BIOLOGICAL BEHAVIOR OF THE RAT MAMMARY ADENOCARCINOMA 13762

By

LEA ROCKWELL GORDON

Veterinariae Medicinae Doctoris

The University of Pennsylvania

Philadelphia, Pennsylvania

1970

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the degree of
MASTER OF SCIENCE
December, 1976

BIOLOGICAL BEHAVIOR OF THE RAT

MAMMARY ADENOCARCINOMA

13762

Thesis Approved:

Andrew W. Hanford
Thesis Adviser

D B Dodgen

J. F. Loyd

Norman N. Durham
Dean of Graduate College

PREFACE

This study is concerned with a syngeneic transplantable mammary adenocarcinoma 13762 in Fisher 344 adult female rats. The primary objective is to describe the biological behavior of this tumor-host system with special emphasis on the response of lymphoid tissues to tumor growth. Thereby, this study will serve as a basis for further studies on the host's response to various manipulations of this system such as surgery, immunotherapy and chemotherapy.

The author wishes to express her appreciation to her major adviser, Dr. Andrew Monlux, for his extreme patience and gentle guidance. Appreciation is also expressed to the other committee members, Dr. David Dodd, for helping to define the goals and proofreading the manuscript and to Dr. Jeffie Roszel for encouragement and enlightenment. Thanks also go to Dr. Robert Chesnut and Dr. Kermit Carraway without whose assistance, materials and laboratory this project would not have been possible, and to the College of Veterinary Medicine Graduate Research Committee for funds to pay for the histologic preparations.

Special thanks go to Mr. Fred Lawson for taking the photographs and making the prints. Also many thanks to Mrs. Rhonda Smith for her careful consideration in typing the manuscript.

Special commendations must go to my colleagues in the necropsy service, Dr. Trenton Schoeb, Dr. Margaret Juliana and Dr. Talmage Brown, for taking up the duties when I was preoccupied with my thesis.

Special thanks also go to Dr. Julia Blue for her moral support and help with the manuscript.

TABLE OF CONTENTS

LIST OF FIGURES

CHAPTER I

THE RESEARCH PROBLEM

Introduction

There is much research today on cancer. It is a very broad field. Studies range from basic research on deoxyribonucleic acid (DNA) to clinical trials in human patients. The ultimate goal is to find ways to prevent and to cure cancer. An approach to a problem of this magnitude is to dissect it into its parts and to study each in detail. Experiments are designed so that as many variables as possible are eliminated. Tumor-host model systems are valuable because they have a minimum of variables when compared with spontaneously occurring tumors in humans. This report concerns a tumor-host model for breast cancer in women, the rat mammary adenocarcinoma 13762 in adult female Fisher 344 rats. It is a syngeneic tumor-host system.

Statement of the Problem

The problem is to define, on morphologic bases, by light microscopy, the syngeneic mammary adenocarcinoma 13762 as a tumor-host system. The definition must include a detailed description of the tumor in its primary and metastatic sites; also, the mode of spread in the body, location of metastases,

and the response of the lymphoid organs such as spleen, lymph nodes and thymus to tumor growth and metastases.

Purpose of the Study

The purpose of this study is to characterize the rat mammary adenocarcinoma 13762 in Fisher 344 rats in the absence of any manipulation, so that this system may be used in future studies with confidence that the biological behavior is predictable and well described. Then, manipulations, such as would be feasible in women with breast cancer, could be applied to this rat-tumor system and one could be confident that changes seen in the host are related to interference with the tumor-host relationship.

Literature Review

The rat mammary adenocarcinoma 13762 in Fisher adult female rats was originally described by Segaloff in 1966 (1). He induced the tumor with 7, 12-dimethylbenzanthracene (DMBA). Transplantation was always successful and all treated rats died from the tumor. In 1974 Bogden et al (2) reported studies they had done that included the effects of surgery, chemotherapy and immunotherapy on metastases of this tumor. These treatments were used alone and in combination. In the untreated group the rats developed large tumors and lived 47.8 ± 6.9 days. When the only treatment was excision of the primary tumor on day 18 after implantation the rats lived 65.5 ± 8 days.

Chemotherapy alone with the 17β-estradiol diester of p=[N, N-bis (2-chloroethyl amino)] phenylacetic acid at 5 mg/kg/day *per os* for 28 days produced oncolysis and cured 25% of the rats of their tumors. In the remaining animals there was regrowth of the tumor, despite continued chemotherapy. These animals lived 87.3 $\pm$ 10.5 days.

When 10 days of chemotherapy was given either before or after surgical removal of the tumor, 75.5% of the rats were cured of their tumors, but if chemotherapy was given for 28 days either before or after surgery the cure rate was reduced to 61%. This effect was attributed to the immunosuppressive action of the drug.

When surgery was followed by one intraperitoneal injection of 10 mg of MSF-MB (Methanol Soluble Fraction-*Mycobacterium butyricum*) as a nonspecific immunological adjuvant 10-20% of the rats were cured of their tumors. Ninety to 100% of the rats were cured when treatment included 10 days of chemotherapy before or after surgery and MSF-MB.

Much more has been done recently to study the immune response to the 13762 rat mammary adenocarcinoma in syngeneic rats in hopes of better understanding the pathogenesis of the tumor growth and metastasis. Most of these effects have been directed toward *in vitro* tests of cell mediated immunity. Fortner *et al* (3) described a microcytotoxicity assay technique that they used to measure cell mediated cytotoxicity (CMC). Their results showed that CMC appeared 4 days after tumor implantation, reached a maximum 2-4 days later, then

suddenly disappeared with continued tumor growth.

Kuperman et al (4) showed that the cytolytic effector cells induced by tumor 13762 are specific killers of that tumor cell line only. In another report Kuperman et al (5) described the existence of suppressor cells and memory cells in spleens from animals bearing the 13762 rat mammary adenocarcinoma and showed that they had a modulatory effect on the expression of cellular cytotoxicity.

More recently Kreider et al (6) studied the 13762 tumor for potential use as a model for BCG (Bacillus Calmette-Guerin) immunotherapy. They found that there was greater suppression of the tumor when the cells were transplanted mixed in the BCG.

Only brief morphologic descriptions of the tumor have been published (2, 6). There are no descriptions in the literature to my knowledge of the cellular changes that occur in the lymphoid tissues of rats during the growth of the 13762 mammary adenocarcinoma even though extensive studies have been done on individual lymphoid cells.

Sass et al (7) reported on the incidence of spontaneous neoplasms in Fisher 344 Rats during their natural life span. Of all tumors seen, the mammary tumors had an incidence of 40.6% in females and 23.1% in males. Sixty two of the 78 mammary tumors in females and 34 of 37 of the mammary tumors in males were fibroadenomas. The rest were adenocarcinomas and adenoacanthomas. No metastases were seen from any of the mammary tumors.

CHAPTER II

METHODS AND PROCEDURES

Introduction

This is basically a histopathological study of the 13762 rat mammary adenocarcinoma and of the lymphoid tissues in the rats with this tumor.

Population

One hundred and twenty-four adult (6-week-old) Fisher 344 female rats were used. They were obtained from Charles Rivers Laboratories in Wilmington, Massachusetts. They were kept in temperature (21°C) and ventilation-controlled rooms and given "Purina Lab Chow" (Purina Co., St. Louis, Missouri) and water ad libitum. Lights were on for 12 hours and off for 12 hours.

Tumor Strain

The rat mammary adenocarcinoma 13762, which is syngeneic in Fisher 344 rats, was used in this study. The tumor was obtained from the Mason Research Institute Tumor Bank in Worchester, Massachusetts, through the courtesy of Arthur E. Bogden. This was originally a DMBA-induced tumor. It has a lag phase of 6-8 days before tumor growth is measurable. The average survival time of a rat with the tumor is 42.1 $\pm$

6.9 days. At the time of death there are metastases in the lungs and sometimes in the abdominal organs.

Instrumentation and Techniques

A young tumor (7-14 days old) was used for transplantation. It was excised from a rat killed by a lethal dose of chloroform. The tumor was trimmed free of surrounding connective tissue and then put in a manual slicer that allowed 0.5 mm-thick slices to be cut. The slices were transferred to small dishes with 1-mm squares etched on the bottom. Pieces of nonnecrotic tumor, 1 mm square, were used for transplantation. The tissue was held in Hepes solution until the time of transplant which was within a few minutes of sectioning.

