point to tumor cells with intracytoplasmic inclusions. Mallory's Phosphotungstic Acid Hematoxylin; x 700.

Figure 2. Tissue culture preparation shows a cell with a distinct outline, a hazy cytoplasm, but no metachromatic granules. Wright's stain; x 721.



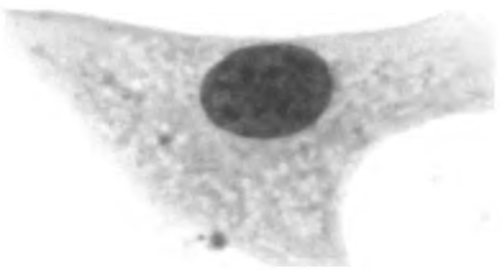
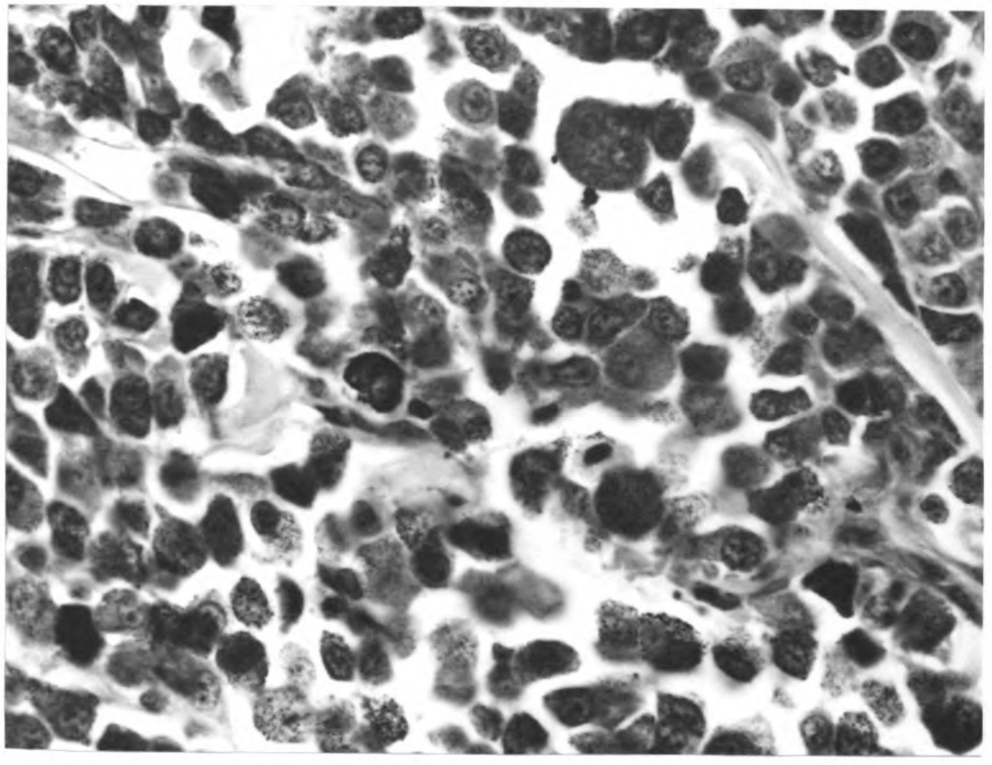


PLATE VII

Tumor number 9

Figure 1. Section showing distinct metachromatic granules in varying concentrations in the tumor cells. Giant multinucleated cells are also present. Pinacyanol Erythrosinate; x 503.

Figure 2. Impression smear demonstrating the presence of distinct metachromatic granules in the tumor cells. Wright's stain; x 911.



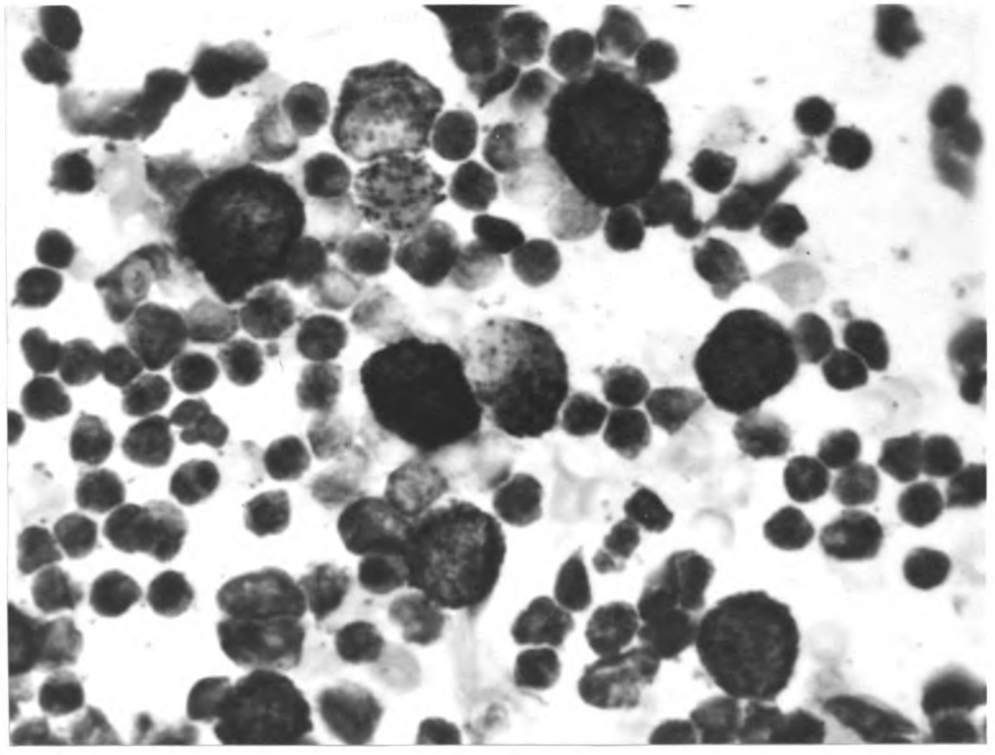
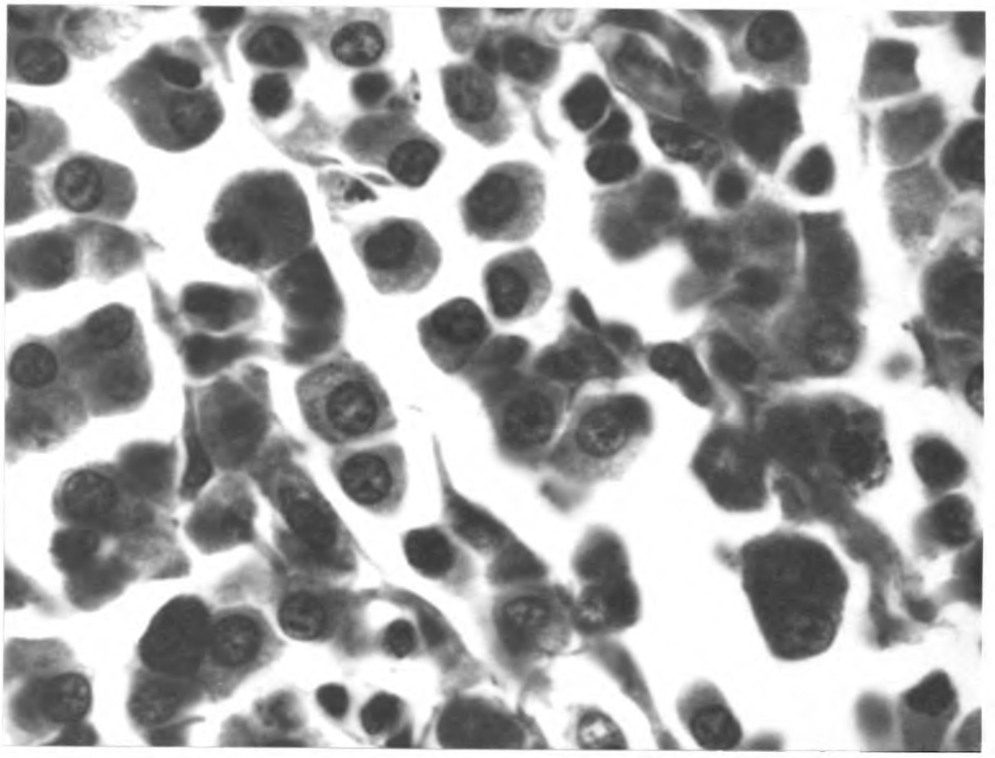