The skin was shaved over the dorso-caudal part of the left flank. The rats were anesthetized with ether and a 1-2 mm incision was made in the skin just in front of the hind leg. A 16-gauge trochar was used to place the 1 mm square of tissue approximately 1 cm anterior to the skin incision. No sutures were used in the skin.

The tumors were measured with a Vernier caliper by three different persons and the average value of the three was used for the chart on the growth rate. The formula used to calculate the volumes is $V = 4/3\pi \left(\frac{d_1}{2} \times \frac{d_2}{2} \times \frac{d_3}{2}\right)$.

Tissues from the rats were fixed for light microscopic examination in 10% buffered formalin. Tissues were dehydrated and embedded in paraffin and sectioned at 6µm. The rou-

tine stain used was hematoxylin and eosin. Special stains included Pollack's trichrome and periodic acid-Schiff (PAS).

Design of Experiment

Thirty-five rats were used to establish the rate of growth of the tumor and five were killed at successive 1-week intervals beginning at 20 days after receiving the tumor.

The remaining 89 rats were from unmanipulated tumor control groups from other experiments not discussed here; for example, the experiment on the effect of surgical removal of the mass on survival time. Some of these tumors were measured and are included in the growth table. Some rats were killed early in the growth of the tumor to try to correlate changes in the lymphoid tissues with the reported appearance and disappearance of cell-mediated cytotoxicity.

The temporal distribution of rats examined histologically after implantation was as follows: 14 rats during the 1st week, 11 during the 2nd, 15 in the 3rd, 23 in the 4th, 28 in the 5th, 10 in the 6th, 9 in the 7th, 8 in the 8th week and 6 between the 8th and 11th week.

CHAPTER III

ANALYSIS OF THE DATA

Introduction

The following is a record of the growth rate and a description of the morphology at the light microscopic level of the tumor growth, metastases and of the concomitant changes in the lymph nodes, spleen and thymus. The tumor-host system must be as well defined as possible before attempts are made to alter the system so that when a change is seen, one knows whether or not it has been caused by the manipulation.

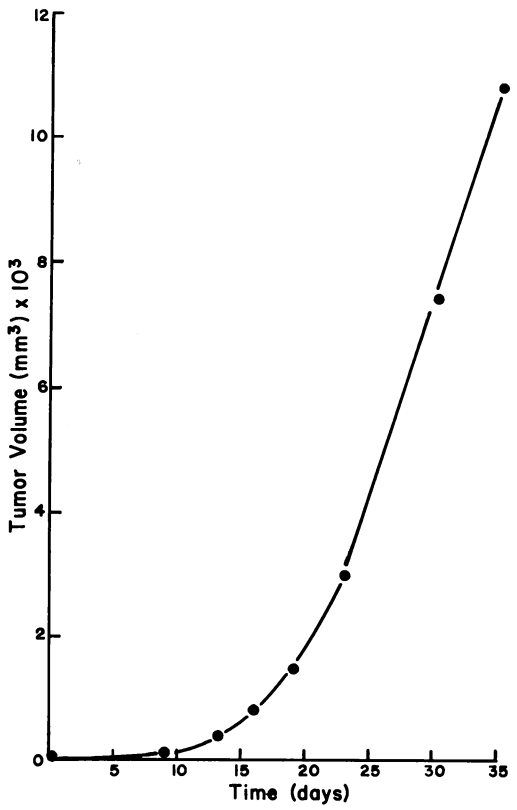
Growth Rate Measurements

It is 6 to 7 days post implantation (PI) before a measurable nodule is detected. By 14 days the mass triples and is about 50 mm^3 and by 28 days the volume reaches approximately 4900 mm^3, an increase of almost 100 fold from the six-day-old tumor. At 35 days, the tumor volume is about 15,000 mm^3 after which time many of the tumors become ulcerated and rupture through the epidermis allowing the liquified necrotic tissue to drain from the area. This changes the dimensions of the tumor, therefore, measurements beyond this time are not recorded (see chart on following page). Lung metastases are not seen grossly until 35 days after implantation at

which time almost all animals have discrete masses in the lungs. At 28 days gross tumor is recognized in the regional lymph node in about 50% of the animals and by 35 days all have tumor in the inguinal node draining the primary tumor site. At 42 days a small number of rats have enlarged spleens and beginning atrophy of the thymus. By 49 days virtually all animals have spleens 1.5-2 times normal size and severe thymic atrophy.

TABLE I

TUMOR GROWTH RATE



The chart is a record of tumor volume growth in $mm^3 x 10^3$ in days after implantation. Note that the tumor is not big enough to measure with a Vernier caliper until about one week after implantation, then there is a logarithmic increase in mass with time for about 2 weeks. By 5 weeks after implantation the tumors are ulcerated so no further measurements are recorded.

Histopathology of the Tumor

The tumor is an adenocarcinoma. The cells are large, pleomorphic and arranged in acini or in sheets. There is a wide range in size and shape of the nuclei. Nucleoli are occasionally seen. The chromatin pattern is light and usually diffuse although there is some clumping of chromatin at the nuclear rim. The cytoplasm is slightly basophilic and frequently has a foamy appearance or contains large single or multiple clear vacuoles (Fig. 1). The cytoplasm is lightly periodic acid-Schiff (PAS) positive. With the trichrome stain the cytoplasm is grey green and the nucleus is red-brown.

There is histologic evidence that the tumor cell is a secreting one because proteinic droplets can be seen at the apices of young cells arranged in acini (Fig. 2) and the cytoplasm is vacuolated in these viable cells. It is not vacuolation associated with degeneration and dying since there are no degenerative nuclear changes seen.

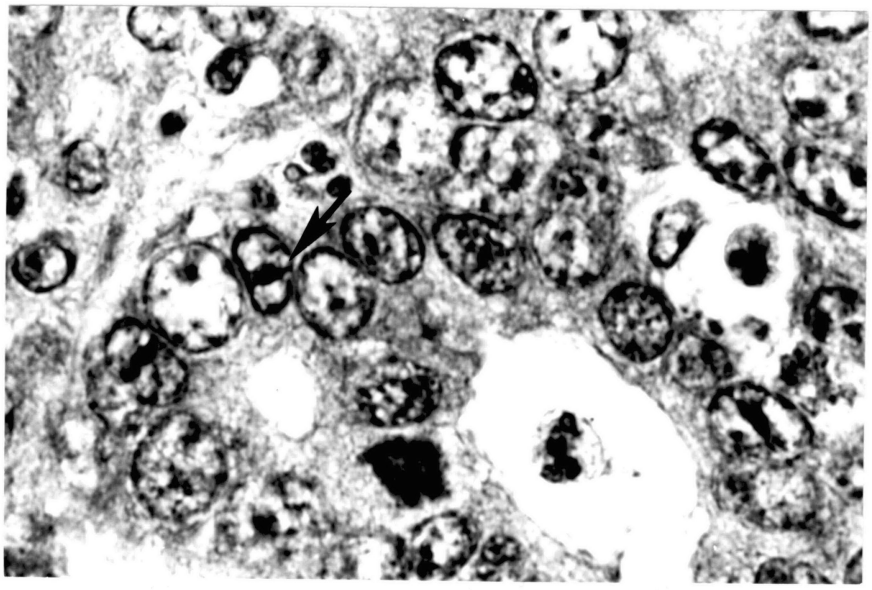


Figure 1. Note the variation in nuclear size, diffuse and clumped distribution of chromatin, occasional nucleolus (arrow) and the foamy cytoplasm. H&E (x1200)

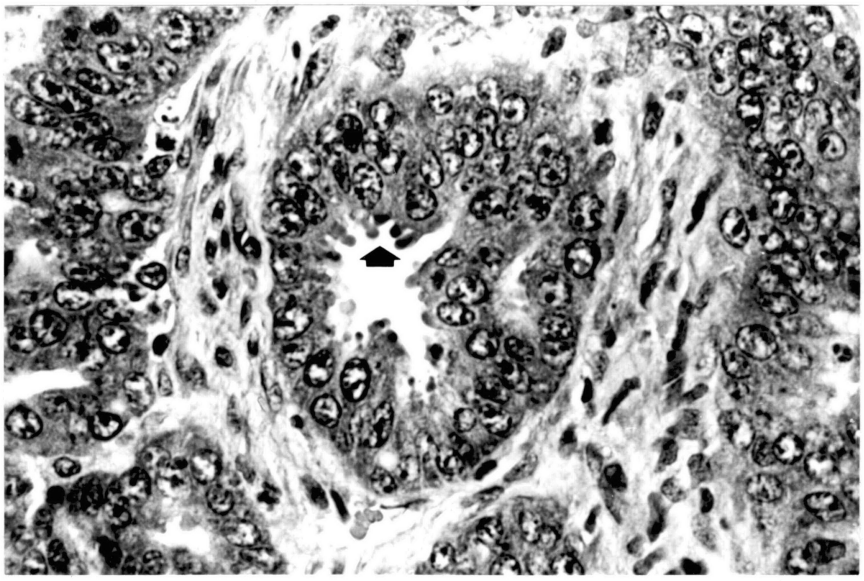


Figure 2. A tumor acinus with secretory globules (arrow) at apices of cells. H&E (x480)

Most commonly the tumor forms acini but occasionally forms sheets as in figure 3.