PLATE VIII

Tumor number 9

Figure 1. Section demonstrating the presence of P.A.S. positive granules. Periodic Acid-Schiff; x 895.

Figure 2. Section showing the lighter metachromatic tumor cells in the lower right hand corner and the intensely metachromatic normal mast cells just below the epithelium of the skin. Pinacyanol Erythrosinate; x 206.



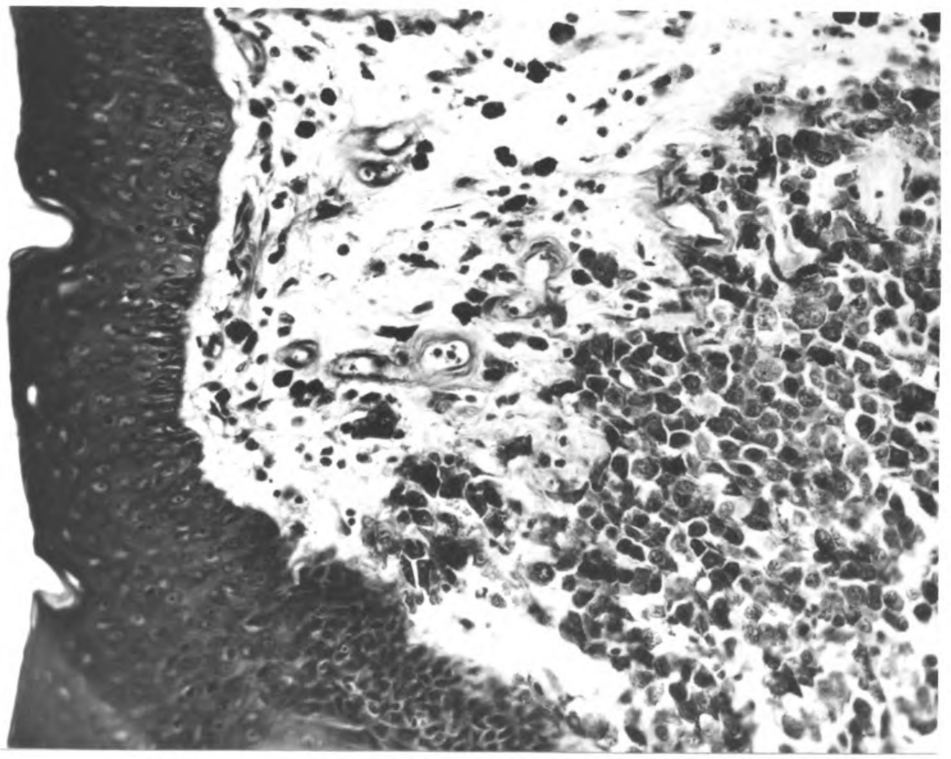
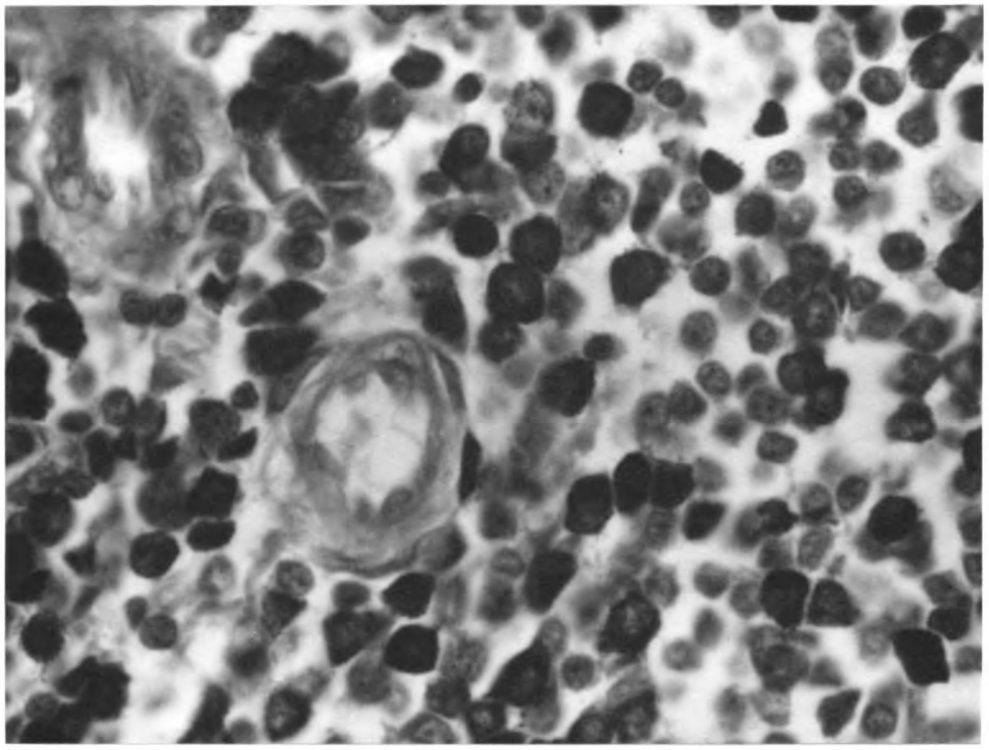