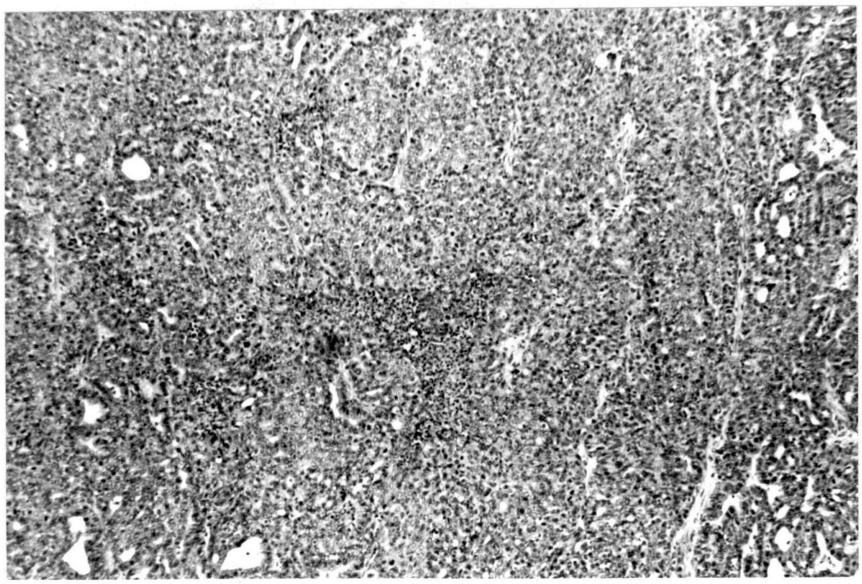


Figure 3. The tumor cells are mainly in sheets. H&E (x75)

Also characteristic of this tumor are the early appearance of necrosis while the tumor is still small ($<1cm^3$) and the high mitotic index (Figure 4).

As the tumor grows it becomes infarcted in the more central areas (Figure 5), presumably because it has outgrown its blood supply. Also it has poorly formed lymphatics that allow for increased hydrostatic pressure within the tumor which predisposes to pressure necrosis.

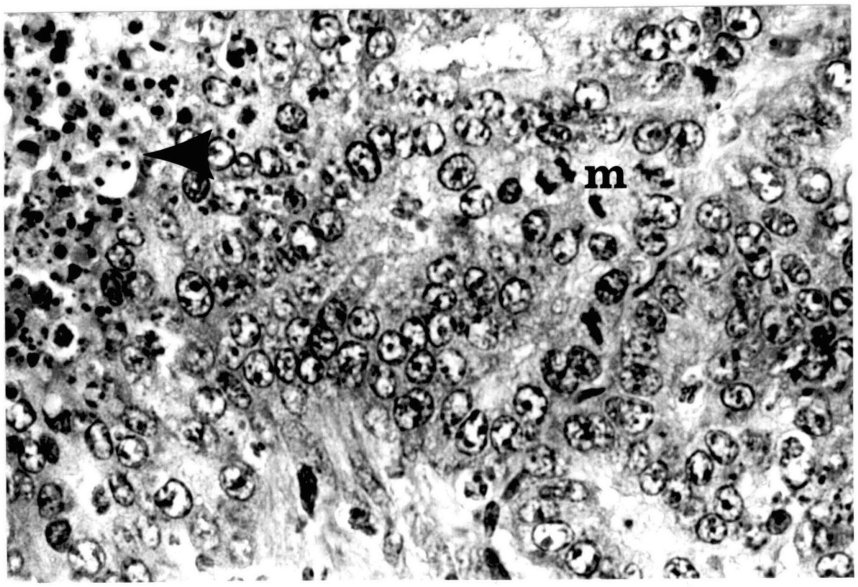


Figure 4. Mitotic figures (m) and areas of necrosis (arrows). H&E (x480)

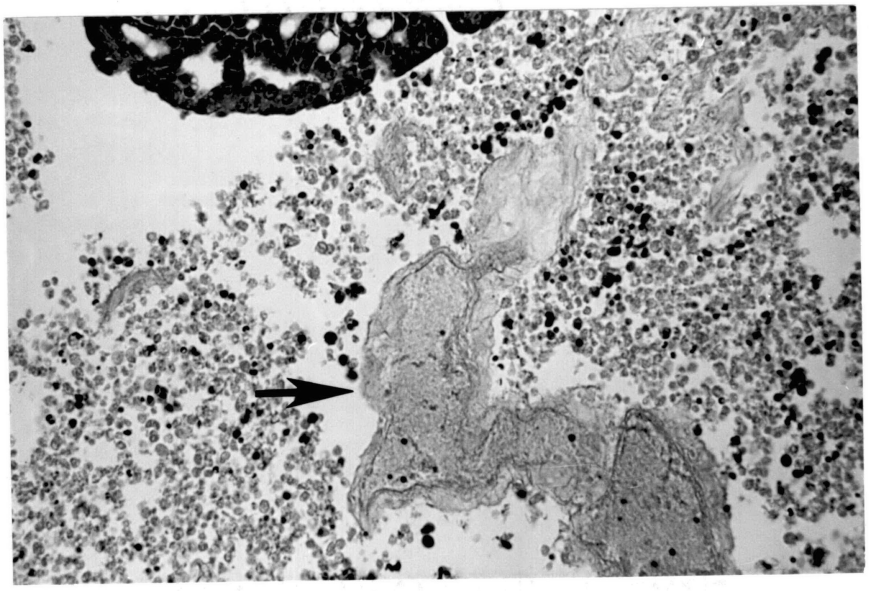


Figure 5. This tumor is 14 days old. Necrotic vessel (arrow) surrounded by necrotic cells and debris. H&E (x75)

Initially the young tumor does not invade the panniculus muscle of the skin but tends to spread laterally in the connective tissue of the subcutis (Figure 6).

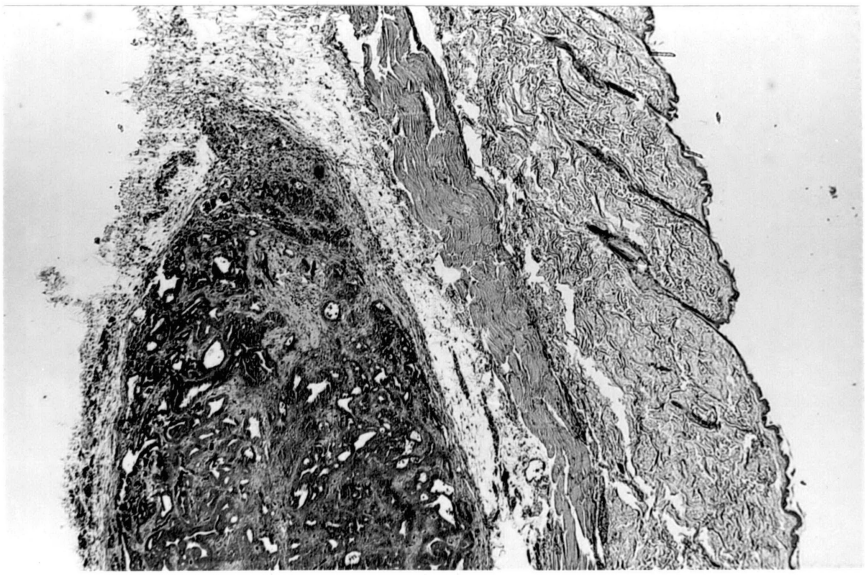


Figure 6. This is a 3-day-old tumor in the subcutaneous tissue. Note the elliptical shape and tendency to grow beneath the panniculus muscle rather than through it. H&E (x75)

Eventually the tumor grows outward through the subcutaneous muscle (Figure 7) and through the dermis and epidermis. It causes necrosis of the skin and ulceration which frequently leaves a gaping hole after necrotic contents have been discharged.

Figure 7. The 12-day-old tumor has begun to invade between the bundles of subcutaneous muscle. H&E (x75)

As the tumor grows, there are increased numbers of blood vessels and lymphatic vessels in the connective tissue surrounding the tumor. The tumor grows by local infiltration and expansion. Strings of tumor cells are seen growing along lymphatic channels (Figure 8).

Histopathology of the Lymph Nodes

Changes in the lymph nodes are predictable. The normal lymph node contains cortical follicles comprised mainly of small lymphocytes (Figure 9). There are very few germinal centers in the follicles.

The normal medullary portion of the lymph node contains stromal reticulum cells, macrophages and a few plasma cells.

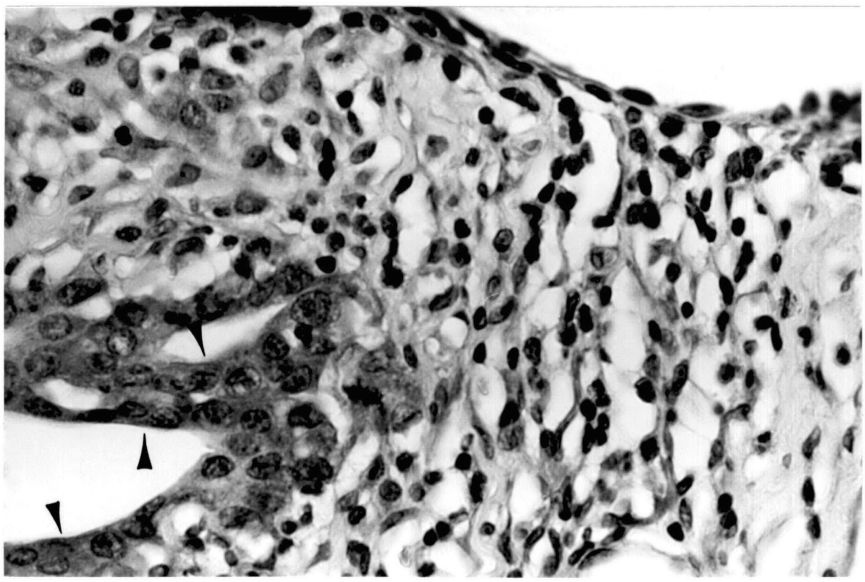


Figure 8. This is subcutaneous connective tissue next to the tumor. Note tumor growing along the wall of a lymphatic vessel (arrows). H&E (x480)

Figure 9. Section of a normal lymph node through inactive cortical follicles comprised mainly of small lymphocytes. H&E (x192)

Concomitant with tumor growth there is progressive generalized lymphadenopathy. This is characterized by increased numbers of germinal centers in follicles (Figure 10) and later diffuse plasmacytosis of the medulla (Figure 11). Cortical hyperplasia was seen initially in the regional node as early as 7 days after implantation of the tumor, and within 20 days after implantation it was seen in the other lymph nodes, both peripheral and visceral.

Figure 10. Notice the germinal centers in the cortical follicles of the lymph node. H&E (x192)

Metastases are first seen in the subcapsular sinus of the lymph node usually by 4 weeks after implantation. The tumor cells are in small clusters that contain mitotic figures and single necrotic cells (Figure 12).

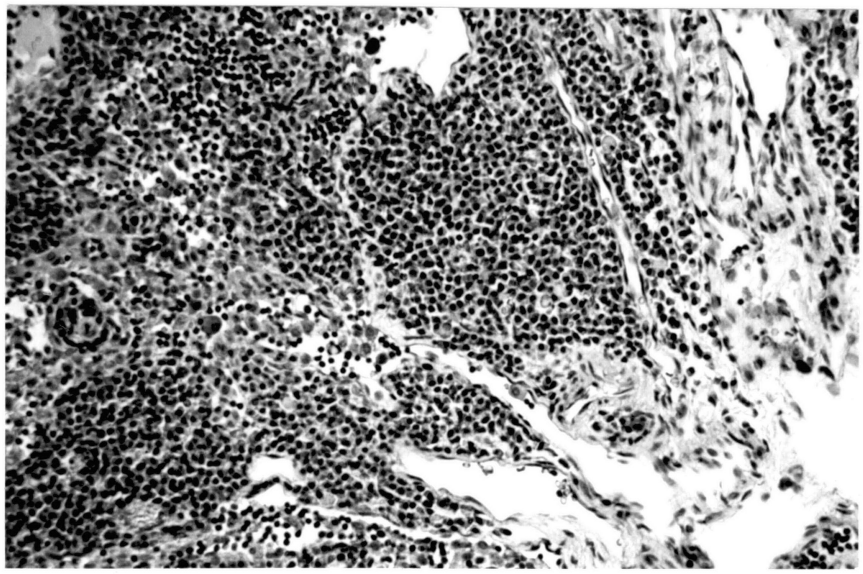


Figure 11. This is a section through the medulla of lymph node with diffuse plasmacytosis. H&E (x192)

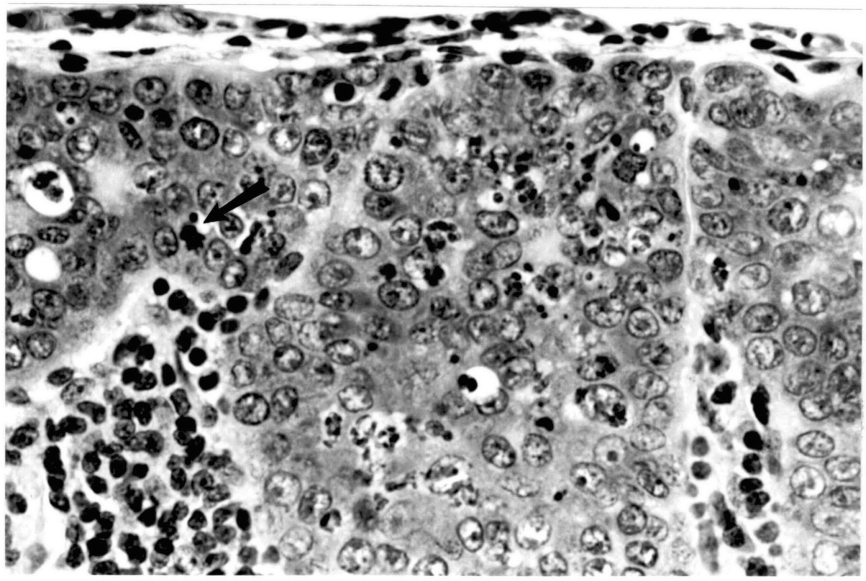


Figure 12. This is a metatasis in the sub-capsular sinus of the regional lymph node Note the mitotic figure (arrow) and the nuclear debris from single-cell necrosis. H&E (x480)

The tumor grows along the subcapsular sinus and then begins to extend into the cortex along the sinus between the follicles (Figure 13). Eventually tumor growth replaces the lymph node.

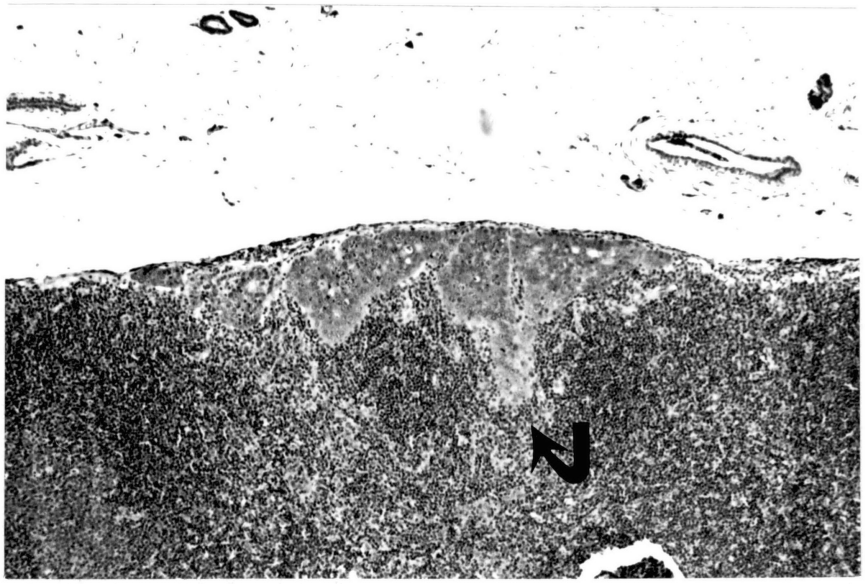


Figure 13. This is a metastasis in the regional lymph node that is growing into the cortex along the sinus between the follicles (arrow). H&E (x75)

The tumor has the same morphologic characteristics in the metastatic sites as it has in the primary implant. In both the lymph node and the lung the tumor forms in sheets and in acini and becomes necrotic.