PLATE IX

Tumor number 10

Figure 1. Section illustrating the very heavy metachromatic granulation in tumor cells. Pinacyanol Erythrosinate; x 680.

Figure 2. Impression smear showing very dense granulation in the tumor cells. Wright's stain; x 695.



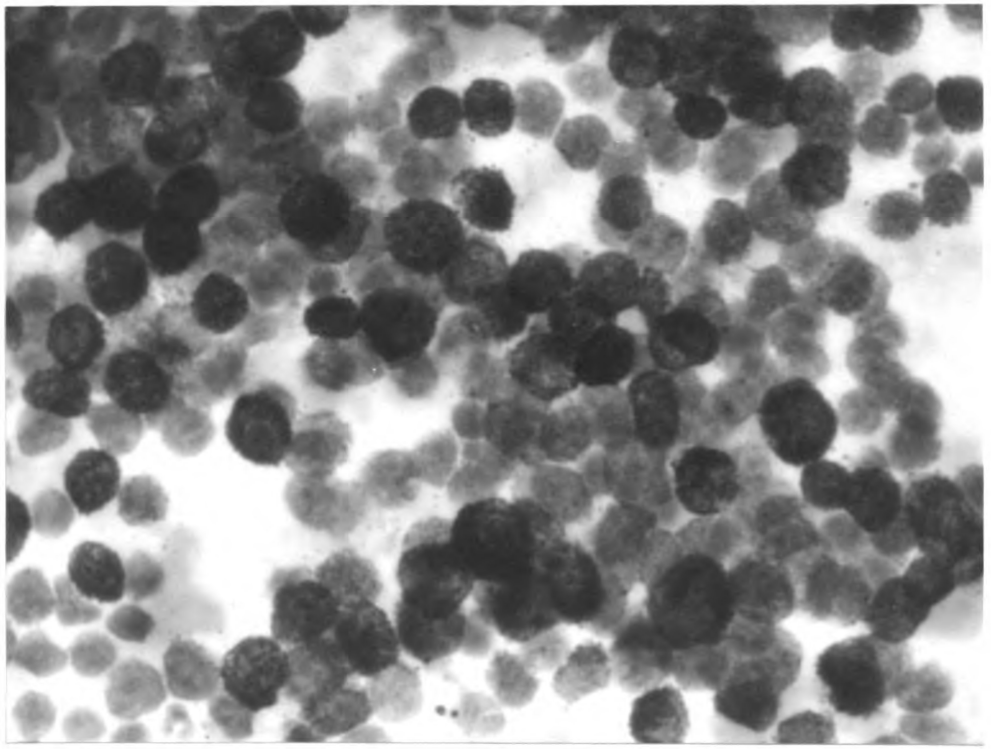
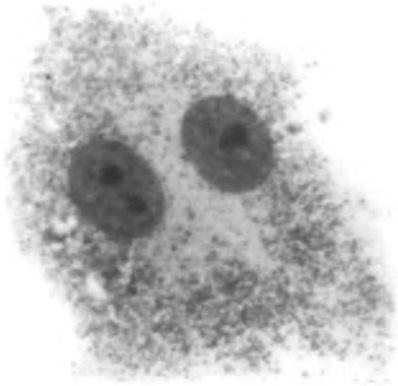