Young metastases in the lung take the form of small clusters of cells or appear to line alveoli. The tumor cells are easily distinguished from alveolar cells by their larger

size, larger nuclei and more basophilic cytoplasm (Figure 14). The lung metastases were seen by 5 weeks after implantation and were most obvious initially in subpleural sites. The number of lung metastases tended to increase linearly with time.

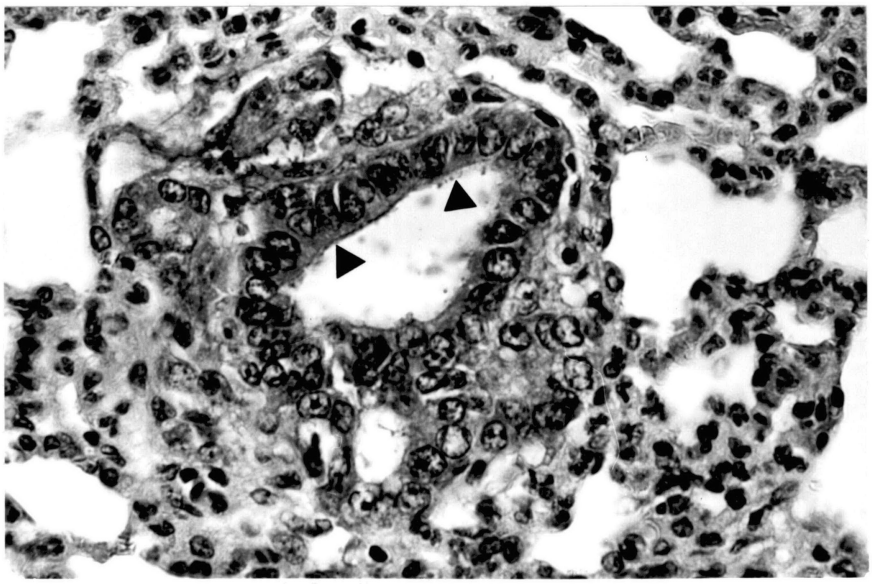


Figure 14. In the lung the metastasis appears to line an alveolus. Note that the tumor cells are larger and darker (arrows) than the adjacent cells. H&E (x480)

Histopathology of the Spleen

The splenic response to tumor growth follows a predictable pattern. The normal spleen in adult female Fisher 344 rats is approximately 1.5 cm long, 6 mm broad across the surface opposite the mesenteric attachment, and 3 mm at its

thickest point which is through the mesenteric attachment. On transverse section, it is triangular. As the tumor grows the spleen gradually increases in size, very slowly at first, then more rapidly until it reaches 3-4 times normal size in rats dying of the tumor. The normal spleen has a fibromuscular capsule and light muscular trabeculae. The rest of the spleen is composed of 1/2-2/3 white pulp or periarterial lymphatic sheaths and 1/2-1/3 is red pulp. The sheaths are oriented around an artery and are composed of several layers of small lymphocytes (Figure 15).

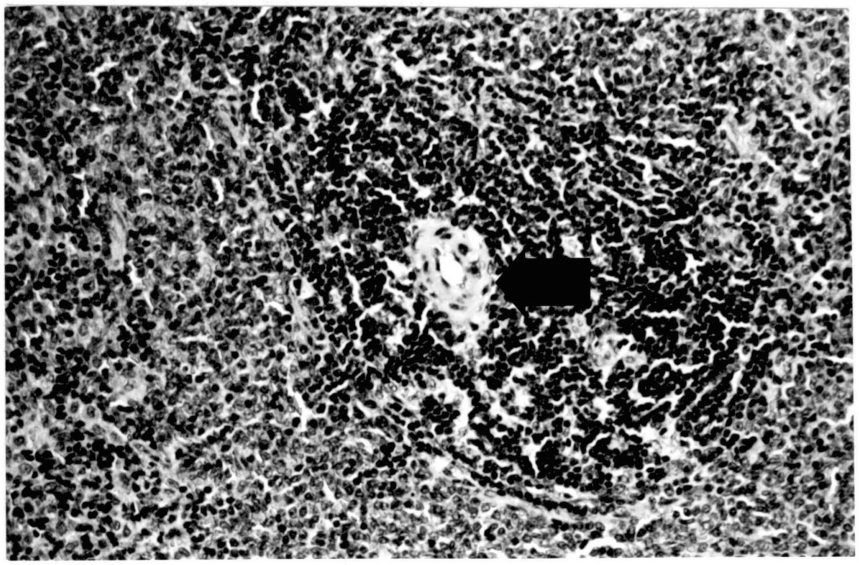


Figure 15. Normal spleen. Note the central artery (arrow) and the periarterial lymphatic sheath composed of small lymphocytes. H&E (x190)

Just peripheral to the central core there is a denser band of reticulum cells and macrophages (Figure 16). The red pulp contains red blood cells, a loose network of vascular channels, hemosiderin-laden macrophages, Reticulum cells and a few red blood cell precursors. Lightly scattered through the red pulp are small lymphocytes singly and in small clusters (Figure 17). Also there are occasional megakaryocytes.

By 12 days after implantation the spleen is slightly plumper than the normal spleen. This increase in size is mainly due to increased mass of the red pulp. The lymphatic sheaths are basically the same as normal except there are a few large lymphocytes centrally as in the beginning of the formation of germinal centers (Figure 18).

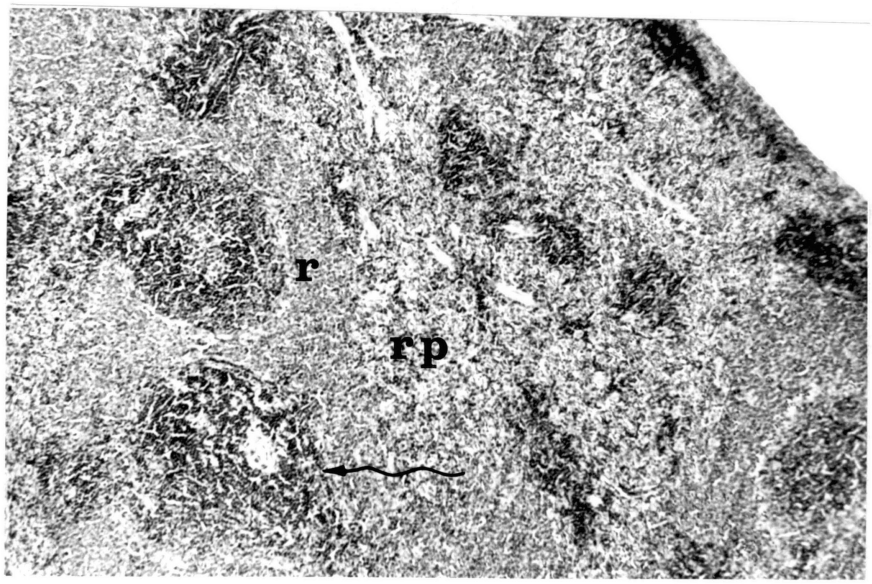


Figure 16. Normal spleen. Note the general organization into lymphatic sheaths (arrow), concentric bands of reticulum cells (r) and red pulp (rp). H&E (x75)

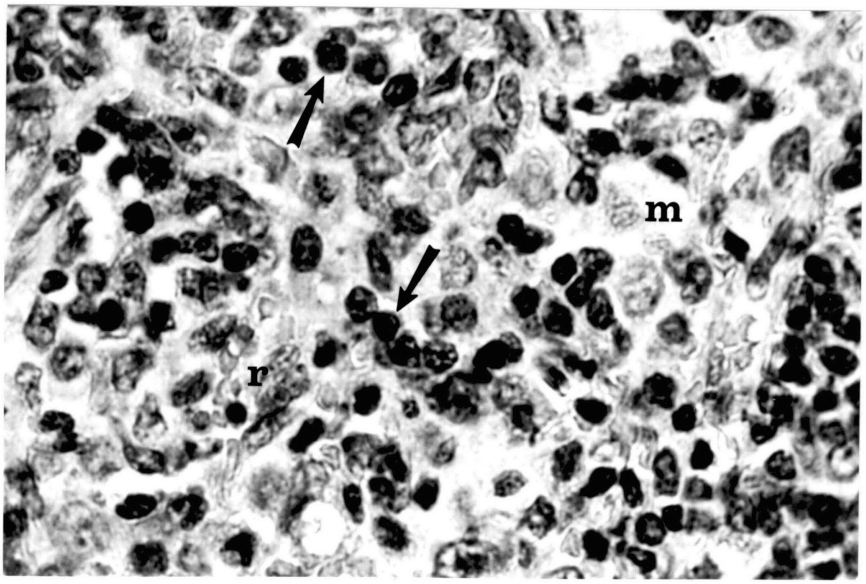


Figure 17. Red pulp of normal spleen. Note the loose arrangement of macrophages (m), reticulum cells (r) and small lymphocytes (arrows). H&E (x780)