PLATE X

Tumor number 10

Figure 1. Tissue culture preparation showing binucleation and many distinct metachromatic granules. Wright's stain; x 744.

Figure 2. Tissue culture preparation from this tumor. Distinct metachromatic granules and a large nucleolus can be seen. Wright's stain; x 921.



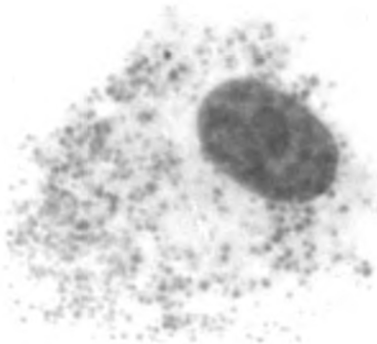
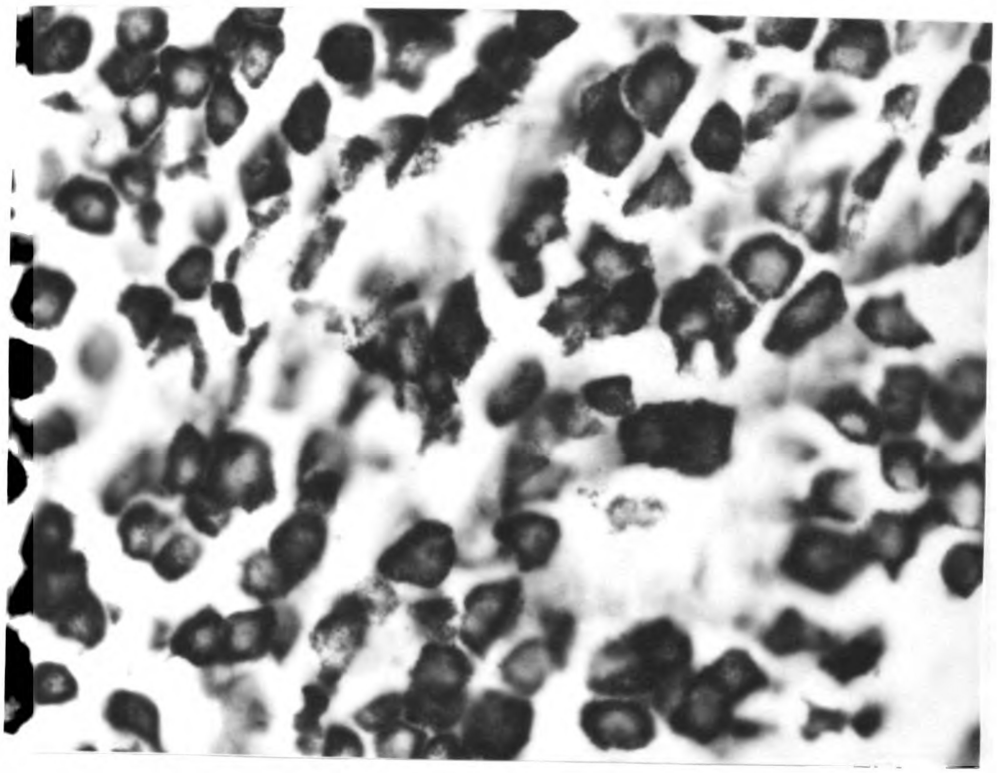