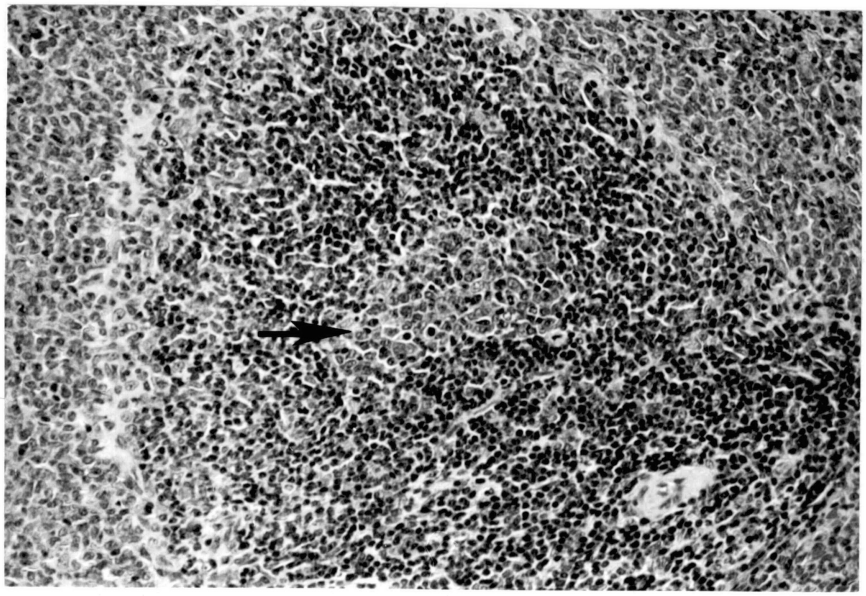


Figure 18. Spleen from a rat 12 days PI. Note the beginning of germinal centers (arrow) in the lymphatic sheath. H&E (x75)

The outer reticulum cell zone of the sheath is slightly larger and less sharply demarcated from the red pulp than in the normal spleen. Neutrophils are noted in the red pulp blood and there are nonsegmented neutrophils in the stroma.

From days 17-25 after implantation, there is no change in the lymphatic sheaths, and in the red pulp there is an increase in the number of clusters of small lymphocytes. There is also an increase in the number of mature and immature neutrophilic leukocytes and a slight increase in the number of megakaryocytes.

By 31 days after implantation of the tumor, the spleen is increased in mass so that instead of being a sharply triangular in cross section, it is more ellipsoid. It has a more uniform thickness from side to side. The red pulp is densely cellular and this accounts for the increase in size. The germinal centers in lymphatic sheaths are larger and more prominent. There is a concomitant decrease in small lymphocytes in the sheaths. In the red pulp there are 2 main cell types, lymphocytes and macrophages mixed in dense clusters. (Figures 19 and 20).

Several mitotic figures are seen in these clusters. There is no difference in the amount of hemato- or granulopoiesis compared with that of the last group.

Gradually the lymphatic sheaths are replaced by reticulum cells and the red pulp becomes a heterogeneous mass of clusters of small and large lymphocytes, macrophages, granulopoietic foci and megakaryocytes (Figure 20).

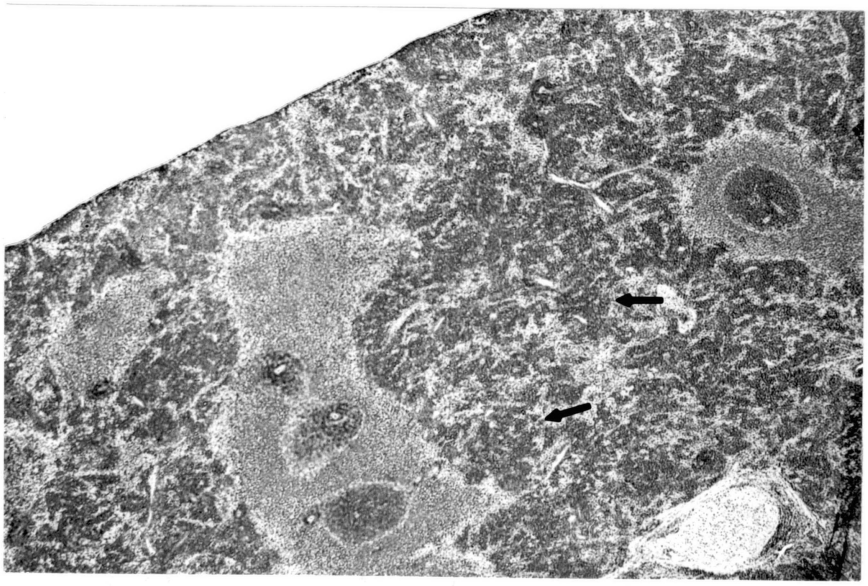


Figure 19. Spleen from a rat 31 days PI. Note the dark clusters of lymphocytes and macrophages in the red pulp (arrows). H&E (x30)
Compare this with the normal spleen in Figure 16.

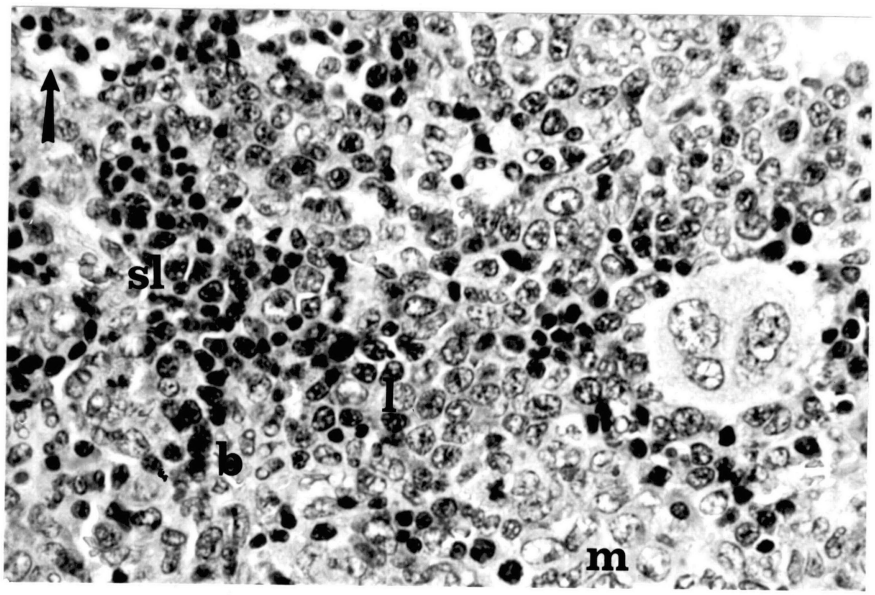


Figure 20. Red pulp of spleen from a rat 55 days PI. The large multinucleate cell is a megakaryocyte. Band shape cells are immature neutrophil leukocytes (b). Small dark cells without cytoplasm are red cell precursors (arrow), macrophages (m), small lymphocytes (sl) and large lymphocytes (l). H&E (x480)

Histopathology of the Thymus

The only significant thymic changes occurred very late in the tumor growth. No recognizable changes occurred in the first 4 or 5 weeks. Only by the time the spleen had become noticeably enlarged did the thymus begin to involute. No proliferative response of any kind was seen.

The normal thymus has a broad outer band of small lymphocytes and a smaller medulla comprised of loose reticulum cells, blood vessels, and macrophages (Figure 21).