PLATE XI

Tumor number 11

Figure 1. Section in which tumor cells show a very heavy metachromatic granulation that often obscures the nuclei. Pinacyanol Erythrosinate; x 922.

Figure 2. Impression smear showing very heavily granulated relatively uniform cells. Wright's stain; x 738.



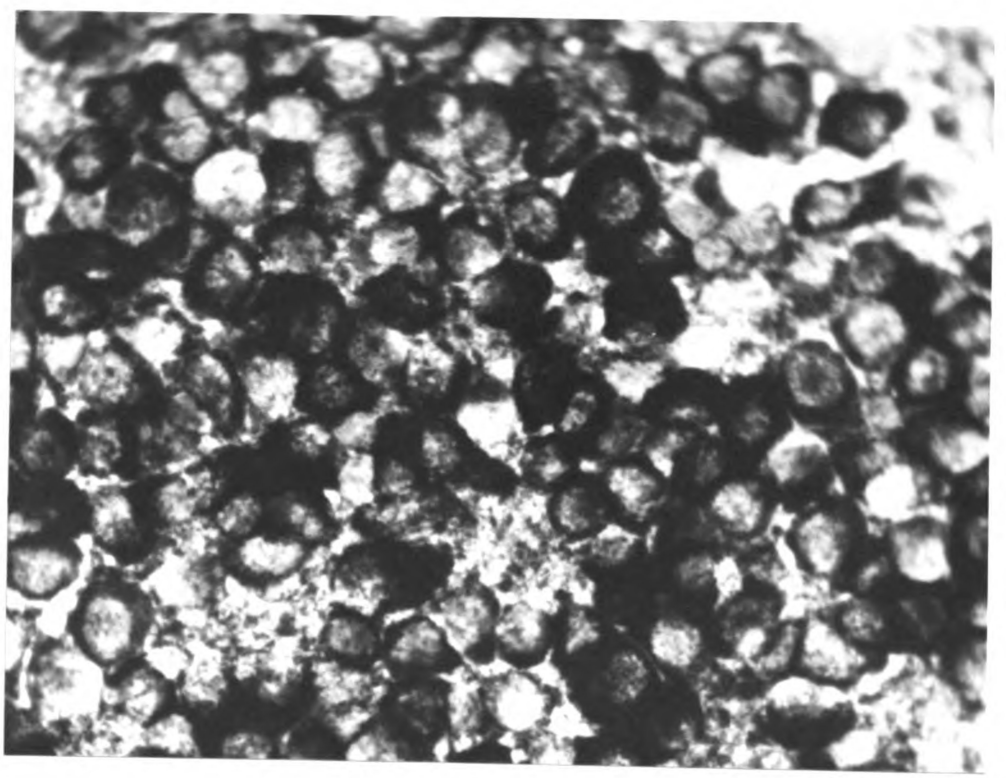


PLATE XII

Tumor number 11

Figure 1. Section demonstrating an intense P.A.S. positive reaction. Periodic Acid-Schiff; x 448.

Figure 2. Tissue culture preparation showing a cell packed with metachromatic granules. Wright's stain; x 1060.