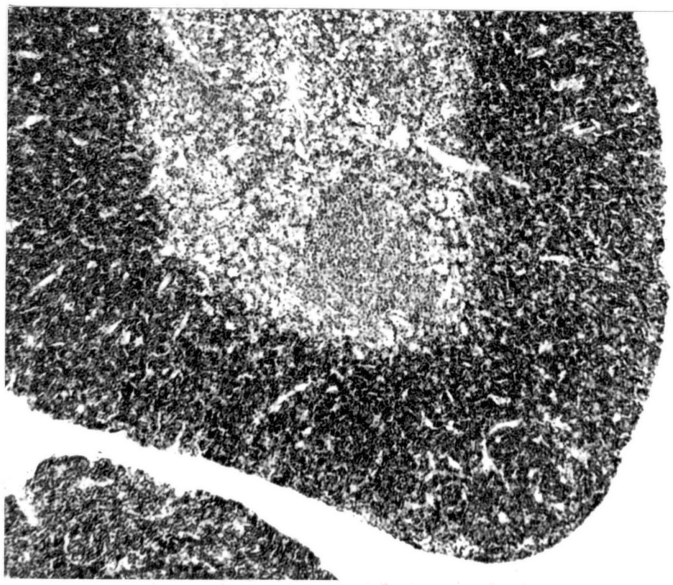


Figure 21. Normal thymus with dense outer zone of small lymphocytes and a loose, less cellular medulla. H&E (x75)

The thymus is very small and hard to recognize in the animal dying of the tumor. Frequently all that is seen

microscopically is stroma, an occasional Hassall's corpuscle and a few small lymphocytes (Figure 22).

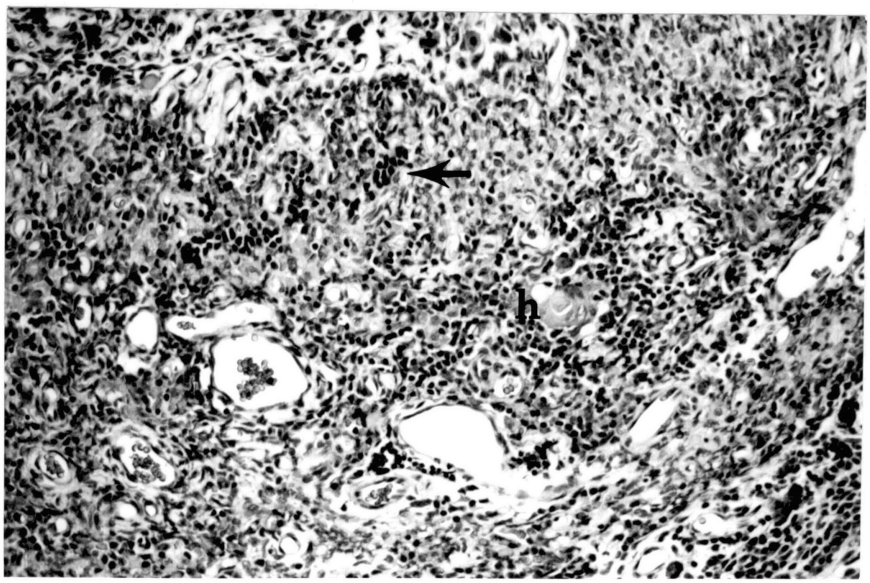


Figure 22. Atrophic thymus from a rat 55 days PI. Note the stromal blood vessels, Hassall's corpuscle (h) and a light scattering of small lymphocytes (arrow). H&E (x190)

Approximately 10% of the rats in this study had pulmonary lesions consistent with mycoplasma pneumonia. No differences were noted in the peripheral lymph node or spleens between these animals and those that did not have pneumonia.

CHAPTER IV

DISCUSSION OF RESULTS

Primary Tumor Growth

In the growth rate table it is shown that the 13762 tumor is a rapidly growing tumor; therefore it is especially suitable as an model because results of individual experiments are obtained in a short time. Although it is not shown in the table the survival time was approximately the same as reported in Bodgen's work (2).

As Kreider reported (6), when the tumors grow to 1-2 cm in diameter there is central necrosis with the formation of fluid filled sacs. These would increase in size until they broke through the overlying skin and discharged their contents.

Tumor Metastases

Lung metastases are not seen as early in this study as in Bodgen's work (2). He reported that by day 18 after implantation all of the animals had metastases to lungs or to other organs. The lungs were not serial-sectioned in this study and it is possible that the early small metastases were missed. Only rarely are tumor metastases seen in organs other than regional lymph nodes and lung and these are in

the liver of rats that lived longer than the average.

Lymph Nodes

Tumor metastases are seen first in the cortical sinus of the inguinal lymph node that drains the tumor. Afferent lymphatics enter the lymph node through the cortical sinus. The proliferation of germinal centers seen in the rats is noted first in the regional lymph nodes by 7 days after implantation. The B cell response (germinal center hyperplasia) seems to be more related to humoral than to local events because germinal center hyperplasia is seen in all body lymph nodes, albeit a week or so later than in the regional nodes. Tumor metastases are observed in the regional lymph nodes with extensive germinal center hyperplasia. The B cell response persists in all lymph nodes through the time that the rat dies of the tumor.

Hunter *et al* (8), in their studies on invasive ductal carcinoma in the human mammary gland, found that sinus histiocytosis in regional lymph nodes was correlated with a very good prognosis and that germinal center hyperplasia was correlated with a very poor prognosis. They hypothesized that the germinal centers may be producing blocking antibody which interferes with cell-mediated immunity. They also noted that in some animal models, including mammary tumors in mice, there was a clear correlation among tumor necrosis, germinal center hyperplasia and the onset of accelerated mammary tumor growth. This agrees with the findings reported

in this study. In none of the rats was sinus histiocytosis of the lymph nodes observed.

In some recent work done on the 13762 rat mammary adenocarcinoma, Lucas (9) found that a blocking factor is produced and its major activity is in the IgG (Immunoglobulin G) fraction of serum (unpublished data). This factor binds preferentially to specific tumor immune lymphocytes and blocks lymphocyte cytotoxicity *in vitro* as well as enhancing tumor growth *in vivo*.

The extreme plasmacytosis of the medulla of lymph nodes is seen after 3-4 weeks of tumor growth and persists through the time that the rat dies of the tumor. This is interpreted as indication of antibody production. This may be an effort to respond to the still circulating tumor antigen. If these are antitumor antibodies they may be ineffective against tumor cells that are coated already by a protective blocking antibody. Since necrosis is an early and consistent feature of this tumor, intracellular antigens may be released into the circulation, and antibodies to these substances may be ineffective in facilitating killing of intact cells. In any case, there is evidence of much antibody production in the face of rapidly growing tumor masses, so one must assume that antibodies are not doing anything related to tumor growth or are ineffective in suppressing tumor growth or that they enhance tumor growth.

Fisher and Fisher (5) showed that the regional lymph node is very important in resistance to tumor growth in

studies with the syngeneic C_3H mammary carcinoma in mice (10). With removal of the regional lymph node there is much less concomitant immunity, even when the animal had been previously immunized for as long as 28 days with a growing tumor. They compared this system with a chemically (methylcholanthrene) induced tumor in which the depressed resistance to tumor challenge was not observed when the regional lymph node was removed. They hypothesized that perhaps in the "less antigenic" C_3H mammary carcinoma system the regional lymph node was more important than in the "more antigenic" chemically induced tumor host systems (11).

Goldfarb and Hardy (12), in their work with the murine mammary adenocarcinoma in C_3H/HeJ mice, measured the phytohemaglutinin (PHA) responses of lymphocytes from regional and nonregional lymph nodes and from the spleen. They found that the regional node was the first lymphoid tissue to be maximally sensitized. The splenic response was later. These reports are brought into the discussion because there was evidence in my work that the regional lymph nodes responded before the nonregional lymph nodes and that perhaps the 13762 mammary adenocarcinoma is less antigenic than some others that are chemically induced.

Bard et al (13) studied a methylcholanthrene-induced sarcoma in mice and showed that although removal of the regional lymph node had no effect on the growth of the tumor or on resistance to subsequent challenge, lymphoid cells from a regional lymph node from a tumor-bearing mouse would adoptively

transfer immunity to a previously unexposed mouse. This suggests that some factor(s) is interfering with the effectiveness of the lymph node response in mice with the rapidly growing tumor.

The Spleen

It is well documented in several different animal tumor models that the spleen plays an important role in both protecting the body from tumor growth and enhancing tumor growth. In the methylcholanthrene induced mammary carcinomas in rats splenectomy increased the incidence of tumor and decreased the latency (14). In the DBA/2J mice with syngeneic mammary adenocarcinomas, there were no metastases without splenectomy (15). Nordlund and Gershon (16) in their work with the Cloudman (S91) melanoma in syngeneic DBA/2 mice, found that with large doses of tumor cells the spleen shortened the latency whereas with low doses of tumor cells the spleen increased the latency.

Bard and Pilch (17) reported on successful adoptive transfer of immunity to tumor challenge using spleen cells from mice with a chemically induced sarcoma.

The most significant change in the spleen in this study is the proliferation of lymphocytes and macrophages. Macrophages are well known for their phagocytic activity and perhaps not so well known for their function as suppressor cells. Kirchner et al (18) reported in 1974 on the suppressor cell activity in spleens of mice with tumors induced by the Maloney

sarcoma virus. In these mice the spleens were enlarged and contained 3-4 times the normal number of mononuclear cells; phagocytic cells were increased to 3 times the normal number and there was a 5-10% decrease in T lymphocytes. The phytohemagglutinin (PHA) response of spleen cells was decreased to 20% of the normal at 15 days after infection and at the same time there was the highest level of specific cytotoxicity. The PHA response could be returned to almost normal by passing the spleen cells through an adherence column. When spleen cells from a mouse with the sarcoma were mixed with normal spleen cells there was a significant drop in the PHA response. These results indicate there is a good possibility in that model system that there are suppressor cells in the spleen and that the cells are the macrophages.

Fortner et al (3) reported that in the 13762 rat mammary adenocarcinoma the cytotoxicity reached a maximum 6-8 days after implantation then abruptly disappeared. They identified the spleen as the source of the cytotoxic cells. They did not test the splenic cells for the PHA response. It is possible that the same type of cellular kinetics is occurring with the spleen cells from rats with the 13762 mammary adenocarcinoma as in the mice infected with the Maloney sarcoma virus.

Thymus

The thymic atrophy was seen very late in the tumor growth. This could have been due to cachexia associated

with chronic illness of several kinds. It is seen in foals for example with bacterial infections that result in death. Perhaps it could be due to a humoral immunosuppressive factor elaborated by the tumor or elaborated by the lymphoid tissues.

When the thymus became atrophied the spleen had considerably increased in mass with large and small lymphocytes and macrophages. Possibly the immunosuppressive factor(s) that allow for tumor growth suppress the lymphoid cells in the thymus.

CHAPTER V

SUMMARY AND CONCLUSIONS

Summary

It was found that the 13762 mammary adenocarcinoma is a rapidly growing tumor that metastasizes to the regional lymph nodes and lungs. The hyperplastic response in the lymph nodes and spleens was characteristic and predictable. The fact that the tumor grows rapidly in the face of this lymphoid response suggests that the response is ineffective in suppressing tumor growth.

Conclusions

The mammary adenocarcinoma 13762 in Fisher 344 adult female rats is a tumor host system that is stable and is one that gives a repeatable pattern of tumor growth and metastases. That there is a characteristic immune response makes it a valuable model for future studies in immunotherapy and chemotherapy.

Recommendations for Future Research

This system is especially suited as a model for breast cancer in women. It is a mammary adenocarcinoma and it metastasizes to local lymph nodes and lungs as do many of

the breast cancers in women. Regional lymph nodes in rats with the tumor have germinal center hyperplasia, which in women is correlated with a poor prognosis. This system therefore would be ideally suited for studies in immunotherapy where efforts could be directed toward changing the responses in the regional lymph nodes.

SELECTED BIBLIOGRAPHY

1. Segaloff, A., "Hormones in Breast Cancer." Recent Progress in Hormone Research, 22, 351-374 (1966).

2. Bodgen, A. E., Esker, H. J., Taylor, D. J., and Gray, J. H., "Comparative Study of the Effects of Surgery, Chemotherapy and Immunotherapy, Alone and in Combination, on Metastases of the 13762 Mammary Adenocarcinoma." Cancer Res., 34, 1627-1631 (1974).

3. Fortner, G. W., Kuperman, O., and Lucas, Z. J., "Immune Response to a Syngeneic Mammary Adenocarcinoma. I. Comparison of Kinetics of Tumor Cell Growth and Cytotoxic Responses in Syngeneic Rats." J. Immunol., 115, 1269-1276 (1975).

4. Kuperman, O., Fortner, G. W., and Lucas, Z. J., "Immune Response to a Syngeneic Mammary Adenocarcinoma. II. In Vitro Generation of Cytotoxic Lymphocytes." J. Immunol., 115, 1277-1281 (1975).

5. Kuperman, O., Fortner, G. W., and Lucas, Z. J., "Immune Response to a Syngeneic Mammary Adenocarcinoma. III. Development of Memory and Suppressor Functions Modulating Cellular Cytotoxicity." J. Immunol., 115, 1282-1287 (1975).

6. Kreider, J. W., Bartlett, G. L., and Purnell, D. M., "Suitability of Rat Mammary Adenocarcinoma 13762 as a Model for BCG Immunotherapy." J. Natl. Cancer Inst., 56, 797-802 (1976).

7. Sass, B., Rabstein, L. S., Madison, R., Nims, R. M., Peters, R. L., and Kelloff, G. J., "Incidence of Spontaneous Neoplasms in F 344 Rats Throughout the Natural Life-Span." J. Natl. Cancer Inst., 54, 1449-1456 (1975).

8. Hunter, R. L., Ferguson, D. J., and Warwick Coppleson, L., "Survival with Mammary Cancer Related to the Interaction of Germinal Center Hyperplasia and Sinus Histiocytosis in Axillary and Internal Mammary Lymph Nodes." Cancer, 36, 528-539 (1975).

9. Lucas, Z., "Natural Mechanisms that Impair the Host's Tumors." Breast Cancer Task Force, Intercom., 6-7 (October, 1976).

10. Fisher, B., and Fisher, E. R., "Studies Concerning the Regional Lymph Node in Cancer. I. Initiation of Immunity." Cancer, 27, 1001-1004 (1971).

11. Fisher, B., and Fisher, E. R., "Studies Concerning the Regional Lymph Node in Cancer. II. Maintenance of Immunity." Cancer, 29, 1496-1501 (1972).

12. Goldfarb, P. M., and Hardy, M. A., "The Immunological Responsiveness of Regional Lymphocytes in Experimental Cancer." Cancer, 35, 778-783 (1975).

13. Bard, D. S., Hammond, W. G., and Pilch, Y. H., "The Role of the Regional Lymph Nodes in the Immunity to a Chemically Induced Sarcoma in C_3H Mice." Cancer Res., 29, 1379-1384 (1969).

14. Kim, U., "Metatastasizing Mammary Carcinomas in Rats: Induction and Study of Their Immunogenicity." Science, 167, 72-74 (1970).

15. Edwards A. J., Sumner, M. R., Rowland, G. F., and Hurd, C. M., "Changes in Lymphoreticular Tissues During Growth of a Murine Adenocarcinoma. I. Histology and Weight of Lymph Nodes, Spleen and Thymus." J. Natl. Cancer Inst., 47, 301-311 (1971).

16. Nordlund, J. J., and Gershon, R. K., "Splenic Regulation of the Clinical Appearance of Small Tumors." J. Immunol., 114, 1486-1490 (1975).

17. Bard, D. S., and Pilch, Y. H., "The Role of the Spleen in the Immunity to a Chemically Induced Sarcoma in C3H Mice." Cancer Res., 29, 1125-1131 (1969).

18. Kirchner, H., Chused, T. M., Herberman, R. B., Holden, H. T., and Lavrin, D. H., "Evidence of Suppressor Cell Activity in Spleens of Mice Bearing Primary Tumors Induced by Maloney Sarcoma Virus." J. Exptl. Med., 139, 1473-1486 (1974).

VITA

Lea Rockwell Gordon

Candidate for the Degree of

Master of Science

Thesis: BIOLOGICAL BEHAVIOR OF THE RAT MAMMARY ADENOCARCINOMA 13762

Major Field: Veterinary Pathology

Biographical:

Personal Data: Born in Hartford, Connecticut, June 16, 1945, the daughter of Lawrence R. and Annette R. Gordon.

Education: Graduated from Rockville High School, Rockville, Connecticut, in June, 1963; pre-veterinary training from the University of Connecticut and the University of Minnesota; received the Doctor of Veterinary Medicine degree from the University of Pennsylvania in 1970; enrolled in Masters program at Oklahoma State University and completed the requirements for the Master of Science degree at Oklahoma State University in December, 1976.

Professional Experience: Resident in large animal pathology, New Bolton Center, School of Veterinary Medicine, University of Pennsylvania, 1970-1972; Visiting Lecturer in Pathology at the University of Bristol, England, School of Veterinary Science, 1972-1973; temporary instructor in pathology, Oklahoma State University, 1974-1975; assistant professor of pathology, Oklahoma State University, 1975-1976.